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Implications of Longterm Diameter-Limit Harvesting: Effects on Radial Growth of Red Spruce (Picea rubens) and Genetic Diversity of White Pine (Pinus strobus)

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IMPLICATIONS OF LONG-TERM DIAMETER-LIMIT HARVESTING: EFFECTS ON RADIAL GROWTH OF RED SPRUCE (*PICEA RUBENS*) AND GENETIC DIVERSITY OF WHITE PINE (*PINUS STROBUS*)

By

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For over 3 centuries, diameter-limit harvesting has been a predominant logging method in the northeastern United States. Silvicultural theory asserts that such intensively selective harvesting can lead to genetic degradation. A decrease in softwood productivity has recently been reported in Maine - has a long history of dysgenic selection degraded the genetic resources of Maine softwoods, contributing to a decrease in growth and productivity? This study examines two aspects of potential implications of diameter-limit harvesting: effects on residual phenotypes of red spruce and impacts on genetic diversity of white pine.

Radial growth of residual red spruce trees in stands experiencing 50 years of fixed diameter-limit harvesting was measured using annual increment rings and compared with residual red spruce trees in positive selection stands. Trees remaining after several rounds of diameter-limit harvesting exhibited significantly smaller radial sizes throughout

their lives, and displayed significantly slower growth rates for the first 80 years of measured growth. These results strongly suggest that the largest and fastest-growing genotypes and their respective gene complexes determining good radial growth have been removed from the diameter-limit stand. Dysgenic selection can be observed in fixed diameter-limit stands, resulting in a diminished genetic resource and decreased residual stand value.

To examine more direct genetic implications of long-term diameter-limit harvesting, microsatellite DNA markers were implemented to study genetic diversity of eastern white pine in Maine. Three age groups of trees were studied: mature trees older than 200 years, juvenile trees 5-30 years old, and embryos. Trees were genotyped at 10 microsatellite loci. Overall genetic diversity levels of eastern white pine in Maine were extremely high, with an average observed heterozygosity of 0.762. Genetic differentiation was minimal among and between all three age groups, although an excess of heterozygotes was shown in the mature and juvenile groups that was not reflected in the embryo group, which actually had a slight heterozygote deficiency. Allele frequencies did not differ significantly between age groups, but did reveal more rare and low frequency alleles in the embryo groups than in the mature group. Overall, low frequency alleles comprise the largest portion of alleles in the sample population, with no common alleles evident overall. These results suggest that significant genetic degradation has either not occurred for white pine, or that the results of dysgenic selection have not yet emerged. It is clear, however, that selective harvesting could result in a loss of low frequency alleles, which are a primary reserve of evolutionary potential in a species.

Implications of these studies affect industrial forestry, regional economics, and ecological concerns for the northeast. Long-term diameter-limit harvesting can lead to a degradation of residual phenotypes, and an overall decrease in stand quality. Potentially, a loss of low frequency, locally adapted alleles could result in a decrease of allelic richness and degradation of the regional genetic resource. Decreased genetic variation can lead to seriously limited evolutionary potential of species and ecosystems, particularly in rapidly changing environments. Based on these findings, I recommend a reassessment of any harvesting prescription that includes fixed diameter-limit removals, particularly for species that have low natural genetic diversity levels or a limited natural range, such as red spruce. Maintenance of a healthy genetic reserve can avoid effects of dysgenic harvesting.

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INTRODUCTION

Worldwide demand for forest products is rapidly increasing as world population grows. In the Northeastern United States, recent reports have projected 5-8% per decade increases in the consumption of pulpwood and sawlogs (Griffith and Alerich 1996). The 1998 Timber Supply Outlook for Maine (Gadzik *et al.* 1998) projects a serious shortfall between net annual growth and projected harvest volume, suggesting that "the current rate of growth in Maine's forests can not [indefinitely] sustain...the current levels of timber harvest." The report indicates that net growth under current forestry practices is approximately 86% of harvest volume over the next 50 years. Compounding this imbalance is the recently reported decline in growth per unit of growing stock for Maine's softwoods over the last 3 inventory periods (Table 1.1).

Inventory Rates

	1959-1971	1972-1982	1982-1995
Softwoods	0.038	0.019	0.016
Hardwoods	0.024	0.025	0.024

Table 1.1. Inventory rates for softwoods and hardwoods in Maine, 1959-95. Growth per cubic foot of growing stock has steadily decreased for softwoods but not for hardwoods in the same inventories. Based on Griffith and Alerich (1996).

Why has softwood growing stock become seemingly less productive over the years? A number of possible reasons for this decline have been suggested, including changes in age class distribution within the growing stock (forest maturation), pollution, and climate change. Anthropogenic forces may be contributing, as suggested by Ledig (1988), Beaulieu & Simon (1994), Rajora *et al.* (2000) and others. Given the long history of selective harvesting in Maine, a factor contributing to the decline in growth may be related to a decline in frequency of the best alleles at genes that control growth

characteristics. Although silviculturists and population geneticists have long warned of the potential for genetic degradation, selection against these "good genes" is very difficult to demonstrate.

Two species of particular regional interest are red spruce (*Picea rubens* Sarg.), which has a relatively limited range in northeastern North America, and eastern white pine (*Pinus strobus* L.), which is economically important throughout northeastern North America. Both of these species have historically been particularly valued for their unique wood properties; white pine for construction lumber and ship masts, and red spruce for furniture and musical instruments. White pine, as an economic mainstay of the region, is more widely studied than red spruce; the dynamics of red spruce as a species are of special interest as the species has regional value as a naturally regenerating timber species. In Maine, both white pine and red spruce are highly valued and have been harvested steadily for centuries. Old-growth red spruce and white pine stands that have remained undisturbed by human impacts are extremely rare, primarily existing only at some fragmented, high elevation sites in Maine and south along the Appalachians; white pine virgin old growth can be found in isolated stands in Quebec and Ontario as well.

Dysgenic Selection - History

European colonization of North America began a long history of heavy resource exploitation. There is evidence that forest ecosystems were altered by native peoples, particularly by burning (Williams 1999), but not until European settlement did these disturbances grow to scales large enough to affect the entire range of particular forest species. Clearing for agriculture and residential use, burning, and harvesting of timber as

an economic resource all began occurring along the eastern coast of North America over 500 years ago. Commercial logging of the northeast was established in the early 17th century. By 1645, regular traffic for white pine ship masts had begun, and in 1710 British parliament passed the Broad Arrow Policy, an act to preserve white pines for use in the masting of Her Majesty's Navy (Williams 1999). According to this act, all non-privately owned trees 24 inches in diameter or more, as measured 12 inches from the ground, were marked with the Royal "R" or a broad arrow, and were thereby reserved for the British navy. The Broad Arrow Policy has been evidenced in some old trees as recently as 1935 (Howard 1986). By 1900, the depletion of the white pine resource had moved west, eventually stripping all merchantable eastern white pine throughout the United States range (Williams 1999). White pine was certainly not the only species of interest to colonists; all forest types in the northeast, including the spruce-fir forests of Maine, have been cut heavily for at least 150 years (Coolidge 1963).

Commercial logging for white pine and red spruce was first recorded in 1623 with the establishment of a sawmill in York, Maine (Howard 1985). Because of limits to felling technology and transportation, only the best lumber was initially valuable enough to justify removal, so lumbermen harvested all of the biggest and best trees in each stand, leaving the rest to grow and re-populate the stand; diameter limit harvesting was a natural decision. After many years harvesting from one area, the larger trees were depleted, and consequently, diameters of desirable trees decreased. In the next wave of harvesting, the best trees remaining in a stand were again removed. As technology improved and harvesting became easier and more efficient, smaller trees became cost-effective to harvest, but only the largest, well-formed trees were of use. This repeated, serial

depletion of the forests using high-grading harvesting techniques continued throughout the centuries, and still occurs widely in the present (R. Seymour pers. comm.). In Maine about 440,000 acres of diameter-limit harvests were reported on industrial land between 1982 and 1995, indicating that this harvesting method was still being practiced extensively during the most recent inventory period (Greenwood *et al.* 2000).

Dysgenic Selection - Theory

It is widely agreed that selection techniques have dysgenic potential, but most arguments thus far have been anecdotal, speculative, or inconclusive; very little scientific evidence is available. Silviculture, by its nature, will affect genetic structure of a population (Wright 1976, Smith *et al.* 1997). Specifically, silvicultural selection can be a very powerful tool in manipulating the genetic composition of a stand. Buchert *et al.* (1994) explain the theory of positive genetic selection as well as the potential for negative selection. Positive selection within a forest stand is the removal of the poorest phenotypes in a stand, allowing the better-adapted trees to grow and reproduce. An example of human-induced positive selection is the increase of white pine resistance to pests through removal of heavily damaged, non-resistant trees, detailed by Ledig and Smith (1981). Conversely, negative or dysgenic selection should occur when the phenotypically best trees for a certain trait are removed from the reproducing pool of a population, leaving inferior trees to regenerate the stand. Theoretically, the best genes and gene complexes could thus be removed from the population, reducing genetic diversity as well as genetic quality of the stand. For positive or negative selection to occur, the traits selected must be heritable. In trees, growth traits such as diameter and height growth are moderately to

weakly heritable, whereas form traits and wood quality are often highly heritable (Howe 1990).

Dysgenic selection has been a topic of discussion for tree improvement scientists as well as silviculturists for several decades, and many of the most prominent minds in each field have expressed serious concern over the consequences. D. M. Smith (1997) believes that selection harvesting is the most likely method of reducing forest productivity by eliminating good genotypes. He suggests that while the effects of dysgenic selection may not be immediately evident, the decrease in genetic potential after several rotations could be significant. Zobel and Talbert (1984) explain that diameter-limit harvesting and harvesting of only desired species in mixed-species forests are among the most damaging practices even during one harvest rotation.

> "When the best trees are removed [from a stand], leaving the inferior ones to produce seed for the next generation, dysgenic selection will result. The most adverse cutting method within a species in even-aged stands is the diameter limit cut in which all trees over a given size are removed and the small diameter trees are left to grow and reproduce the stand (Trimble 1971). The diameter limit cut is a type of dysgenic selection that is widely practiced throughout the world and results in succeeding generations of poorer-quality, slower-growing stands." (p219)

Effects of dysgenic selection harvesting are explained by Zobel and Talbert:

> "Since such characteristics as disease resistance or straightness of tree bole are strongly inherited, a few generations of dysgenic selection can result in 'minus-type' stands."

Although Kang (1979) mathematically demonstrates the potential for negative selection to alter the genetic structure of a population, experimental evidence is sparse and

polemical. Ledig (1992) uses the example of eastern white pine to explain the potential for genetic degradation through selective exploitation, and continues by mathematically examining harvest scenarios with a model of various heritable traits under varying selection intensities; this demonstration clearly shows the potential for selection to negatively alter genetic composition.[1] He concludes that while the short-term effects of dysgenic selection may be ephemeral, the long-term consequences could be irreversible. Beyond theoretical considerations, there is varied experimental evidence of anthropogenic selection pressure changing the genetic make-up of forest tree populations. Because results and conclusions of these types of investigations are so widely disparate, a comprehensive and critical review of literature is a formidable task; several excellent reviews have been published (Ledig 1992, Savolainen and Karkkainen 1992, Howe 1990).

Regeneration is an important aspect of successful selection, as successful trees must pass on allele complexes to their progeny in order to increase the frequency of these genes; methods of regeneration can also be very useful in demonstrating effects of selection. In tree-improvement programs, seed sources are collected from positive-trait trees and grown in nurseries; both of these treatments may be selective processes, potentially resulting in genetic changes. Decreases in genetic richness have been reported for seeds and progeny of phenotypically selected trees (Cheliak *et al.* 1988, Hamrick 1991, Gomory 1992, Rajora 1999), but in many cases, no change in genetic composition resulted from selection of seed sources (Neale 1985, Knowles 1985, Williams *et al.* 1995, Adams *et al.* 1998, Schmidtling *et al.* 1999). See Chapter 2 Introduction for a more detailed review.

Indirect examinations of historical data reveal some changes of genetic resources attributable to harvesting. The impacts of selective harvesting in the Mediterranean throughout civilization have been debated, with some evidence pointing to severe dysgenic selection of Cedar of Lebanon (*Cedrus libani* Loud.) (Thirgood 1981, Savolainen and Karkkainen 1992). Ledig (1986) suggests that selective cutting of the phenotypically elite trees may have degraded pitch pine (*Pinus rigida*) and loblolly pine in the eastern United States. Modern populations of southern pines such as longleaf pine (*P. palustris*) may have been degraded by two centuries of high-grading leaving many areas populated by inferior genotypes (Schmidtling and Hipkins 1998). In harvested old-growth white pine stands in Ontario, reports indicate a substantial loss of genetic diversity in the stand after a harvest removing most of the best trees (Buchert *et al.* 1997, Rajora *et al.* 2000).

In the forests of Maine, stands are often mixed-species and uneven-aged, and because there can be plentiful regeneration, some assume that diameter-limit harvesting may not affect the genetic potential of the stand. However, Zobel and Talbert (1984) point out " the largest trees are harvested, with the incorrect assumption that the smaller trees are younger and are therefore genetically as good as the harvested trees." Especially in the case of uneven-aged spruce-fir forests, the smaller trees may not be simply younger, but instead the ones of poorest vigor and more likely to be poorer genotypes. In addition, they continue "One of the most serious types of dysgenic selection is the harvesting of desired species from mixed stands, leaving only undesired species. Vast changes in land productivity and timber quality have resulted and are still being produced

by this policy. ... It is especially bad in the tropics, in the northeastern part of the United States, in central and eastern Canada...".

In natural populations of red spruce and white pine, where harvesting has extended unchecked and unregulated throughout nearly the entire range of the species for over 400 years, the effects of selection could range from negligible to dramatic and may be difficult to predict. Particularly, because these species are long-lived and selection has occurred for only a few generations, negative impacts could yet be incipient; the sum consequences of which have not yet manifested. The genetic systems of forests and the genetic implications of harvesting are poorly understood. Has selection against better growers altered growth patterns of trees or genetic composition of forest stands? Has this affected the overall softwood productivity, contributing to the recently reported decline? There are several approaches to evaluate this problem. Most research has focussed on the effects of positive selection in either form or growth traits, or for seed orchard use, including use of range-wide seed collections. As tree improvement programs show, artificial phenotypic selection can affect the phenotypes of progeny. Similar effects of negative selection have been observed, but not well quantified. An important key to evaluating impacts of selective harvesting is to ascertain whether specific phenotypic traits are being changed as a result of selection.

The first project examines differences in the radial growth patterns of residual red spruce trees after 5 decades of fixed-diameter selection harvesting. Because few spruce genes have been mapped and few molecular markers exist with which to study genetic dynamics more directly, this study can only report trends in phenotype changes after selective harvesting. Genotypic changes are very difficult to study, particularly as the

conifer genome is one of the largest known and few trait-linked genes have been identified. An examination of genetic impacts of harvesting can be accomplished by studying genetic diversity. Levels of genetic variation in a species, a population, and an individual stand, as well as the potential for replenishing diversity in stands are each important aspects of the genetic resource. The second project applies microsatellite DNA markers to directly measure levels of genetic diversity in 2 white pine stands, and to compare older trees with progeny--younger regeneration and seeds. Although the levels of genetic variation are shown to be very high for this species, a comparison of age groups suggests a potential for dysgenic selection to occur.

CHAPTER 1:
IMPACTS OF LONG-TERM DIAMETER-LIMIT HARVESTING ON RESIDUAL STANDS OF RED SPRUCE IN MAINE

ABSTRACT

Diameter limit harvesting has long been suspected as a dysgenic forestry practice, but conclusive, practical evidence on the effects of this selection technique is lacking. After several centuries of high-grade harvesting, red spruce populations (*Picea rubens* Sarg.) in Maine may have experienced a degradation of their natural genetic resource. Because red spruce displays low levels of genetic variation, it may be particularly susceptible to effects of artificial selection, which could be a contributing factor to the recently documented decline in softwood productivity. To determine the effects of several rotations of diameter-limit harvesting on the residual population of trees, I investigated 100-year radial growth patterns of residual trees in these stands in comparison with residual trees in a positive selection stand. I measured annual increment rings to determine cumulative radial growth at progressive ages, as well as radial growth rates over time.

Results suggest a difference in average phenotypes in diameter-limit residual stands. After nearly 50 years of fixed diameter-limit harvesting, the residual trees of these stands were nearly 40% smaller and 32% slower-growing than residual trees in positive selection stands. Diameter-limit residuals were initially smaller in radius, and remained significantly smaller than positive selection residuals throughout their life span, despite major release events. After release, the diameter-limit residual trees did show response to release by increasing growth rates, but the increase was relatively small.

Growth rates were consistently and significantly lower for fixed-diameter residuals until the final 20 of 100 years when rates became similar to positive selection stands.

In comparison to residual 5-year selection stands, fixed diameter-limit harvest treatments result in residual stands that contain smaller, slower-growing trees that do not respond as well to release. Based on these results, it appears that residual trees in diameter-limit stands are phenotypically inferior compared to residuals in positive selection stands. Long-term diameter-limit harvesting over a greater area could lead to genetic degradation, as more successful phenotypes are removed, and poorer phenotypes predominate. These poor phenotypes may be the result of poorly adapted genotypes, which will be the primary source of reproduction in these stands. Red spruce stands subject to diameter-limit harvesting will result in progressively less valuable growing stock with less future growth potential.

INTRODUCTION

Picea rubens is an economically and ecologically important forest tree species in Maine. Its particular growing properties create a specific niche in 13 ecotypes and as a major component of five forest cover types in northeastern North America (Seymour 1995, Blum1990). The species ranges from upper elevations of North Carolina, north to southeastern Ontario, and east to the Canadian Maritime Provinces. Maine lies within the middle portion of *P. rubens* natural range and makes up a significant part of the total species population (Figure 1.1). Red spruce is a genetically uniform species, with genetic variability estimates of 6.9%, and has been suggested to have limited adaptive potential (DeHayes and Hawley 1992). A reduction in range has been reported of 1/5 to 1/10 of the species' former distribution in terms of population sizes and numbers (NRC-CFS 1999). Recently, increased mortality and a decline in growth has been reported range-wide (Hornbeck and Smith 1985, Scott *et al.* 1985) with little conclusive evidence of the cause. Several factors may be influencing this decline such as forest maturation, pollution (McLaughlin *et al.* 1987), or climatic change (Johnson *et al.* 1988). Red spruce has a long history of high-grade logging, which has fragmented populations and may be impacting the growth characteristics of the remaining populations. The long history of heavy and repeated selective harvesting of red spruce may be a factor in the observed growth decline. Unfortunately, although dysgenic selection has long been considered a risk to the genetic well being of a forest tree population (Zobel and Talbert 1984), there is very little concrete, quantitative evidence of the impacts.

This study uses annual ring increment measures to examine radial growth patterns of residual red spruce trees in selectively harvested spruce-fir stands. Two types of

harvesting were compared: fixed diameter-limit harvests and 5-year selection harvests. Different growth patterns for the trees remaining after harvest may be an indication that diameter-limit harvesting is a direct genetic selection pressure, selecting against fast-growing, well-adapted genotypes and thereby reducing the genetic vigor of the stand. Residual trees are the reproductive resource of the stand, and are important for future growth and harvesting. Dysgenic selection could seriously impair the growth or survival potential of the stand.

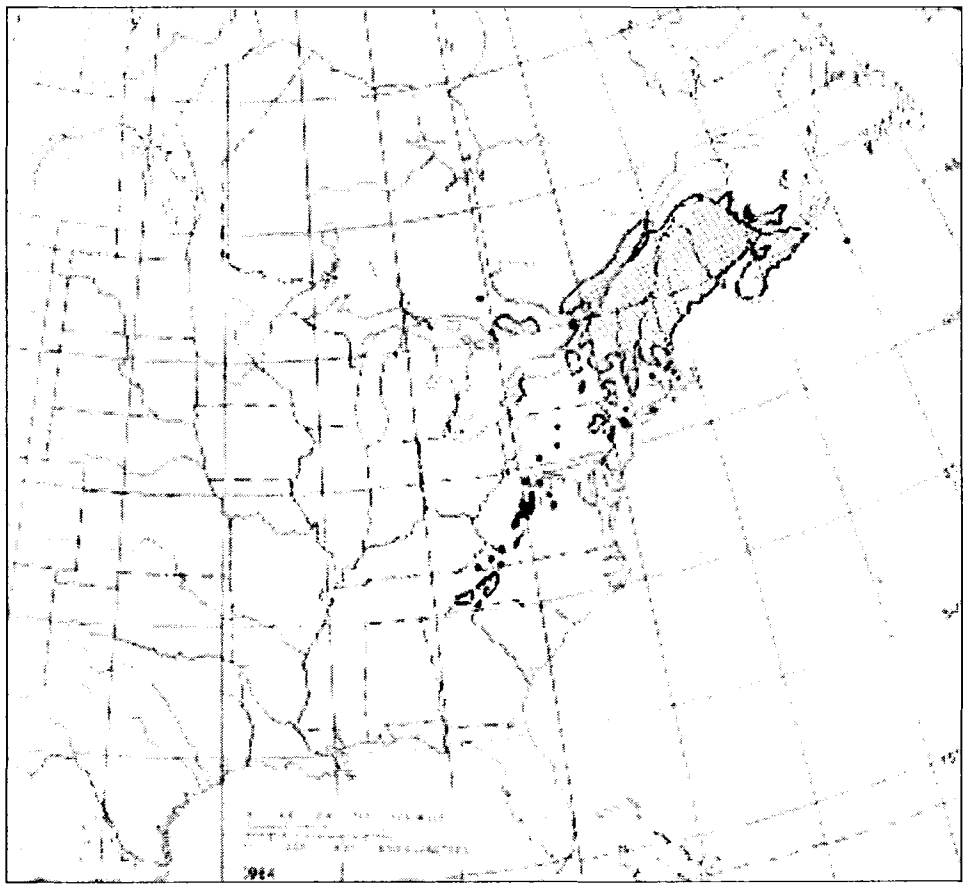

Figure 1.1: Distribution map of *Picea rubens* (Blum 1990).

MATERIALS AND METHODS

Stands were located in the Penobscot Experimental Forest (PEF), a USDA Forest Service installment in Bradley, Maine (Figure 1.2). This 3800-acre forest has been under long-term experimental management since the 1950's, although the land now belongs to the University of Maine. The PEF is located in the Acadian Region, dominated by *Tsuga canadensis* (L.) Carr., *Abies balsamea* (L.) Mill., and *Picea rubens* in mixture with hardwood and other softwood species. Four stands, approximately 10-ha each have been managed according to specific silvicultural treatments for nearly 50 years. Two replicates each of spruce-fir stands representing two methods of harvesting were studied: compartments C4 and C15 represent fixed diameter-limit harvesting (FDL) and compartments C9 and C16 have been managed in a 5-year selection harvesting (S05) system (Figure 1.3). The USDA Forest Service notes that FDL is not technically considered a silvicultural treatment. FDL stands have undergone five harvests with fixed removal guidelines of all red spruce trees greater than or equal to 9 inches (22.86cm) diameter at breast height (DBH) . S05 stands have been harvested nine times with a combination of single-tree and group selection cutting using structural goals (see Seymour and Kenefic1998 for details). The S05 stands serve as a comparison treatment in which no diameter-limit harvesting has been implemented for at least 50 years. Although historical harvesting details are not documented, over the past century these stands have been harvested moderately as evidenced by old stumps.

Trees greater than pole-size (>5 cm) at breast height were identified along random stand-wide transects. Increment cores were collected from breast height, sanded, and amount of radial growth measured by width of annual rings (Figure 1.4). To smooth out

effects of annual variation affecting radial growth throughout the stands, 20-year increments were measured for each tree from the pith to the bark. For cores that did not reach the precise center of the pith, a ring chart was used to correct for missing rings. Annual ring data for trees in compartments C9 and C16 were generously provided by Laura Kenefic, USDA Forest Service (Seymour and Kenefic 1998). Over 600 total trees were included in the measurements. To ensure that the trees analyzed were pre-treatment residuals of harvesting treatments, and to obtain enough 20-year measurement increments in each tree, only trees 100 years or older were analyzed. The final sample size was N = 113.

Statistical analysis was performed using SYSTAT package (SPSS Science, Chicago, IL). Two harvest treatments were analyzed with 2 replicates each: 5-year selection (S05) and fixed diameter-limit (FDL). Five growth periods (GP) were used for analysis: 0-20 years (GP_20), as measured from the pith to the 20^{th} annual growth ring, 20-40 years (GP_40) measured from the 20^{th} ring to the 40^{th}, and so on for GP_60, GP_80, and GP_100. These growth periods can represent either the amount of radial growth occurring during a 20-year time frame, or cumulatively, the amount of growth that has occurred up to the final measured growth ring, which is also a measure of radial tree size. For each 20-year growth period, comparisons of harvest treatments were made using paired t-tests to examine radial tree sizes and average growth. Analysis of Variance (ANOVA) tests were performed using repeated measures to analyze overall tree sizes, tree size over time (five 20-year age groups), and radial growth (mm) over time in the two treatments.

Figure 1.2: Location of PEF sites. The USDA Forest Service Penobscot Experimental Forest (PEF) is located in the towns of Bradley and Eddington, Penobscot County, Maine.

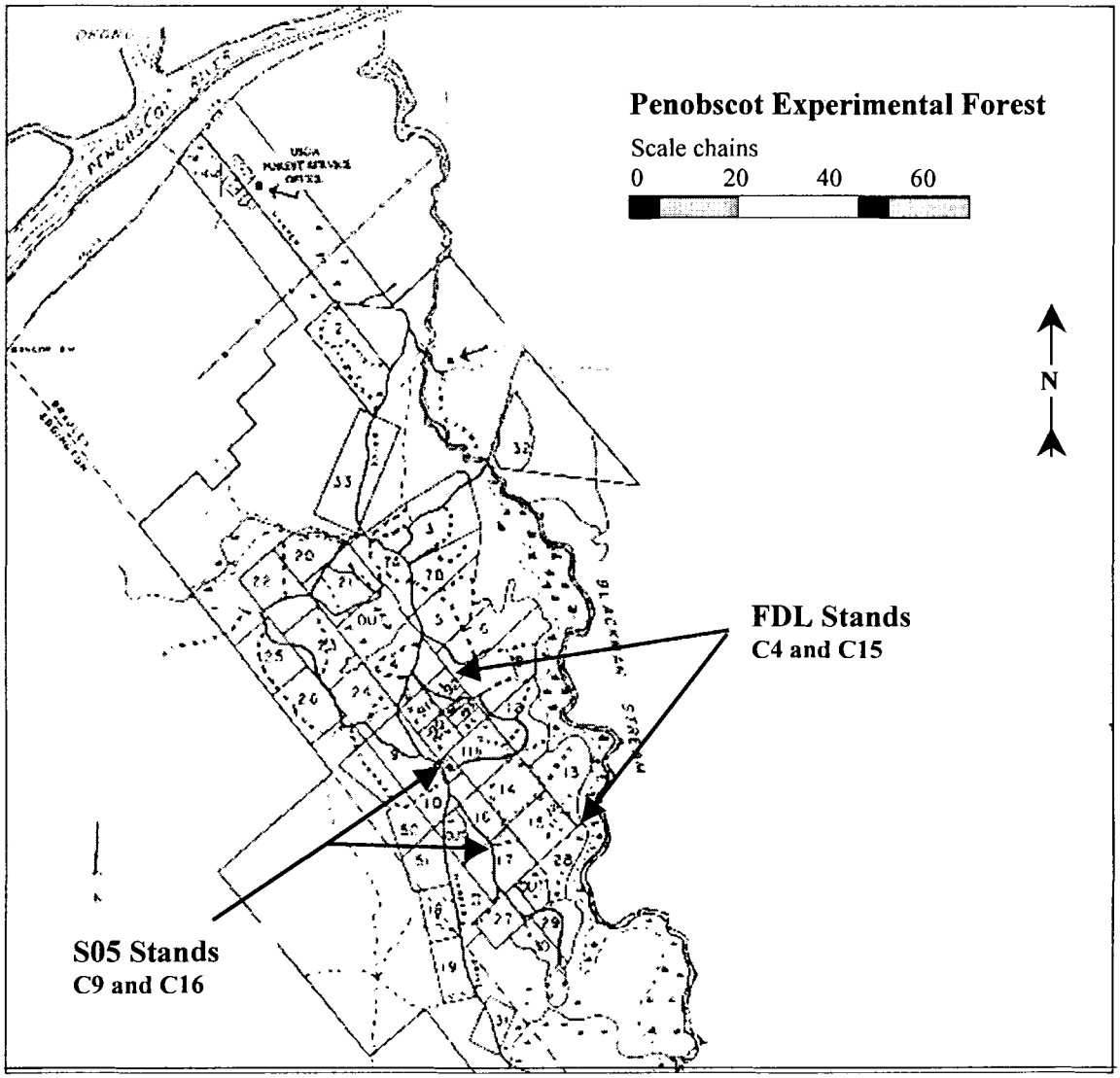

Figure 1.3: Map of four study compartments in the PEF. C4 and C15 have been harvested for over 50 years using fixed-diameter limit (FDL) guidelines. C9 and C16 have been managed using a 5-year selection system (S05) and represent spruce-fir stands in which no fixed-diameter limit harvesting has occurred within the last 50 years.

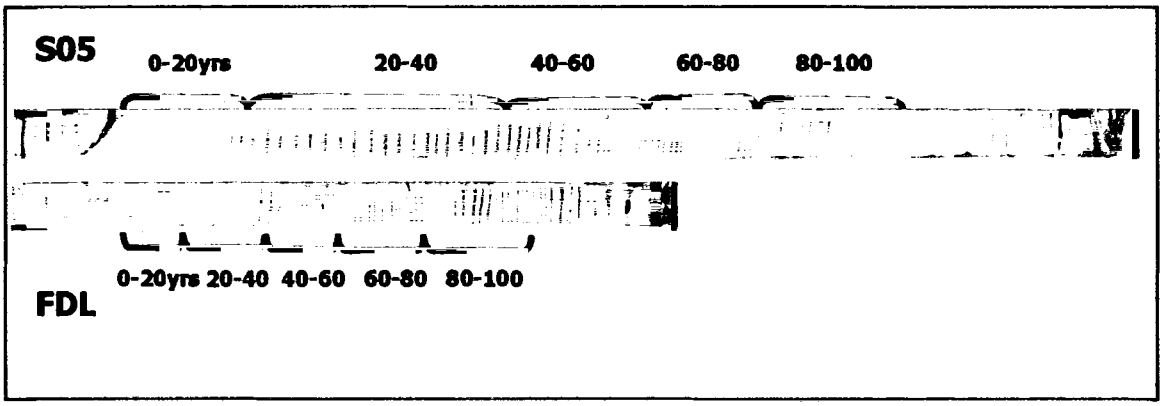

Figure 1.4: Red spruce increment core examples. Cores were collected from breast height, then sanded and analyzed. Annual rings were measured by hand in 20-year growth periods starting from the pith, ending at the 100th ring. Growth periods are depicted in brackets for an example of an S05 core and an FDL core.

RESULTS AND DISCUSSION

Paired t-tests showed no significant differences ($P < 0.01$) between the harvest treatment replicates, so data for replicates was pooled into treatments S05 (5-year selection harvests) and FDL (fixed-diameter limit harvests). Charted data points can be viewed in figures 1.5 and 1.6. Summary statistics for samples and sample groups are listed in table 1.2. Data reveal two specific trends; FDL residual trees are smaller and slower growing than S05 trees. Tree size can be measured by using cumulative radial growth over time (over 5 growth periods spanning 100 years) as if looking at snapshots of increasing tree sizes at progressive ages. Growth rates can be compared by analyzing non-cumulative radial growth during each period as if looking at each growth period as an independent snapshot of the amount of growth attained within that period. Of course, larger trees that may have better canopy position have the opportunity to grow more than small, suppressed trees, so growth is not independent of tree size. Additionally, although diameter can be correlated to height, height growth can not necessarily be inferred from radial growth.

Tree Size

Comparison of treatments for cumulative radial increment growth, or overall tree size, over the five growth periods show that the average radius at breast height for residual trees in FDL stands after 100 years of growth is much smaller than the S05 residual trees (Figure 1.7). These differences are highly significant at $P<0.01$ both among treatments and among the five 20-year growth periods. Average cumulative growth shows clear evidence that residual FDL trees are consistently smaller than residual S05 trees throughout at least 100 years of their life spans (Figure 1.9).

	20		40		60		80		100	
	S05	**FDL**	**S05**	**FDL**	**S05**	**FDL**	**S05**	**FDL**	**S05**	**FDL**
N	53	60	53	60	53	60	53	60	53	60
Minimum	0.50	0.50	6.00	6.00	12.5	12.00	18.92	16.00	24.21	29.50
Maximum	56.65	28.50	102.35	64.00	153.44	83.50	199.33	119.00	235.67	162.00
Mean	23.256	12.12	41.55	23.51	62.04	36.54	88.35	55.08	113.27	79.81
St Error	1.72	0.90	2.98	1.73	4.23	2.30	5.63	3.16	6.61	4.28
St Deviation	12.50	7.00	21.72	13.43	30.76	17.81	40.95	24.47	48.09	33.12

	20		40		60		80		100	
	S05	**FDL**	**S05**	**FDL**	**S05**	**FDL**	**S05**	**FDL**	**S05**	**FDL**
N	53	60	53	60	53	60	53	60	53	60
Minimum	0.50	0.50	3.00	2.00	3.62	3.50	1.80	3.50	5.40	6.50
Maximum	56.65	28.50	55.81	39.00	59.94	34.00	63.24	53.00	63.91	79.80
Mean	23.256	12.12	18.30	11.39	20.49	13.03	26.31	18.54	24.92	24.74
St Error	1.72	0.90	1.50	1.00	1.76	1.00	1.91	1.47	1.79	2.04
St Deviation	12.50	7.00	10.90	7.75	12.78	7.76	13.90	11.42	13.04	15.83

Table 1.2: Summary statistics for annual increment ring measurements (mm). Tree size is shown above (cumulative radial growth at 20-year periods), and tree growth (non-cumulative incremental radial growth over each 20-year growing period) is shown below.

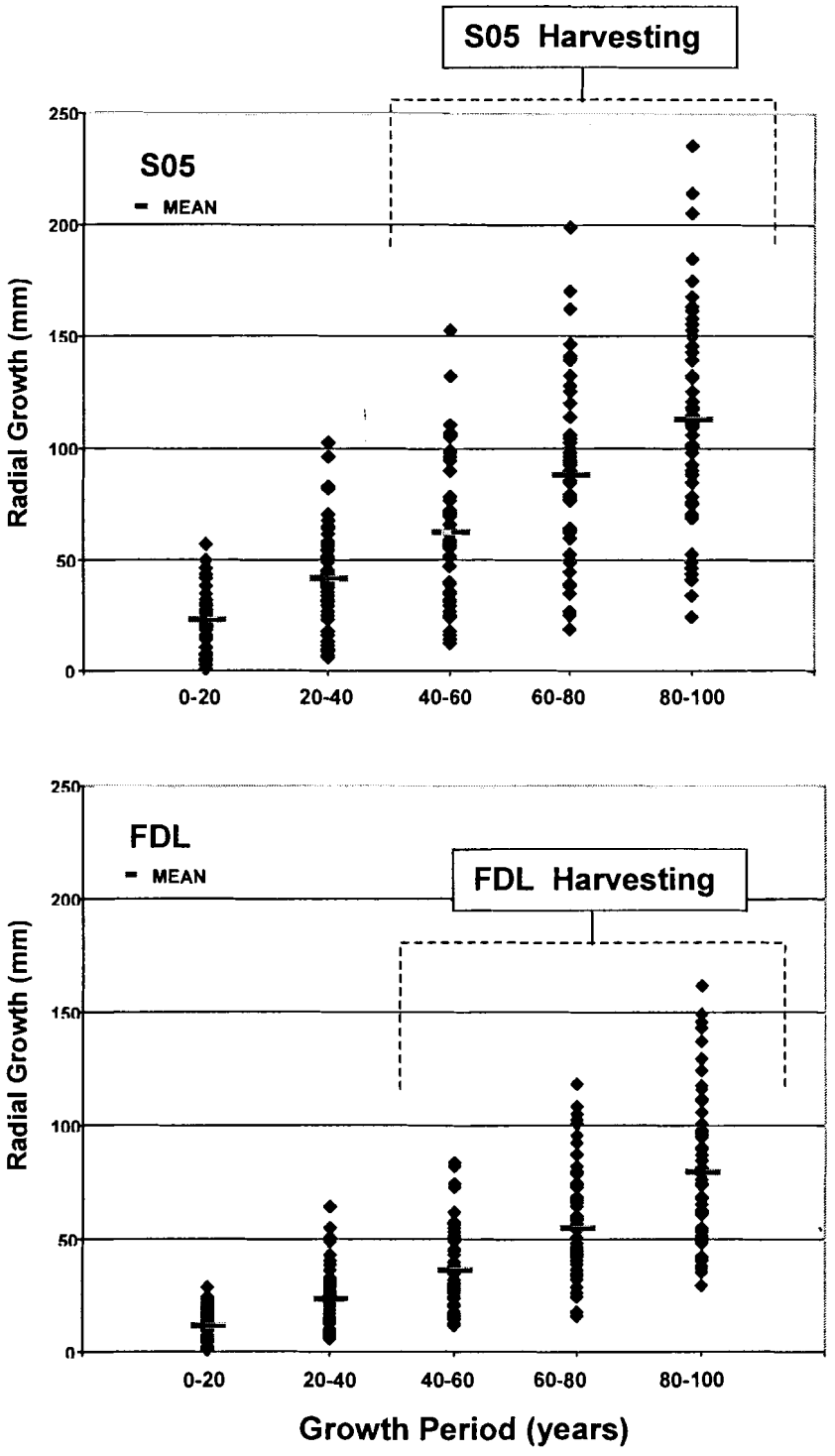

Figure 1.5: Data points for cumulative radial growth. Growth of trees at each 20-year growth period in S05 stands (top) and FDL stands (bottom). Means are indicated by dashes and treatment harvesting period is noted. The S05 trees show a broader range of sizes and much larger trees at each age. There is an abundance of very small trees even in the 100-year age group; small, suppressed trees over 100 years old are common in red spruce.

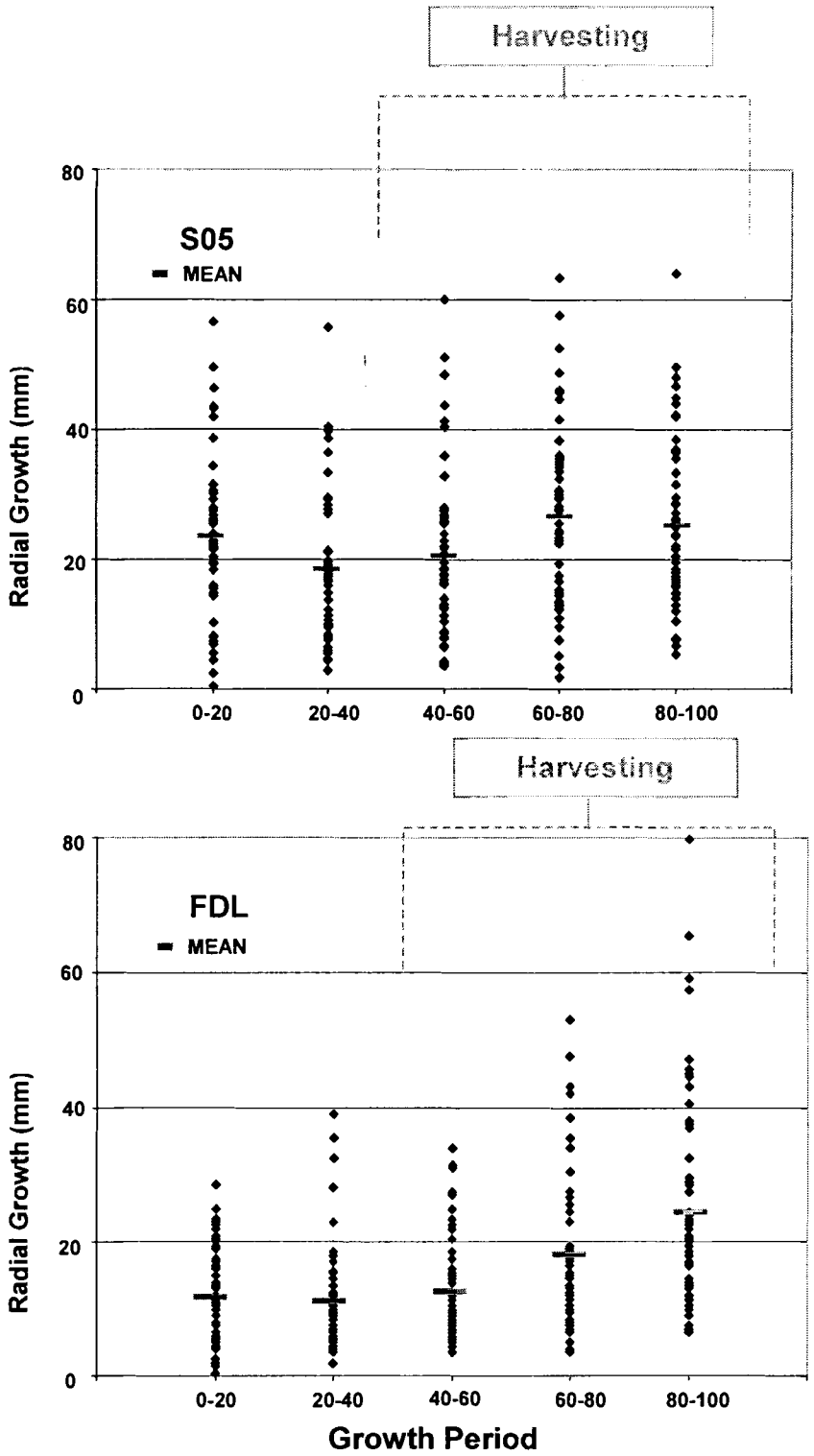

Figure 1.6: Data points for radial growth. Growth of trees during each 20-year growth period in S05 stands (top) and FDL stands (bottom). A larger range of data points and a higher frequency of large radial increment growth exist in S05 stands. The upper range outliers in the 80-100 year period for FDL stands affect mean size and growth.

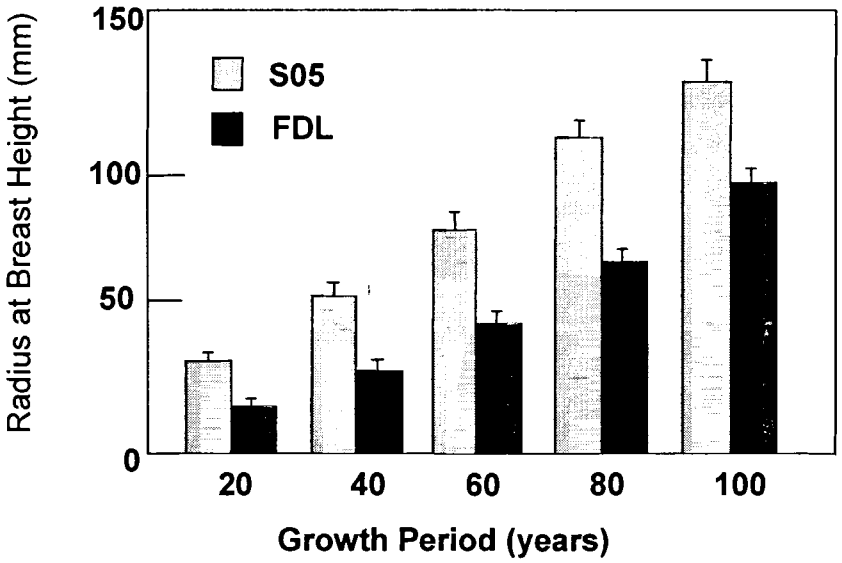

Figure 1.7: Average radius of residual trees. Residual trees older than 100 years in S05 and FDL stands shown at each growth period. Throughout 100 years of growth, FDL residual trees maintain a smaller radial size.

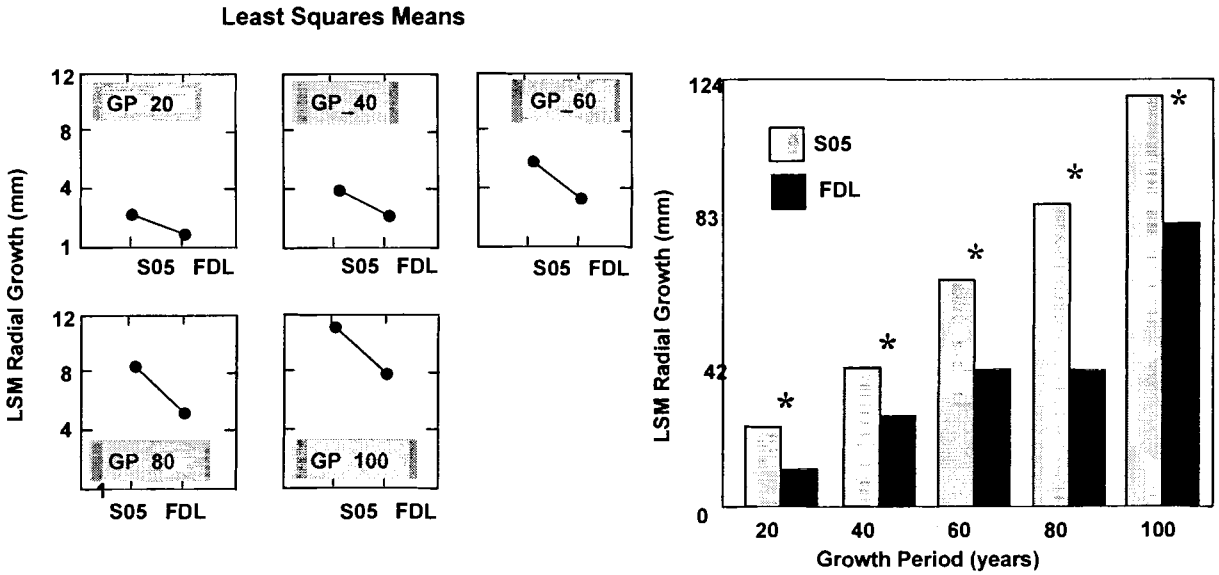

Figure 1.8: Least squares means for each growth period (GP). Corrected for variation in the data, significant differences in average tree sizes at each growth period are apparent between the two treatments. * indicated significance at P<0.001.

In their first 20 years, trees in the FDL treatments started much smaller than S05 trees. At each subsequent 20-year period, the FDL trees remained smaller than S05 trees (Figure 1.7), which is particularly significant when the natural growth characteristics of red spruce are closely considered. Red spruce relies on advance regeneration (Seymour 1995), growing as suppressed trees very slowly for long periods in the understory until a release event, at which time they begin to grow more rapidly. Even very small red spruce, 2-4 feet high and just a few centimeters in diameter may be anywhere between 10 and 100 years old (Kenefic, pers. Comm., Morris, 1948). This means that any one of the trees sampled might be very large or very small, very old or very young, which accounts for the great variation in the size averages at any of the 20-year growth periods, as each tree is measured from the pith. Larger release events, such as complete removal of all large trees in FDL harvests, should offer suppressed trees a better opportunity for rapid growth than smaller group selection harvests. Despite this, all residual FDL trees were much smaller during the initial 20 years of growth, and despite potential release events (harvests of all larger trees), they remained relatively smaller throughout the next 100 years (Figures 1.5 and 1.7).

ANOVA analysis reveals strong statistical significance for the effects of harvest treatment and growth period on tree size, as well as an interaction between the two factors (Table 1.3). An interaction is expected, as each 20-year measurement is not independent of the previous years' growth; tree size, canopy position, and live crown ratio could all be affected each progressive measurement of growth. Least squares means taken as a comparison of treatments at each growth period show the dramatic size

differences at each age, with significant differences between the treatments at $P<0.001$ (Figure 1.8).

	Source	SS	df	MS	F-ratio	P-value
Between subjects	**TRT**	**36.487**	**1**	**36.487**	**24.019**	**0.000**
	Error	**168.618**	**111**	**1.519**		
Within subjects	**Time (GP)**	**242.146**	**4**	**60.537**	**785.677**	**0.000**
	Time*TRT	**2.010**	**4**	**0.503**	**6.522**	**0.000**
	Error	**34.210**	**444**	**0.077**		

Table 1.3: ANOVA table for effects of treatment and growth period on cumulative radial growth.

Tree Growth

In contrast to cumulative radial growth, which indicates overall tree size, the amount of radial growth disassociated from previous growth allows for comparison between growth periods, giving a picture of how trees grow at different ages. The two treatments present distinct growth patterns (Figure 1.9). S05 trees have higher growth levels throughout the 100 years, but demonstrate a different pattern of growth; where FDL trees continue to increase the amount of radial growth at each interval, S05 trees experience decreases in growth at GP_40 and later at GP_100. Paired t-tests comparing average radial growth during each growth period show significant differences at each of the first GPs, up to 80 years, but no significant differences between the treatments at the final period of 80-100 years. Growth for FDL residual trees remains lower throughout the first 4 periods, but then the averages become quite similar. The similarity in growth

at GP_100 can be seen in figures 1.9 and 1.11. Using a grouped t-test that pools all of the GPs in each treatment, there is a significant difference between the overall average growth, with more FDL residuals experiencing overall lower levels of growth, and more S05 trees in a large range at higher levels (Figure 1.10). The range of data points is particularly large for S05 stands, which may indicate high levels of natural variation in these stands.

Examination of the patterns of growth over time for the two treatments reveals a significant difference in the rate of growth and the changes in rates (Figures 1.6 - 1.12). Residual trees in the FDL stands grow less during each 20-year period, and experience less of an increase in growth between each period. This may be an indication that the residual trees are not as well adapted to site conditions as the S05 residuals, and are not prepared to take advantage of release from suppression. The number of very suppressed, older trees in S05 stands confirm that better average growth of S05 trees is not due to the fact that they are inherently more established trees and therefore are larger and have better canopy position. There are enough small and mid-range trees in both treatments to represent a full spectrum of canopy positions (Figure 1.5). Despite this, there is no clear indication that FDL residuals responded to release (overstory harvest) by significant growth increases compared to previous growth (Figures 1.7 and 1.8). Moreover, the harvest events in the FDL stands were inherently more severe for removal of overstory (as all the biggest trees were removed), so residuals should experience significant response to release and less overstory competition than those in S05 stands. Looking at the changes in growth between each 20 year period, it is evident that release events did not trigger periods of faster growth in the FDL stands during the time of documented

harvesting (Figure 1.7). Because successful trees in FDL are serially removed, it is possible that successful trees differentiate themselves only after 100 years of growth after reaching breast height, and that these trees would begin to make up the size and growth deficit in FDL stands after 100 years. The similarity of growth during the final 20-year period could indicate such a trend; because the FDL trees are much smaller at this 80-100 year period, however, they would require much higher growth than that seen in S05 trees to attain similar overall tree size.

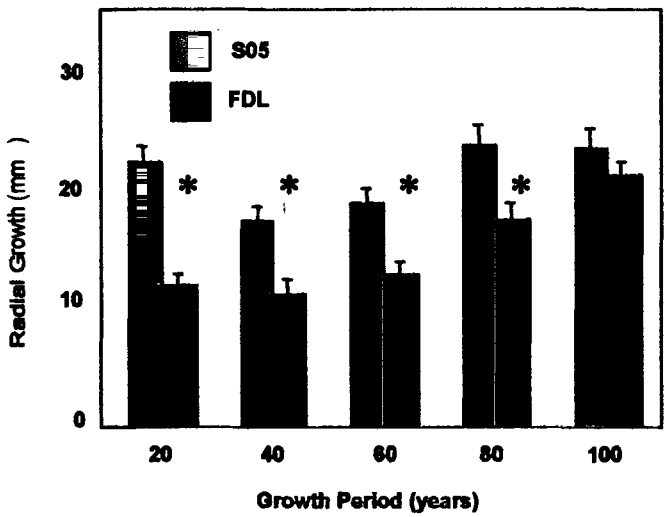

Figure 1.9: Bar chart of radial growth during each growth period. Radial growth in the two treatments shows significant differences during the first 80 years, but growth from 80 to 100 years from the pith shows FDL growth matching S05 growth. * indicates significant difference at P<0.001.

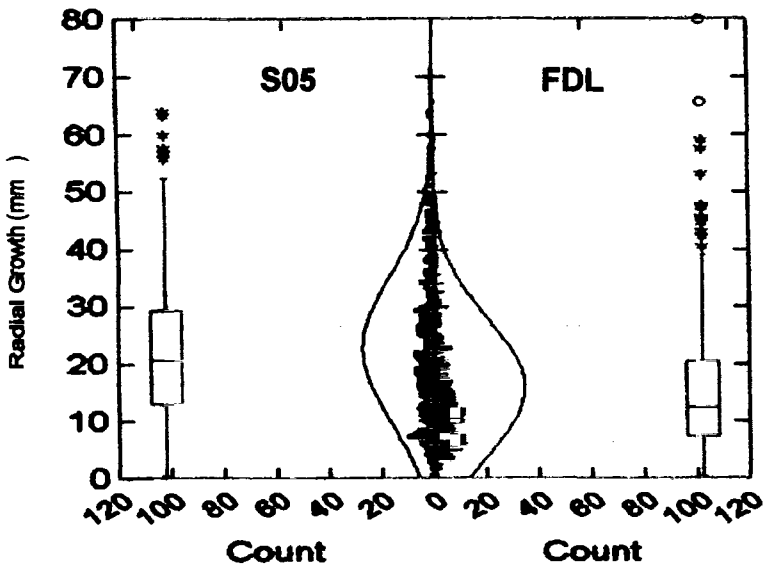

Figure 1.10: Frequency box plot showing grouped t-test comparing all GPs in S05 and FDL samples. A higher frequency (count) of FDL trees experiencing lower growth and a higher frequency of S05 trees experiencing higher levels of growth are demonstrated by the frequency curves, with box plots showing ranges and means. Both treatments have large ranges of radial growth during 20-year periods.

	Source	SS	Df	MS	F-ratio	P-value
Between subjects	**TRT**	**21.921**	**1**	**21.921**	**18.299**	**0.000**
	Error	**132.970**	**111**	**1.198**		
Within subjects	**Time (GP)**	**25.763**	**4**	**6.441**	**25.096**	**0.000**
	Time*TRT	**6.114**	**4**	**1.528**	**5.956**	**0.000**
	Error	**113.946**	**444**	**0.257**		

Table 1.4: ANOVA table for growth increments in each growth period.

ANOVA shows highly significant effects on radial growth from growth period or time (GP), and harvest treatment (TRT), as well as an interaction between GP and TRT (Table 1.4). These results indicate a strong influence of harvest treatment on the types of trees left to grow in respective stands. In this case, the effect of growth period on radial growth is expected, as older trees are more likely to have higher crown-to-stem ratio and better crown position, allowing for more growth in successive growth periods. The interaction is also expected, as each growth period is not entirely independent of previous growth. Treatment and growth period effects are clearly seen in Figure 1.7, and are also demonstrated by charted least squares means (Figures 1.9 and 1.10). S05 stands have a clear growth advantage at all growth periods except the final 80-100 year period (GP_100). At this final 80-100 year stage, both stands showed very similar mean radial growth increments of about 25 mm (Table 1.5). The maximum value for radial growth was actually higher in FDL stands than in S05, at 79.80 mm and 63.91 mm respectively (Table 1.6). This is caused by two FDL outlier trees that displayed significant growth in the final 2 growth periods measured (see Figure 1.6).

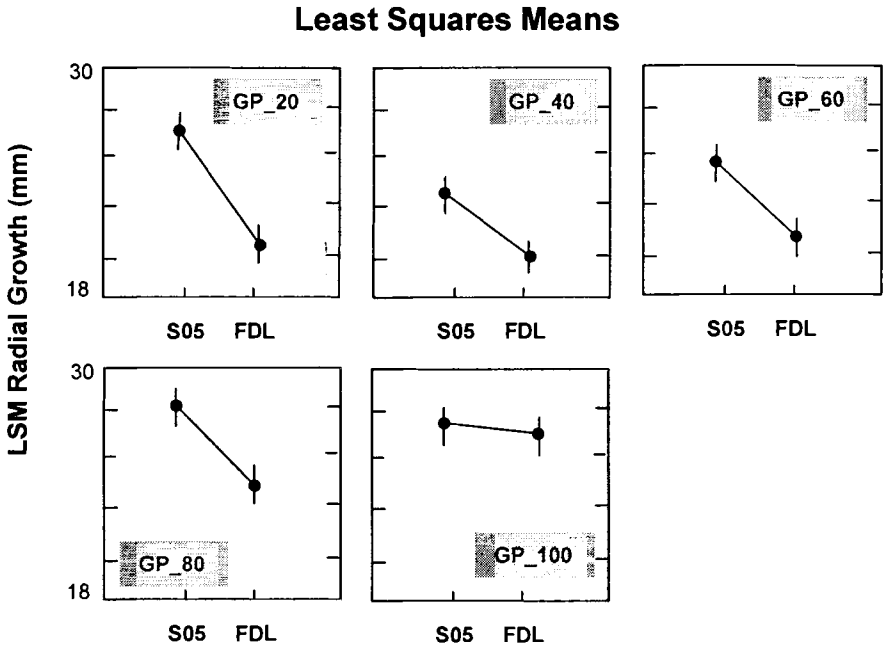

Figure 1.11: Least squares means for radial growth during each growing period. S05 stands have significantly higher means than FDL stands at each growth period except for GP_100, where they are nearly identical.

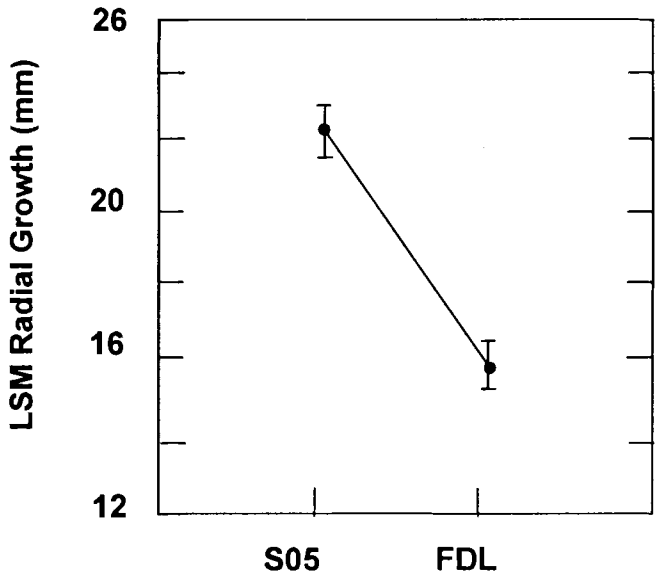

Figure 1.12: Least squares means of all growth periods grouped into treatments. A significant deficit in radial growth is seen in the FDL stands compared with S05 stands, illustrating the overall better growth in stands not subject to diameter limit harvesting.

The considerable range of sizes and growth patterns of S05 residuals suggests that this stand consists of a wide array of phenotypically variable trees, particularly when compared with the more concentrated pattern of the FDL residuals (Figure 1.10). Although no morphological measurements were applied, this could be a display of reduced genetic variation after selection, which has been supported by Rajora (1999) and Cheliak *et al.* (1988), who show that phenotypically selected parent trees contain less allelic richness than natural populations. Adams *et al.* (1998) show that the regeneration after only one round of shelterwood harvesting in which the smallest trees were removed resulted in loss of rare alleles and suggests that it is these very alleles that cause reduced tree size. Neale (1985) observed very few divergences from Hardy-Weinberg expectations in the regeneration after a shelterwood harvesting of Douglas-fir. The residual tree density was high which may indicate that selection against the smallest, least productive trees was very low. A study of eastern hemlock conducted in the same PEF compartments as the present study reports that genetic variation was not affected by diameter-limit harvesting, but that more rare alleles were present in FDL stands than in S05 stands (Hawley, DeHayes, and Brissette 1994). The authors suggest that these rare alleles may cause poor growth, and that deleterious rare alleles are more likely to be present at higher frequencies in FDL stands because these stands consist of trees with poorer fitness.

Regeneration may play a role in ameliorating the effects of selection. Rajora (1999) demonstrates that phenotypically selected white spruce parents produce genetically depauperate progeny, even when maternal trees are open-pollinated; however, red spruce are known to rely on advance regeneration (Seymour 1995), with understory

trees remaining suppressed for many years before taking advantage of a release event. It has been suggested that these suppressed trees survive in a sort of suspended state where they do not progress toward maturity with passing years, but only after a release event occurs allowing them a second birth. Morris (1948) suggests that in advance regeneration, total age should not be considered as the factor inducing maturity, but rather the age after release of suppression. If this is the case, it is possible that genetically superior trees that have been removed from the site may still be contributing to the genetic pool of the stand long after their removal, as the advance regeneration progeny from those trees are released and grow to reproductive maturity. Studies examining different forms of regeneration and their effects on genetic integrity address only the differences between seedlings planted artificially or seeds germinated from local source after harvest (see review of literature in Chapter 2). There is no information on alleles persisting in a population from advance regeneration. In order for advance regeneration to survive and flourish, care must be taken not to disturb the small trees. If the trees are damaged and they lose initial height advantage over fast-growing hardwoods, they will quickly be overtaken by competition and this potential re-infusion of genes will be lost. In PEF stands, harvesting techniques are preferentially applied during winter when snow cover protects advance regeneration and soil, so damage should not be a significant issue.

If regeneration is playing a role in maintaining genetic integrity in FDL stands, there may be a trend towards better growth at some point in the next century as those higher fitness progeny take over. This effect is doubtful, as the suppressed regeneration from more than 50 years should have contained the original genetic potential as that contained in the S05 stands, but the FDL data show no significant rebound toward

closing the gap. The long-term effects must be monitored to ascertain whether advance regeneration serves as a repository of genetic variation. The short-term effects of FDL harvesting seem clear: a residual stand stocked with smaller trees that are less suited to their local environments, trees that can not respond well to release, and that do not grow well at any point in the first century of growth. In addition, residual FDL stands are less valuable than the residual S05; Kenefic (2001) shows that in these same PEF FDL stands, regeneration density decreases and residual species compositions shift towards less economically desirable hardwoods and balsam fir. In addition, standing volume is significantly less valuable in FDL than in selection stands, with less potential for appreciation (Kenefic 2001). The long-term economic picture may be even bleaker if residual trees represent a degraded genetic resource that will regenerate continuously poorer FDL stands.

A significant factor in considering these comparison results is the lack of a control plot that has not undergone any type of harvesting for the past 50 years. Unfortunately, the natural areas contained in the PEF consist of very different forest types growing under different environmental conditions and are unsuitable for use as a control. Because the historical harvesting record of the area is not well documented, it is difficult to find any set of stands that are perfectly matched for direct comparison of harvesting effects. Study of a comparable control stand would better elucidate the distinct effects of negative versus positive selection, although such a study would not be as directly applicable to the forestry community.

Some shortfalls of this study include the potential to overlook very early and very small annual rings and potentially miss significant years of growth, particularly as red

spruce can remain suppressed for many years. Collecting increment cores from the base of the stump might help avoid missing early growth rings, but this method is time-consuming and requires a comparison breast height core to correct for irregular root collar growth, followed by cross-dating, which can cause measurement inaccuracies. Another possible method of avoiding this problem would be to take the advice of Morris (1948) and begin measurement from the year of initial release; however, this assumes that all trees began as advance regeneration and were suppressed at least until they reached breast height, which may not be the case. Additionally, release effects are not conclusively evident from the annual ring growth pattern, so a decision of the exact point of release would be subjective. Environmental effects such as long-term drought or temperature shifts may be interfering with measures of growth; a sliding window of 20 year periods starting from bark instead of pith would avoid many of these effects lasting less than 20 years total, but would have been much more time consuming. Although diameter at breast height (DBH) is a more common measurement for tree size, radial measurements were not transformed into diameter, as data were collected from only one core and corrections for measured DBH would have been complex. Two cores taken from different sides of each tree would have offered a more accurate picture of true diameter growth, but would have been time-consuming.

Initially, this study was intended to be followed by a study of genetic variation in FDL residual stands compared with S05 and unmanaged stands of red spruce using Random Amplified Polymorphic DNA markers (RAPDs). Unfortunately, an endophytic fungal contaminant (Camacho *et al.* 1997) interfered with this method, and other markers were not yet available to easily measure genetic variation.

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CHAPTER 2:
INVESTIGATION OF GENETIC DIVERSITY OF EASTERN WHITE PINE (*PINUS STROBUS*) USING MICROSATELLITE DNA MARKERS: IMPLICATIONS FOR ARTIFICIAL SELECTION

ABSTRACT

Intensive forestry practices, including tree improvement and genetic modification have become increasingly important as mechanisms to increase yields on decreased land area. Although a solid base of natural genetic diversity is valuable as both a reserve and a source of adaptable genotypes for future use, very little is known about the genetic impacts of many forestry practices. A more complete understanding of genetic dynamics and the effects of management are critical to ensure that loss of genetic resources does not inadvertently occur. Genetic effects of forest management are difficult to measure directly, particularly as trees are long-lived and slow to reach reproductive maturity. Provenance testing and breeding, followed by examination of quantitative traits is effective but time-consuming. Molecular markers can be used to measure and observe changes in genetic diversity, allele frequencies of mature trees, and their immediate reproductive products (gametes, seeds, and seedlings). To examine the status of genetic diversity of conifers in Maine, I applied 10 microsatellite DNA markers to 2 stands of eastern white pine with three age groups in each stand: mature, juvenile, and embryos (seeds). I compared the data from three age groups to determine: 1) how much diversity existed in stands 250-275 years ago; 2) How much of that diversity is reflected in the recent regeneration; and 3) the existence of significant differences in the structure and proportioning of diversity exist between age groups.

Heterozygosity values indicate extremely high levels of genetic diversity in both stands and in all three age groups. The values of average heterozygosity obtained in this study are higher than other reported values for white pine but are supported by the high variation generally seen in conifers. Overall, the stands exhibited Hardy-Weinberg equilibrium, although individual alleles within age groups did show heterozygote excesses in the mature and juvenile groups, and heterozygote deficiencies in the embryo group. No significant differences in heterozygosity values emerged between age groups, although allele frequencies did display some differences between the age groups. More private alleles were found in embryos than in juvenile or mature trees, and common alleles were only exhibited by the juvenile trees. All alleles present in mature trees were represented in the regenerating populations. Overall, the majority of alleles were rare and low frequency alleles, with very few common alleles present. Genetic differentiation was extremely low for all groups, indicating a lack of structure of diversity; nearly all the total variation was within-population.

Implications for forestry include a potential loss of rare and low frequency alleles if harvesting does not account for the genetic resources of a population, particularly if artificial selection is used. These lower frequency alleles represent much of the genetic potential required for adaptation to changing environments; a loss of such genetic variation could be adaptationally limiting. Selection could also result in a shift of gene frequencies, increasing some deleterious alleles under regimes such as diameter-limit selection. The past four centuries of exploitative harvesting of white pine does not appear to have affected the genetic diversity resources in this region of Maine.

INTRODUCTION

Molecular markers

Molecular markers are extremely useful tools in the analysis of genetic diversity in and among individuals and populations. In particular, the PCR-based strategy using microsatellite DNA variability offers an opportunity to assess genetic diversity definitively, efficiently, and inexpensively. Microsatellite loci of *Pinus strobus* have been identified and characterized and have been used to determine genetic diversity levels for comparison among and between populations and age groups.

Genetic diversity can be measured by either direct measures of trait variation or by using molecular markers to examine alleles. Alleles, variable genetic traits governed by a particular locus, can be informative by determining genetic diversity parameters such as: number and frequency of alleles at gene loci, proportion of polymorphic loci (P), observed and expected heterozygosities (H_o and H_e , respectively) of the population, and Nei's (1978) diversity measures (Yeh 2000). Traditional approaches to examining genetic variability within tree populations have included long-term provenance trials which have the advantage of directly measuring variation in gene expression and inheritance of traits. Provenance trials are particularly useful when combined with molecular markers to establish linkage with genetic loci and potentially uncover quantitative trait loci (QTLs). These trials are extremely valuable, but can be time-, cost-, and labor-intensive, relying on qualitative traits that may be strongly influenced by environmental effects. In the past few decades, molecular markers have been developed into increasingly useful tools, offering the opportunity to examine direct genetic information quickly, inexpensively, and more definitively than traditional methods.

Biochemical markers such as isoenzymes are limited by their need for large amounts of DNA, and in the finite number of loci available for analysis, as well as being potentially influenced by selection pressures on genetic expression. RFLPs (Restriction Fragment Length Polymorphisms) offer a wider selection of loci, but are time consuming and require large amounts of genetic material. PCR-based methods require very little DNA and, like RFLPs, deal directly with raw genetic material, thereby avoiding selection effects. RAPDs (Randomly Amplified Polymorphic DNA fragments) offer unlimited loci and are very fast, but can be difficult to replicate and do not show simple Mendelian inheritance (Tsumura *et al.* 1996). Some success has been reported by combining RAPD-RFLP to examine species with particularly low genetic variation (DeVerno and Mosseler 1997). PCR-dependent marker systems relying on microsatellites have proven to be valuable in mapping and diversity studies because they are abundant, extremely variable, highly conserved, and easy to identify (Tautz 1989, Echt *et al.* 1996).

Microsatellites, or simple sequence repeats (SSRs), are short, tandemly repeated sequences abundantly distributed throughout the genome, which have been found to show Mendelian segregation in conifers (Tsumura *et al.* 1996, Wang *et al.* 1994). Sequence repeats display high levels of allelic diversity through variability in repeat numbers due to strand slippage (Schlötterer and Tautz 1992). Sequences flanking SSR loci are conserved among species, making these loci unambiguous, discrete markers (Echt *et al.* 1996). Polymorphisms within microsatellite loci can be used to examine sequence variations within species, but flanking sequences must be known for primer synthesis, which can be a time-consuming and expensive process. The strand slippage mechanism of microsatellite DNA mutation (see Schlotterer and Tautz 1992 for review) creates discrete

alleles over a finite size range. Back-mutation and large insertions and deletions can occur as well (Richards and Sutherland 1994). The size of new alleles depends on the allele from which it originated. These factors compromise the accuracy of standard statistical measures (Hoelzel and Bancroft 1998), so slightly modified measures must be calculated. Statistical software has been created to deal with microsatellite data (PopGen32, Yeh and Boyle 1997), so reported parameters are comparable to those of other molecular markers.

In forest trees, simple sequence repeats have been used for population studies and breeding (Scotti *et al.* 2000, Khasa *et al.* 2000, Rahman *et al.* 2000), but less work has been focused on diversity (see more detail below). Chloroplast microsatellite loci were used by Echt *et al.* (1998) to uncover chloroplast genome diversity in *Pinus resinosa*, a species known for largely homozygous nuclear genomes. Vendramin *et al.* (1998) also used chloroplast microsatellite loci to examine genetic diversity and evolutionary history in several conifers, finding the markers much more sensitive than isozymes or RAPDs in determining diversity. SSRs resulted in higher diversity estimates than those reported using isozymes for *Pinus strobus* (Echt *et al.* 1996). Five microsatellite loci were used in concert with RAPD markers by Echt and Nelson (1997) to construct the first framework genome map for soft pine. Marker development for eastern white pine has been underway for several years and has resulted in the current availability of primers for 20 polymorphic loci (Echt *et al.* 1996). The availability of these primers (MapPairs, ResGen, Madison, WI) makes microsatellite markers the ideal system for determining genetic diversity within and between white pine populations. Because the loci have already been

characterized and have definitive allele assignments (Echt *et al.* 1996), the method is fast, inexpensive, and highly informative.

Genetic Diversity of Conifers

Gymnosperms are among the most genetically variable plants (Hamrick and Godt 1990, Hamrick *et al.* 1992), indicating a potential resilience to environmental changes. Most conifers exhibit very high levels of genetic diversity as observed in morphological, chemical, and genetic variation; see reviews in El-Kassaby (1991) and an extensive list in Ledig (1998) (Table 2.1).

	Species	**A**	**P**	**H_o**	**Reference**
Isozymes	*P.strobus*	1.96	50.6%	0.176	Beaulieu & Simon, 1994
Microsatellites	*P.strobus*	5.4	91.0%	0.515	Echt *et al.*, 1996
	P.strobus	9.43	92.3%	0.522	Rajora *et al.*, 2000
	P.radiata	6	100%	0.625	Smith & Devey, 1994
	Picea abies	13	100%	0.789	Pfeiffer *et al.*, 1997
	P.resinosa	2.67	100%	0.618	Echt *et al.* 1998
	Picea glauca	8	75.0%	0.493	Rajora *et al.*, 2001
	Populus tremuloides	7	85.7%	0.30	Rahman *et al.*, 2000

Table 2.1: Genetic diversity estimates for selected conifers. Conifers compared with an angiosperm, *Populus tremuloides*. A = Alleles per locus, P = Percent polymorphic loci, H_o = Average observed heterozygosity.

In some important conifer species there is a paucity of genetic diversity. Torrey pine (*Pinus torreyana*), which has only 2 populations range-wide, has been reported to show no detectable variation for 59 isozyme loci (Ledig and Conkle 1983). Red pine

(*Pinus resinosa*) has been shown to have extremely low levels of genetic variation, detectable only by sensitive genetic marker techniques such as RAPD-RFLP (DeVerno and Mosseler 1997). Red spruce (*Picea rubens*) has exhibited low variability in survival traits, morphology, and chemical variation (Gordon 1976), and the moderate variability expressed in growth traits is closely related to the degree of introgressive hybridization with black spruce (*Pinea mariana*) (Morgenstern *et al.* 1981), which may indicate low levels of natural genetic variation. It has been suggested that genetic bottlenecks have occurred as a result of small glacial refugia populations within the last several thousand years (DeVerno and Mosseler 1997). Even though red pine and red spruce have subsequently spread to sizable ranges, they have not regained significant genetic diversity over the thousands of years since bottleneck events, provoking the question: once genetic variation is lost, how long might it take to re-evolve?

It is now widely agreed that genetic diversity and natural adaptation must be preserved as protection against future biotic and environmental stress. Ledig (1988) extensively reviewed the arguments surrounding the importance, scope, and implications of maintaining genetic potential in forest trees. In forestry, genetic diversity is an even more complex subject because of the large and long-lived nature of forest trees and the complex interactions of ecosystems and environment. Namkoong (1992), Ledig (1986, 1988, 1992), Buchert (1994, *et al.* 1997), Savolainen and Kärkkäinen (1992), and others have concluded that a more complete understanding of the implications of the effects of management on the dynamics of genetic variation is critical for future use of the resource.

Several factors intricately interact to affect genetic variation in forest trees. In natural populations, genetic variation is generally increased by mutation and gene flow,

and decreased by selection and genetic drift. Mutation and gene flow compound each other as mutations from one population occur and then potentially migrate to other populations. Selection can be caused by natural pressures such as changing climate, or by human-induced pressures such as logging or breeding. Genetic drift generally occurs in small populations where random fluctuations in allele frequencies shift the composition of alleles in the next generation, leading to fixation or loss of alleles, thereby reducing diversity (see Yeh 2000 for a full review). Drift can be considered to be an effect of sample error, and inherently occurs in tree breeding programs, with subsequent loss of "unfavorable" alleles (Yow 1992, Ledig 1998). In general, long-lived woody plants maintain high levels of variation within populations, but less variation among populations (Hamrick and Godt 1990, Hamrick *et al.* 1992). Part of this trend is explained by the combination of life-history and ecological characteristics that ensure the preservation of genetic diversity, including large, continuous populations in widespread regions, large size, long lives, outcrossing breeding systems, and long distance pollen movement (Hamrick *et al.* 1992). The development of variation occurs over the evolution of the species as mutations randomly arise and are selected for or against. Mutational processes in trees may be an overlooked source of significant variation (Ledig 1986), considering that these gametes derive from vegetative cell lines which have undergone many more cell divisions (and therefore DNA replication events) than those leading to an animal gamete or even gametes from smaller plants. Ledig (1986) reviews the high potential for mutation accumulation in pines compared to other well-studied organisms.

Certainly, the natural course of evolution will result in both gain and loss of some genetic variation and shifts in the structure and organization of the gene pool of the populations. Rapid erosion of diversity is rare in tree species and can be much more destructive than gradual change (Ledig 1992). Rapid reduction of genetic variation can occur naturally or unnaturally as a result of sudden extreme reductions in population size, or bottleneck events. Red pine and torrey pine are believed to have undergone bottleneck events after glacial advances swiftly reduced the natural population sizes, with only small, isogenic glacial refugia populations remaining in each species. These refugia populations are believed to have founded the current species as they migrated first elevationally, then north following glacial retreat. The range of torrey pine may have been spread considerably farther than the existing range today, as it has been reported to have been cultivated for seeds by prehistoric groups (Shipek 1989), but the cause of the range contraction is unknown. Even though red pine has a large population with a moderate range, it has remained genetically uniform since the Holocene (Mosseler *et al.* 1992). These natural reductions in variation occurred over an evolutionary time scale and have not yet been naturally restored; what permanent changes might drastic reductions in diversity over only hundreds or thousands of years effect? Forestry practices may be more localized than glacial effects, but they can potentially lead to similar bottlenecks. Ledig (1992) reviews mechanisms and theoretical effects of several human-effected bottlenecks, such as logging, forest fragmentation, and exploitation of particular species over other species. In the case of northeastern North America, rapid colonization and resource exploitation led to forest fragmentation (see Main Introduction), which may have interrupted normal gene flow. Compounded by heavy harvesting, the effects of

logging may have seriously altered the genetic dynamics of the region by decreasing migration and increasing selection.

Genetic diversity is a balance between the influx of variation via migration and mutation, and the reduction of variation through selection and genetic drift. Migration and mutation are considerable and highly fluctuating in conifers, which makes them difficult to measure. Drift occurs in isolated or small populations and is problematic to identify. Selection is also difficult to identify and quantify; however it is slightly better understood using information from breeding programs. Selection can certainly have a dramatic effect on the phenotypes of forest trees (see Zobel and Talbert 1984), but whether these effects can be mitigated over time by migration and mutation is unknown. Effects of anthropogenic selection on the balance of gene flow have not been well studied. Strong selection may throw the natural equilibrium into an unbalanced state that is difficult to stabilize, and may have a deleterious impact on genetic variation, particularly in species that do not inherently maintain very high levels of diversity or have otherwise compromised genetic dynamics such as fragmentation.

Eastern White Pine

Eastern white pine (*Pinus strobus* L.) is an important part of the forests of northeastern North America and historically has played an integral role in the economic development of the region, but has recently experienced dramatic population changes. White pine is a long-lived species, regenerates readily, and is one of the most rapidly growing northern conifers, with a natural range extending from the foothills of the Southern Appalachians in Georgia, through the northeastern and Great Lakes states, and

northwest into Quebec and Ontario (Figure 2.1). White pine is a keystone species in old-growth white pine ecosystems (Wendel and Smith 1991) and has remained an economically important resource for the region. In the New England states, "white pine comprises 43% of the region's cubic volume in the white pine/red pine type group", a forest cover type that covers 16% of the region's timberland (Leak *et al.* 1995). Several hundred years ago, European colonization began a long history of exploitation, with diameter limit harvesting for timber as well as stand clearing for agriculture and residential use (see Introduction). Once a dominant tree species throughout northeastern North America, white pine has been reduced to a minor forest component in fragmented populations in many areas of its natural range (Buchert *et al.* 1997). Following heavy harvesting and land-clearing, a reduction in regeneration and ingrowth has been observed in the Northeast, and the declining trend is predicted to continue (Leak *et al.* 1995). Old-growth stands that have remained undisturbed by human impacts are extremely rare, primarily existing only in isolated stands in Quebec and Ontario (Rajora *et al.* 2000).

White pine has historically been a valuable silvicultural species and is consequently one of the most widely planted trees in northeastern North America. Several studies have examined the genetic structure and variation of the species, demonstrating it to be genetically highly variable, with a diversity of growth characteristics and pest resistance (Genys 1991, Buchert 1994, Beaulieu and Simon 1994, Rajora *et al.* 1998). Several programs are currently involved in selection of white pine seed sources for silvicultural applications, but little information exists about the genetic implications of silvicultural management techniques.

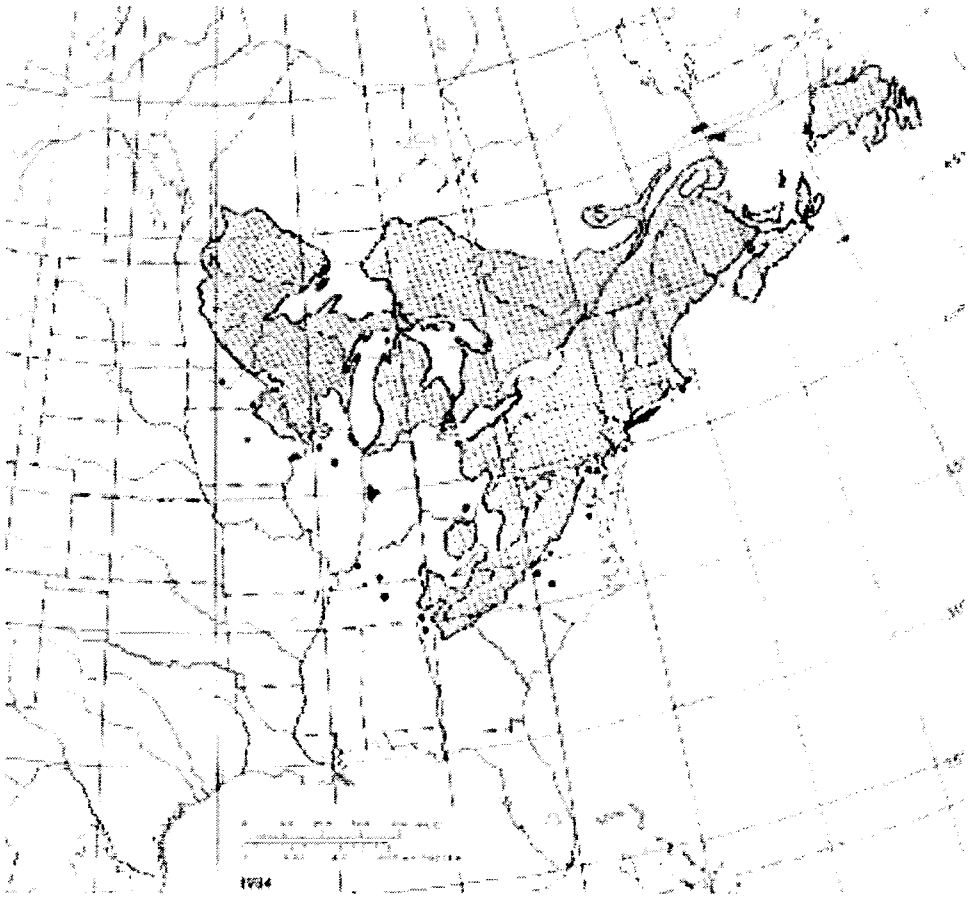


Figure 2.1: Range of eastern white pine (*Pinus strobus*).

Pines are highly variable plant species; variation has been widely observed for morphological and growth traits (see references in Zobel and Talbert 1984, Ledig 1998), at isozyme loci (Hamrick and Godt 1990), and in genetic sequences(Echt *et al.* 1996, Rajora *et al.* 2001). *Pinus strobus* has been long reported as a highly variable species, as discussed below. Like many other conifers, inbreeding depression has been reported to be high for the species (review by Mitton 1992), which may be a driving factor for high variation (Ledig 1986). As a widespread conifer ranging over diverse environments, eastern white pine shares many of the characteristics associated with preservation of variation, and has been shown to be a very genetically diverse species

(Fowler and Heimburger 1969, Genys 1987). High levels of dispersal of pollen (Wright 1953) and winged seeds that can travel over 20 m (Critchfield 1980) lead to high levels of gene flow within and among populations (Beaulieu and Simon 1995); however, spatial genetic structure within stands has also been found to be an important factor in maintaining the genetic integrity of natural white pine stands (Brym and Eckert 1983, Epperson and Chung 2001). Morphological and growth variation in *P. strobus* has been reported as highly variable. Significant geographical variation has been reported in adaptation and growth traits (Demeritt and Kettlewood 1976, Fowler and Heimburger 1969, Abubaker and Zsuffa 1991, Genys 1991, Beaulieu and Simon 1994). The first white pine seed source experiments were initiated in 1955 by the US Forest Service and reported wide geographic variation in height growth, DBH, and volume; with as much as 56% of the variation due to differences among seed sources (Pauley *et al.* 1955, Demeritt and Kettlewood 1976, Abubaker and Zsuffa 1991). In a set of range-wide experiments established in the early 1960s, Genys (1987) also demonstrated geographic variation in growth and survival rates of white pine.

Molecular markers have been used to examine *P. strobus* populations throughout the natural range of the species (Table 2.2). Isozymes have been used to measure geneticpolymorphisms. Most authors report allozyme variation in *P. strobus* comparable to other conifer species such as *Pinus taeda* (Eckert, *et al.* 1981, Ryu and Eckert 1983, Beaulieu and Simon 1994). Eckert *et al.* (1981) found genetically variable isozyme loci in provenances. Ryu and Eckert (1983) applied isozymes to discover high levels of variation over the natural range of *P. strobus*, and to uncover ecotypic variation in provenance sources. Brym and Eckert (1983) and Beaulieu and Simon (1994) both

demonstrated regional variation in diversity levels as well as within-stand structural variation. Rajora *et al.* (1998) examined genetic diversity in isolated white pine populations of Newfoundland and found levels to be comparable to those reported for other parts of the range despite small population size and long-term isolation. Beaulieu and Simon (1994) examined several Quebec populations of white pine and discovered low isozyme diversity levels in a historically harvested region of the St. Lawrence region, but also found other isolated populations to have moderately high diversity levels. Rajora *et al.* (1998) reported high levels of genetic diversity in isolated Newfoundland populations, with little differentiation from inland populations. In a study of an old-growth Ontario populations of white pine, Buchert *et al.* (1997) used isozymes to uncover high diversity levels which were later supported by even higher values reported in the same populations using microsatellite markers (Rajora *et al.* 2000).

Method	N trees	N loci	A	P	H_o	Reference
Isozymes	300	12	1.96	50.6%	0.176	Beaulieu and Simon 1994
Isozymes	95	54	2.37	75.9%	0.126	Buchert *et al.* 1997
Isozymes	102	20	1.75	47.8%	0.215	Rajora *et al.* 1998
SSRs	16	16	5.4	91.0%	0.515	Echt *et al.* 1996
SSRs	102	13	9.43	92.3%	0.522	Rajora *et al.* 2000

Table 2.2: Genetic diversity estimates for eastern white pine. Values show high levels of genetic variation.

Diversity values are similar across studies. Values obtained with microsatellite markers are much higher than values obtained from allozyme data (Echt *et al.* 1996, Buchert *et al.* 1998, Rajora *et al.* 2000), which is a trend also seen in other conifer species, attributable to the increased sensitivity of microsatellite markers.

Effects of Selection on Genetic Diversity of Forest Trees

Regeneration is an important aspect of successful selection, as parents must pass on gene complexes to their progeny in order to increase the frequency of these genes. Methods of regeneration can also be used to demonstrate effects of selection. Several regeneration methods have been studied to determine genetic effects of positively selected seed sources. These seeds are frequently collected from positive-trait trees and grown in nurseries, which may both be selective processes, potentially resulting in reduced genetic variation; however, it is expected that seed orchards and the artificial regeneration stemming from them will display higher variation because they have generally been collected from range-wide sources (El-Kassaby 1992). Seed orchard clones were found to have higher genetic diversity levels than natural populations of sitka spruce (*Picea sitchensis*), western red cedar (*Thuja plicata*), and Douglas-fir (*Pseudotsuga menziesii*) (see review in El-Kassaby 1995). In a study comparing genetic implications of natural and artificial regeneration, Adams *et al.* (1998) found little difference in successful offspring populations, although the seedling stocks used in artificial regeneration planting had significantly greater levels of diversity than natural regeneration. Selections from seed orchard clones of loblolly pine (*Pinus taeda*) displayed higher isozyme diversity and heterozygosity than natural stands, although the seeds collected from both groups did not significantly differ in diversity measures (Schmidtling *et al.* 1999).

Differences have not been evident in all reports of selected regeneration, and in some cases, genetic losses have been documented after selection. In jack pine (*Pinus*

banksiana) and black spruce (*Picea mariana*), allele frequencies at 5 isozyme loci revealed no significant differences between mature stands, natural regeneration, plantation, and seed orchard clones (Knowles 1985). Cheliak *et al.* (1988) phenotypically selected white spruce (*Picea glauca*) from a natural population and compared isozyme diversity with a randomly selected group from the same population. Although there was no significant difference detected in heterozygosity or allele frequencies, only 75% of the alleles were represented in the selected group. Because this study represents only one round of phenotypic selection and the method may not be very sensitive, this loss of 25% of isozyme alleles may represent a considerable change. No significant loss of genetic diversity was found in advanced-regeneration breeding populations of loblolly pine (*Pinus taeda*) (Williams *et al.* 1995); however, in the same species, seed orchards were reported to have lost 10-38% of the genetic diversity found in natural populations (Hamrick 1991). Evidence of negative impacts of breeding populations and planting stock is supported by the significantly reduced genetic diversity of planted stands of Norway spruce (*Picea abies*) compared with virgin forests or naturally regenerated stands (Gomory 1992). Despite a wide collection range, Rajora (1999) reports a significant reduction of genetic diversity in black spruce plantations and phenotypic tree-improvement selections compared with old-growth and natural regeneration black spruce. This study used 51 RAPD loci, which may be more sensitive than the 5 isozyme loci used by Knowles (1985). Shelterwood harvesting in old-growth Douglas-fir (*Pseudotsuga menziesii*) was reported to have no negative effects to either genetic variation of regeneration (Neale 1985) or to mating systems (Neale and Adams 1985). Four stages of life-cycle phases showed no significant differences at 10 isozyme

loci, which may be a result of the high variation in the species, as well as the high within-stand genetic variation levels found in Douglas-fir. The authors warn, however, that leave-tree density was high in this shelterwood study, and that different results might occur if leave-tree density were too low.

Effects of Selection on Genetic Diversity of Eastern White Pine

Buchert *et al.* (1994) examines the theoretical genetic effects of altering gene flow and selection pressures; mating systems, population structure, and local adaptation are all shown to be vulnerable to silvicultural pressures. Although white pine is a highly variable species, local variation within stands and local populations is a significant portion of total genetic diversity (Ryu and Eckert 1983, Brym and Eckert 1983, and Beaulieu and Simon 1994). In addition, stand structure is an important part of how the species maintains diversity (Epperson and Chung 2001). The loss of genes during one harvesting event may be partially reversible through gene flow from pollen produced in nearby stands, but white pine has been heavily and consistently exploited throughout its range. Compounding the heavy logging is the increased fragmentation of forests, which may inhibit outcrossing, and decrease local diversity pools. Loss of variation over several locally adapted stands could lead to a loss of long-term adaptability. In addition, because white pine grow as primarily shade intolerant pioneers in mixed-wood forests with competitive hardwoods, the effects of selection for one desired species in a mixed forest described by Zobel and Talbert (1984) could dramatically increase the dysgenic impacts, resulting in a significant loss of economic and environmental resources.

The impacts of selective harvesting on forest species have been extensively argued (see Section 1 Introduction). White pine has elicited a particular concern in debate and research of selective harvesting. Ledig (1994) uses white pine as a theoretical example of the potential effects of range-wide selective harvesting. Several experimental results have suggested residual effects of selective harvesting in white pine, but are not conclusive. In a possible explanation for reduced genetic diversity found in white pine in Quebec, Beaulieu and Simon (1994) suggest that the human logging activity of the past 400 years could be considered to act as small bottlenecks in the local populations, decreasing gene flow and increasing differentiation. In contrast, in Newfoundland populations, which also experienced at least one cycle of heavy harvesting in addition to a long geographical isolation, Rajora *et al.* (1998) found high levels of diversity and a heterozygote excess as opposed to deficiency, and further found very little among-population differentiation between isolated Newfoundland populations and inner-continental Ontario populations. High gene flow rates and low levels of inbreeding success were suggested as an explanation of the apparent lack of genetic differentiation in this small, isolated population. In conifers, most genetic variation is found within populations (Ledig 1986), as is the case in *P. strobus* (Ryu and Eckert 1983, Beaulieu and Simon 1994), which could also help explain the maintenance of similar diversity levels and lack of differentiation even in isolated populations. Only a small amount of gene flow is necessary to prevent the decay of variation (Ledig 1986); long-distance pollen movement may account for high heterozygosity maintained in small or isolated white pine populations.

Although few white pine populations have been designated as low-diversity areas, the erosion of the natural genetic structure of the species has been noted by Buchert (1994) in a review of patterns of white pine genetic variation and the factors affecting that variation. The author points out the potential loss of variation directly from historical logging practices and notes that the additional effects of reduced population sizes and forest fragmentation create a situation where variation must be specifically managed to sustain genetic remnants of natural populations. Several studies have supported the loss of variation in white pine directly as a cause of harvesting, but the results are not comprehensive explanations of the mechanisms of loss and are inconclusive in the quantitative effects of selective harvesting. Buchert *et al.* (1997) and Rajora *et al.* (2000) examined the potential for genetic diversity loss in an undisturbed (old-growth) white pine stand after a partial cut harvest. Two virgin white pine stands were designated for timber harvesting. Prior to harvest, 222 scattered white pines with an average age of 250 years were distributed in both stands - 120 trees in one stand and 102 in the other. The stands consisted of mature white pines in a mixed-wood understory with little natural white pine regeneration. The harvest was a partial cut removing 75% of the stand, leaving residual white pines for regeneration potential, and using site preparation to ensure natural regeneration. Trees were removed under positive selection recommendations, where residual trees are chosen specifically for positive traits, the opposite of dysgenic selection. Although the cutting prescriptions were not specifically phenotypically selective, there were two types of trees specified as residuals (not for harvest): half of the residuals were chosen for seed production potential and half for old-growth attributes which include nesting cavities and dead or dying tops. Because of the

relatively low number of total trees, 30 and 22 trees respectively postharvest, and the requirements for half the residuals to be very old, potentially dead or dying trees, this leaves only about 26 residual seed trees, which were not specified for positive growth or form traits. Cone and foliage samples were obtained from all trees pre-harvest. Fifty-four isozyme loci were used in the first investigation to assess genetic diversity levels, and 13 microsatellite loci were applied to the same genetic material several years later. The authors used isozymes (Buchert *et al.* 1997) as well as microsatellite DNA markers (Rajora *et al.* 2000) to compare diversity levels in the original intact stands with levels remaining post-harvest and found a substantial loss of diversity as a result of harvesting. While heterozygosity did not change, the number of alleles per locus decreased by 25% and the proportion of polymorphic loci also decreased by 25%. Forty percent of all low frequency alleles, which accounted for 36% of all assayed alleles in pre-harvest stands, were lost in the harvest, and 80% of rare alleles were lost. The authors conclude that the harvest intensity of the study reduced the latent genetic potential of the gene pool by one half and resulted in a reduction in long term evolutionary potential. Post-harvest gene flow effects are unknown, but have been suggested to be high in white pine (Beaulieu and Simon 1994). In the work by Buchert *et al.* (1997) and Rajora *et al.* (2000) however, all surrounding stands had also been harvested, indicating the possibility of permanent loss of alleles. Post-harvest mating systems are also unknown, making the true post-harvest gene diversity in the stand difficult to estimate. Because this study is unique in its focus of total allele loss from a harvesting event in undisturbed forests, it is difficult to extrapolate the implications of the conclusions to other situations, particularly to diversity of stands that have already experienced several harvests. However, it serves as a

benchmark for diversity levels of natural white pine ecosystems and for first-rotation harvest effects. In addition, no examination of seeds or regeneration was reported in this study, so the potential for maintenance of diversity through regeneration is unknown.

In the case of white pine, the potential for genetic degradation as a result of historical exploitation may have led to a significant alteration of natural genetic dynamics. Before an investigation of diversity equilibria can be undertaken, several steps are necessary to establish baseline measures of genetic diversity within the species and populations, and to characterize "normal" gene flow. Because assessing the loss of specifically well-adapted genes or alleles is presently difficult and time-consuming, levels of genetic diversity instead should be measured. In this study, genetic diversity of white pine was measured using microsatellite DNA markers. Two stands were examined, with three age groups in each stand: mature trees over 200 years old, juvenile trees 10-30 years old, and seeds from mature trees. Although white pine is known as a highly variable species, local variation has not yet been assessed using molecular markers, and gene flow characteristics, including effects of artificial selection, remain unclear.

MATERIALS AND METHODS

Sites and Sample Trees

Two sites were chosen for study in University of Maine forests located on Marsh Island, in Penobscot County, Maine (44°N 68°W) (Figure 2.2). Sites were chosen specifically for presence of white pine cohorts older than 200 years to obtain baseline diversity estimates for the populations. White pine is historically prevalent in the surrounding area. The first site is located in the University of Maine Dwight Demeritt Forest in Old Town, Maine. The stand consists of mixed hardwood and softwood with uneven age distribution resulting from nearly a century of managed harvesting. No agricultural clearing is apparent. White pine (*Pinus strobus*) and eastern hemlock (*Tsuga canadensis*) dominate the canopy, with white pines as the most significant emergents. Foliage was collected from eight trees, spaced between 0.5m and 20m apart. Diameter at breast height (DBH) was recorded for each tree (Table 2.3). Several trees were cored to determine age using annual rings; four of five cores displayed some wood deterioration, one core was intact to near the pith and annual increment rings counted to determine an age of 275 years. Comparable DBH in the other seven trees at this site indicate an equivalent age. No other white pine older than 175 years are found in the area, although aged stumps and younger trees indicate a history of white pine presence. No juvenile trees were found within a 15m radius of the mature trees. All juvenile trees were sampled within a 30m radius of the mature trees. Juvenile trees were selected only for available foliage; twenty trees were estimated between 10 and 30 years of age based on number of branch nodes. Cones were collected in September 2000, a productive seed year for the eastern white pine in Maine. Twelve cones were collected directly beneath mature trees

the morning after a windstorm. Four to ten fresh seeds were removed from each viable cone, labeled, and processed for DNA extraction within 2 days. A total of 20 seeds were genotyped.

The second site is located approximately three miles away, in the Woodland Preserve on the University of Maine campus in Orono, Maine. The stand is approximately 2.4ha of forest, designated as a preserve in 1967. The stand is comprised of a white pine/hemlock canopy with mixed hardwood understory. The site has not apparently been cleared for agriculture and has been lightly harvested early in the past century. There are abrupt edges on the west and south boundaries, and more gradual edges on the east and north sides where the forest gradually changes to a different composition. Twenty-four of the roughly 50 individual trees all generated after a stand-replacing disturbance between the years 1800 and 1840 were sampled. Trees were selected primarily for ease of collection, with average spacing between individuals 5-20 meters. A total of 34 *Pinus strobus* trees were tagged and DBH was recorded (Table 2.3). Several trees were cored at each site to examine age and growth patterns (Figure 2.3), although many cores were not intact to the pith. Missing rings are not taken into account and no cross-dating was done, so all ages and time of establishment are minimum values. Mature foliage was collected directly from each tree by a professional arborist, climbing and pruning the most accessible branches. Trees were selected for age according to increment cores, with the purpose of examining genetic variation of trees between 175 and 250 years old. Foliage was immediately put on ice and later stored at 20° C. Very few juvenile trees

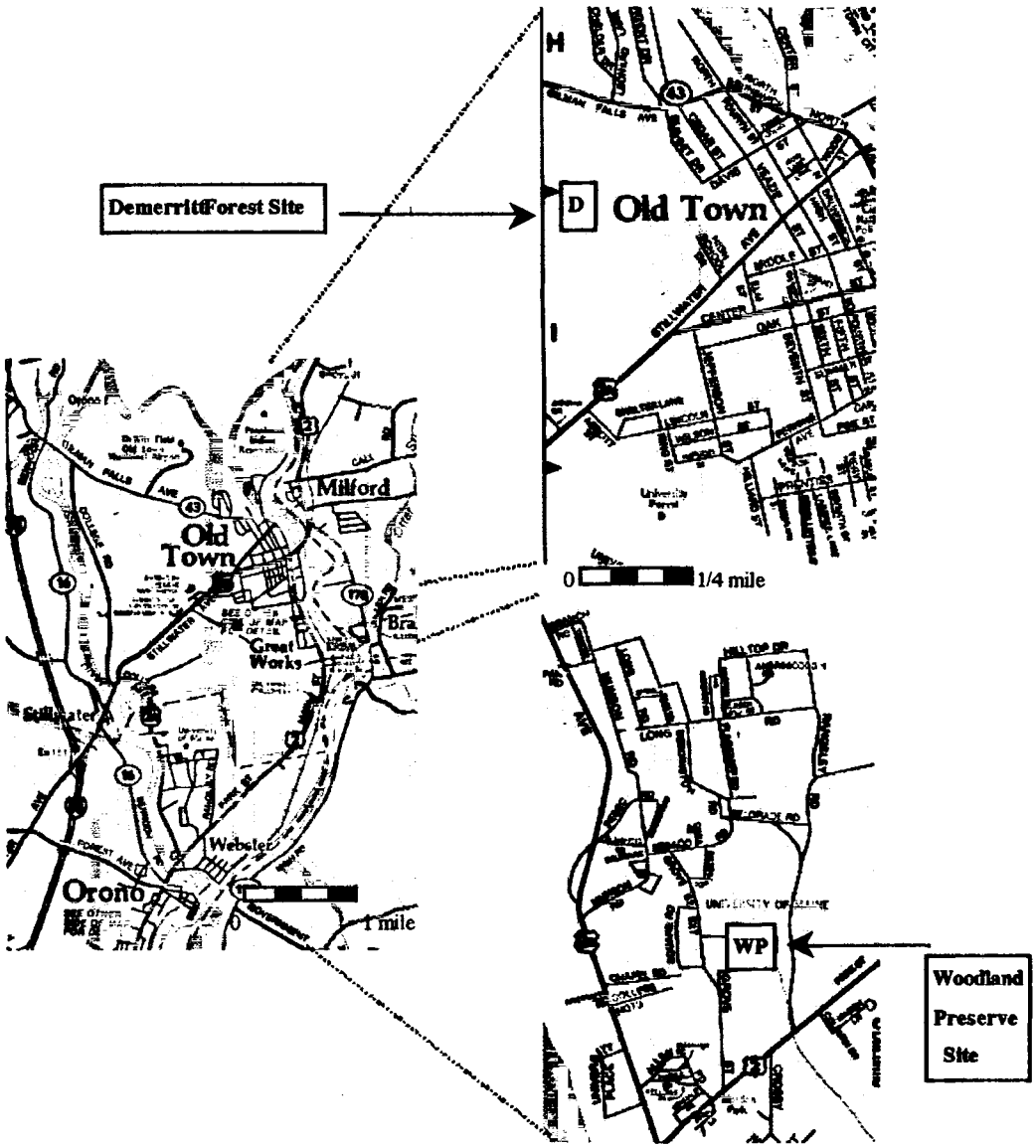


Figure 2.2: Location of stands on Marsh Island. Stands are located near the University of Maine in Orono, Maine, and are separated by less than five miles.

are present at this site; foliage was sampled from ten non-reproductive trees between the estimated ages of 10 and 30 years. As in the Demerritt site, cones were collected in September of 2000 after a windstorm from beneath the mature trees. Thirty cones were collected, but because of labor and time constraints, seeds from only ten cones were removed. A total of 42 seeds were genotyped.

MATURE FOLIAGE			
TREE ID	DBH (cm)	TREE ID	DBH (cm)
mfD-1	120.0	mfW-1	60.3
mfD-2	127.2	mfW-2	51.1
mfD-3	110.7	mfW-3	49.9
mfD-4	122.2	mfW-4	55.8
mfD-5	112.1	mfW-5	62.5
mfD-6	127.7	mfW-6	61.8
mfD-7	100.3	mfW-7	53.2
mfD-8	98.5	mfW-8	47.2
		mfW-9	50.0
		mfW-9b	66.5
		mfW-10	58.6
		mfW-11	40.2
		mfW-12	68.5
		mfW-13	69.2
		mfW-14	41.9
		mfW-15	110.2
		mfW-16	62.8
		mfW-17	112.5
		mfW-18	57.0
		mfW-19	61.8
		mfW-20	55.3
		mfW-21	66.5
		mfW-22	51.5
		mfW-23	60.0
		mfW-24	60.2
		mfW-25	45.7

JUVENILE	
TREE ID	TREE ID
jfD-1	jfW-1
jfD-2	jfW-2
jfD-3	jfW-3
jfD-4	jfW-4
jfD-5	jfW-5
jfD-6	jfW-6
jfD-7	jfW-7
jfD-8	jfW-8
jfD-9	jfW-9
jfD-10	jfW-10
jfD-11	
jfD-12	
jfD-13	
jfD-14	
jfD-15	
jfD-16	
jfD-17	
jfD-18	
jfD-19	
jfD-20	
jfD-21	
jfD-22	
jfD-23	
jfD-24	
jfD-25	
jfD-26	
jfD-27	
jfD-28	
jfD-29	
jfD-30	

EMBRYO		MEGAGAM.
	CONE/ SEED ID	
D1-0E	W1-1E	D2-1M
D1-1E	W1-2E	D2-2M
D1-2E	W1-3E	D3-1M
D1-1E	W1-4E	D4-0M
D2-1E	W2-1E	D5-1M
D2-2E	W2-2E	D6-1M
D2-3E	W2-3E	D6-2M
D3-1E	W2-4E	D7-3M
D3-3E	W2-5E	D8-1M
D4-0E	W3-1E	D8-4M
D4-1E	W3-2E	W2-1M
D4-3E	W3-3E	W2-2M
D4-4E	W3-4E	W2-3M
D5-1E	W4-1E	W2-4M
D6-1E	W4-2E	W2-5M
D6-2E	W5-1E	W3-1M
D6-3E	W5-2E	W3-2M
D6-4E	W6-1E	W3-3M
D7-1E	W6-2E	W3-4M
D7-2E		
D7-3E		
D8-1E		
D8-4E		

Table 2.3: List of all white pine samples collected and DBH (cm) of mature trees. Mf=mature foliage, jf=juvenile foliage, E=embryo, M=megagametophyte, D=Demeritt, W=Woodland Preserve

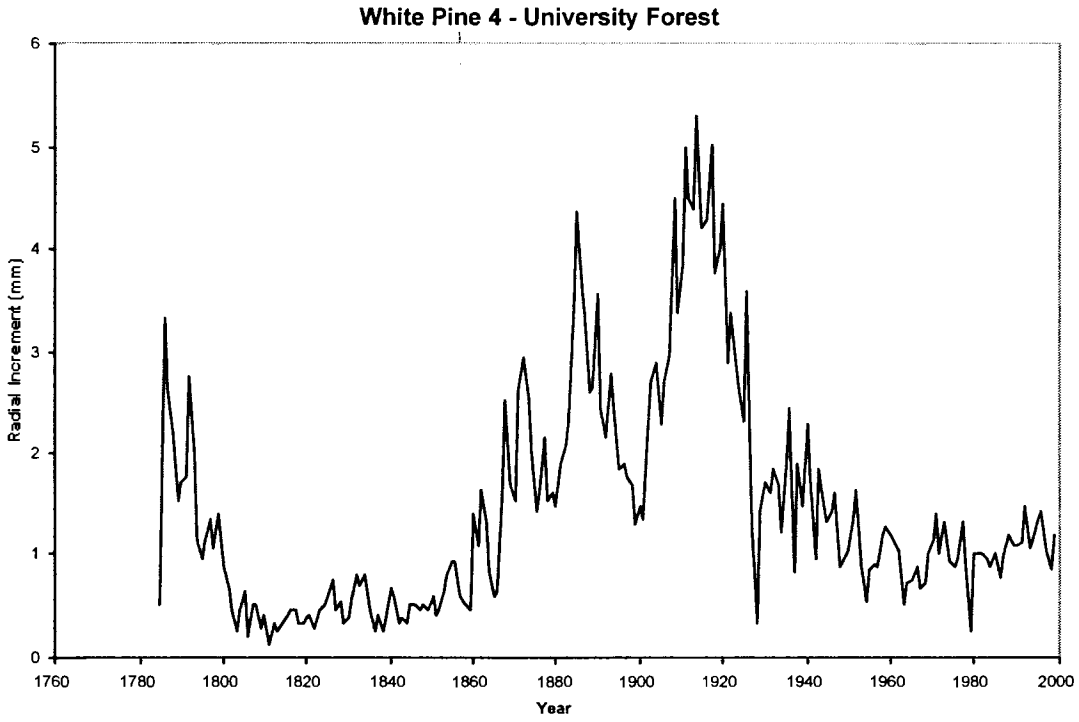


Figure 2.3: Growth pattern of a typical sample mature white pine.

DNA Isolation and PCR Amplification

Genomic DNA was extracted from foliage samples within four days of collection using a modified CTAB method (Doyle and Doyle 1987). Fresh needles (0.5 – 1.0 gram) were frozen in liquid N_2 and ground to very fine particles using sterile mortar and pestle. The tissue was ground again in the presence of 1-2 mLs CTAB extraction buffer at 60°C (100mM Tris HCl pH 8.0, 1.4M NaCl, 20mM EDTA, 2% CTAB, 1% PVP-40, 0.2% ß-mercaptoethanol). Additional extraction buffer was added to a total volume of 10 mL/g of tissue and samples incubated at 60°C for at least 30 minutes, with gentle agitation every 10 minutes. An equal volume of chloroform:isoamyl alcohol (24:1) was added. The solution was inverted repeatedly to ensure mixing and set on a rocking platform for 20-30 minutes for protein separation. Samples were centrifuged; all centrifugation steps performed in a chilled J-2 rotor at 15°C. The separated aqueous phase was decanted from the organic phase using cut-tip 1000µL pipettors, and the DNA precipitated by adding 2/3 volume of ice cold (-20°C) isopropanol. After a 20-minute incubation at –20°C, DNA was either pelleted by a 10-minute centrifugation or spooled onto a sterile glass rod. The DNA pellet or spool was gently washed with 70% EtOH and left to dry completely at room temperature. DNA was resuspended in 1/10 volume TE buffer and RnaseA added. The solution was incubated at 37°C for 30 minutes. DNA was washed using one of two methods. A double wash was initially preferred using first 1/4 volume ammonium acetate with 2 volumes ice cold 100% EtOH, followed by mixing, precipitation, drying, and resuspension in TE, then washing again in 1/20 volume sodium acetate with 2 volumes ice cold 100% EtOH. A single wash became more useful during high-volume DNA extraction procedures using 0.3M NaCL with 2 volumes ice cold 100% EtOH.

Samples were repeatedly inverted and placed at –20°C for 20 minutes after each wash to fully wash and precipitate DNA. DNA was again spooled, washed with 70% EtOH, allowed to dry completely, then resuspended in 250-500uL sterile TE buffer. Suspended DNA was stored at –20°C.

Seeds were gently washed with 10% hydrogen peroxide and soaked on moistened filter paper in petri dishes for 24 hours. After dissection and complete removal of seed coat, embryos and megagametophytes were separated and placed in tubes on ice. CTAB DNA extraction proceeded as described above using a total volume of 1mL CTAB. Modifications include using no liquid N_2 and crushing using glass pestles fitted to 1.5mL microcentrifuge tubes, as well as using a benchtop microcentrifuge.

Precise DNA concentrations were not estimated; instead, a set of dilutions was tested to determine the optimal concentration for PCR reactions. All DNA samples used in PCR were 50:1 dilutions. DNA was amplified using polymerase chain reaction (PCR) protocols modified from Echt *et al.* (1996), Rajora *et al.* (2000), and Kutil and Williams (2001). Prepared primers were obtained from ResGen (Madison, WI) (Table 2.4). Although 14 primers were initially screened, only 10 provided reliable results and were subsequently used in this investigation.

Genomic DNA was amplified in either 12.5 or 25µL volumes containing 50mM Tris-HCl, pH 9.0, 20mM $(NH_4)_2SO_4$, 200µM dNTPs, 200nM primer, 0.04Units/µL Taq DNA polymerase (Perkin-Elmer), 1-2ng/µL template DNA, and molecular grade water to final volume. Some primer pairs required hotstart technique; master mix was prepared without $MgCl_2$, which was added to each sample reaction after a preliminary heating of 90°C for 5 minutes. A touchdown protocol (Echt *et al.* 1996) was run on an M-J

Research thermocycler. Samples of PCR products in each reaction set were horizontally electrophoresed alongside a low DNA mass ladder (Perkin-Elmer) on 1% agarose gels stained with ethidium bromide to determine product concentration.

PAGE Visualization and Silver Staining

Denaturing stop solution with loading dye (Promega) was added to reactions after amplification in varying concentrations depending on intensity of bands in 1% agarose gel (0.5- 1.0 volumes of reaction mix). Products were visualized using 6% denaturing polyacrylamide gel electrophoresis (PAGE) according to Promega Silver Sequencing Kit. Fifty grams urea were heated and dissolved in 28mL ultrapure H_2O and 15mL 40% 1:1 acrylamide:bisacrylamide, then deionized for 5 minutes using 150 mg ion-exchange resin beads. The solution was allowed to cool slightly, then was filtered through 0.2 micron Whatman filter paper and degassed for 5-10 minutes using a water tap apparatus. Five hundred microliters freshly made 10% ammonium persulfate was added with gentle swirling and 50μL TEMED was quickly added. Solution was carefully poured into previously assembled gel apparatus (Gibco BRL/Life Technologies S2 vertical electrophoresis set-up with gel casting boot and 0.4mm spacers/sharktooth comb) and allowed to polymerize for an hour. Three to four microliters of sample were loaded in each well, 20 samples at a time with a 5-minute break to electrophorese samples into the gel to avoid well leakage. PGEM markers (Promega) were loaded between each 20 samples group for length reference. The upper buffer was 1X TBE and the lower buffer was 2/3X TBE with 0.5M sodium acetate (pH 7.0). Samples were electrophoresed at

constant power of 60-95W to maintain constant temperature of 50°C, 2-3.5 hours depending on length of amplified sequence.

Product was visualized using a silver staining kit and slightly modified protocol (Promega). All solutions were made using ddH_2O. Gels affixed to the short glass plate were fixed in a solution of 10% acetic acid for 20 minutes to 8 hours. After 3 ddH_2O washes, gels were stained for 30 minutes in a solution of 6mM silver nitrate containing 0.056% formaldehyde with constant rocking. After a 5 second ddH_2O wash, stain was developed in a freshly prepared solution of 0.28M sodium carbonate, 0.056% formaldehyde, and 8μM sodium thiosulfate with gentle agitation by hand. Developing was stopped after 3-7 minutes depending on band resolution by immersing the gel in a separate 10% acetic acid bath for 5 minutes. Both developer and stop solution were chilled to 4° C prior to use. After a 2-minute ddH_2O wash, plate and gel were immersed in a 4% sodium hydroxide solution for 30-60 minutes to loosen the gel from the plate, then washed for another 2 minutes. Gels were removed form the plate and dried on Whatman paper, photographed, then scanned to obtain permanent digital images. Gel Imager (Dyer 2001) program was used to compare PGEM (Promega) size standards with product sizes (band locations) and determine allele lengths. Gels were scored by hand according to product length (Figure 2.4). See Appendix B for a full discussion of allele scoring methods. Microsatellite loci profiles for rps50 and rps6 demonstrate multiple,

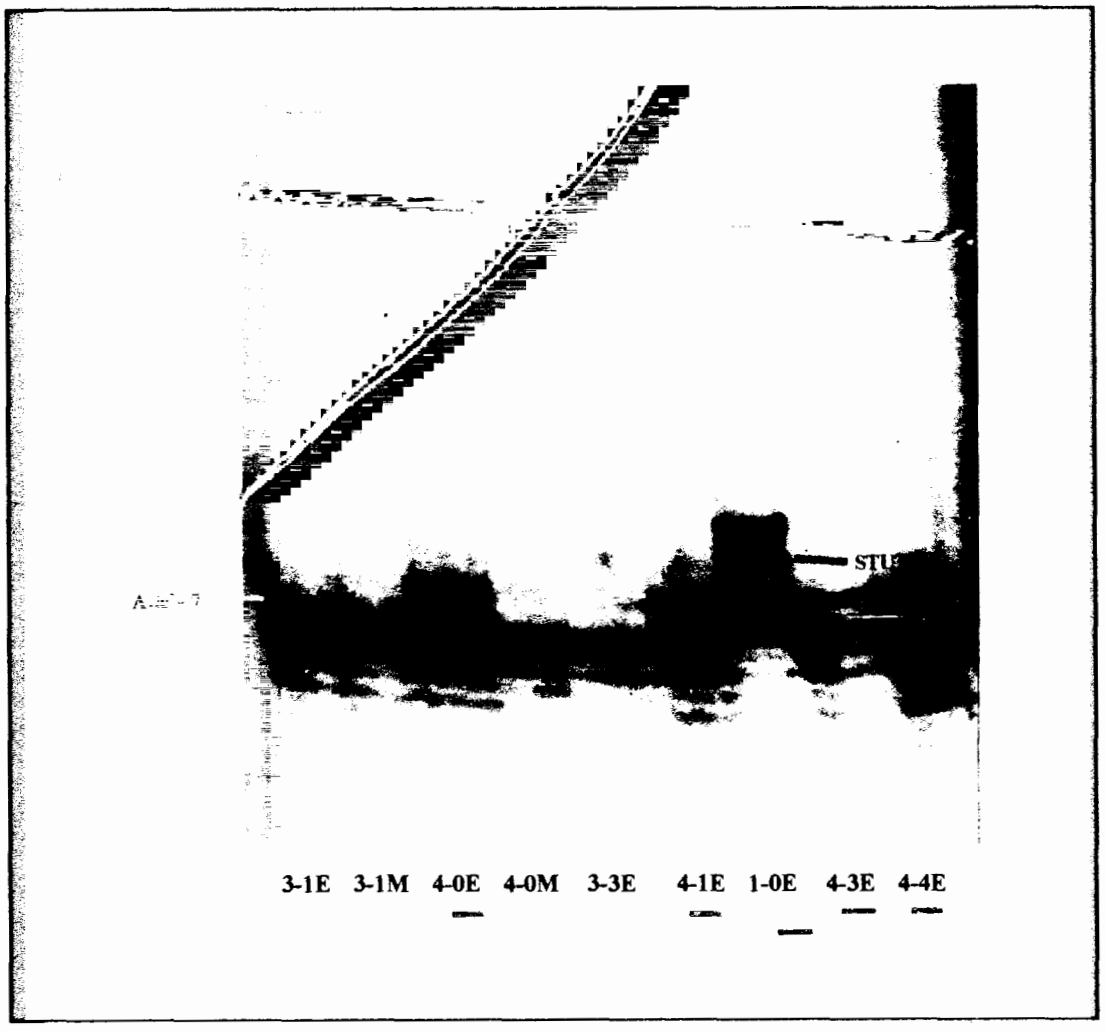


Figure 2.4: Digital image of a PAGE gel. Gel shows three alleles (of 8 total for this marker). In this case, each allele is 2 basepairs longer than the previous allele (size standards are not shown). E denotes diploid embryos, M denotes haploid megagametophytes, and the first ID number refers to cone ID, so sample 3-1 and 3-2 are both seeds from the same tree. Stutter bands can be easily identified by the lighter band intensity.

highly variable alleles in individuals and few alleles in individuals respectively. To cross check scoring, megagametophyte tissue corresponding to embryos was amplified at 8 loci; the genotype data from this haploid tissue were compared with corresponding embryos and mature foliage for verification of amplification and scoring procedures, and are not included in any other analyses. No null alleles were seen in these comparisons and are accounted for in any other analysis under the assumption that individuals showing only one allele were homozygous (Ciofi *et al.* 1998). Stutter bands were easily diagnosed and could be ignored in scoring (Ciofi *et al.* 1998).

Statistical Analysis

Statistical analysis was performed using diploid co-dominant settings in the POPGENE program, version 1.32 (Yeh & Boyle 1998). Standard genetic diversity parameters were calculated, including number of alleles per locus, allele frequencies, Nei's (1978) unbiased estimates of mean expected and observed heterozygosity (H_e and H_o), as well as Nei's (1978) unbiased genetic distances. Departures of observed allele frequencies from Hardy-Weinberg expectations for each locus in each population were determined using χ^2 tests of expected and observed heterozygosities. Wright's F-statistics (1951) were used to determine heterozygote excesses and deficiencies. Nei's (1978) Gst gene diversity statistic was used to obtain estimates of the distribution of genetic variation and amount of genetic differentiation. Alleles were assigned to one of four frequency classes adapted from Rajora *et al.* (2000) and Marshal and Brown (1975): common ($P \geq 0.75$); intermediate ($0.75 > P \geq 0.25$); low ($0.25 > P \geq 0.05$); and rare ($P < 0.05$). Comparisons were made for allele frequency distributions using χ^2 tests. Heterozygosity

may naturally increase with age as deleterious recessive homozygotes are selected against (Ledig 1986). To test the differences between age groups (mature, juvenile, and embryo), paired t-tests and χ^2 tests were performed on heterozygosity estimates, numbers of alleles per locus within groups, and allele frequencies.

		Echt *et al.* (1996) N=16		Rajora *et al.* (2000) N=238	
Locus	**Repeat**	**# alleles**	**size range bp**	**# alleles**	**size range**
rps2	(AC)15	4	149-171	10	145-171
rps6	T6 (AC)n T6	4	159-164	*	
rps12	(AC)17	11	163-209	21	153-195
rps18	(AC)n(A)6	4	162-166	*	
rps20	(AC)16(AT)6	8	138-174	16	100-162
rps25b	(AC)17 AG(AT)9	9	97-115	10	101-125
rps34b	(AC)14	3	145-149	6	141-151
rps39	(AC)17	2	172-174	6	160-180
rps50	(AC)17	8	160-188	13	152-184
rps60	(AC)19(AT)7	8	261-279	17	247-279
rps84	(CT)10(AC)n	5	145-163	*	
rps90	(AC)n	5	138-164	*	
rps118b	(AC)23	7	148-164	19	130-166
rps124	(AC)n	4	147-153	*	
rps127	(AC)10(AT)5	2	194-196	3	191-195
rps119	(AC)10(AT)5	*		2	203-205

*not reported

Table 2.4: *P. strobus* microsatellite loci.

RESULTS

Fourteen *Pinus strobus* microsatellite loci were tested; 12 amplified reliably, but only 10 were verifiably scored across all samples. These ten loci were used to genotype all of the mature and juvenile foliage samples (see Appendix C). Only 8 loci proved reliable in amplification of lower-concentration embryo DNA. All of the microsatellites used for analysis were polymorphic in both stands and in each age group (Table 2.5, 2.6). The small sample sizes of the Demeritt (D) mature trees and the Woodland Preserve (WP) juvenile trees required both stands to be combined for most analysis. At several individual loci, χ^2 tests for Hardy-Weinberg equilibrium show significant differences between the two stands, but this is most likely an effect of small population sizes in the mature Demeritt and the juvenile Woodland Preserve group, which inherently discourage Hardy-Weinberg equilibrium. As the population increases (as group data are combined), Hardy-Weinberg equilibrium is observed in more loci. In overall data, no detectable difference was evident between either heterozygosity estimates or overall allele frequencies between the two stands, so data were pooled into one representative population for further analysis except where noted.

The total number of alleles detected for all loci ranged from 3 to 14 alleles, with a total of 76 alleles in the study and an average of 7.5 alleles per locus. No single generation group contained all 76 alleles; the embryo group had the highest percentage of alleles represented (96.7%) and the mature group the least (65.3%) (Table 2.7). Differences between observed and expected heterozygosities were not significant for any of the generation groups, indicating the populations are within Hardy-Weinberg

		Echt *et al.* (1996) N = 16		Rajora *et al.* (2000) N = 238		Sokol (2001) N = 116	
Locus	**Repeat**	# alleles	size range bp	# alleles	size range	# alleles	size range
rps2	$(AC)_{15}$	4	149-171	10	145-171	6	145 -171
rps6	$T_6 (AC)_n T_6$	4	159-164	*	*	4	159-164
rps20	$(AC)_{16}(AT)_6$	8	138-174	16	100-162	12	124-164
rps25b	$(AC)_{17} AG(AT)_9$	9	97-115	10	101-125	9#	101-125
rps39	$(AC)_{17}$	2	172-174	6	160-180	4	160-178
rps50	$(AC)_{17}$	8	160-188	13	152-184	10	154-188
rps60	$(AC)_{19}(AT)_7$	8	261-279	17	247-279	14	251-279
rps84	$(CT)_{10}(AC)_n$	5	145-163	*	*	5#	145-163
rps90	$(AC)_n$	5	138-164	*	*	8	138-162
rps127	$(AC)_{10}(AT)_5$	2	194-196	3	191-195	3	192-196

*not reported

#not used for embryo genotyping

Table 2.5: Microsatellite loci used for genotyping. All 10 loci were used to genotype mature and juvenile foliage tissue; 10 loci were used to genotype haploid megagametophyte tissue for scoring comparisons; 8 loci were used to genotype embryos.

8 Loci

Diversity Parameter	OVERALL (8 loci)	MATURE	JUVENILE	EMBRYO
No. of Trees	116	34	40	42
Total no. of alleles over all loci	75	49	63	59
Mean no. alleles per locus*	7.75	6.125	7.13	7.375
Mean effective no. alleles per locus*	4.843	4.075	4.242	4.968
Mean heterozygosity (observed)*	0.759 (0.084)	0.772 (0.121)	0.775 (0.187)	0.729 (0.091)
Mean heterozygosity (expected)*	0.752 (0.105)	0.711 (0.112)	0.708 (0.169)	0.769 (0.094)
Mean heterozygosity (Nei's expected)*	0.749 (0.105)	0.700 (0.120)	0.699 (0.168)	0.759 (0.039)
Mean F_{IS}	-0.0544	-0.1153	-0.1212	0.0311
Mean F_{IT}	-0.0175			
Mean F_{ST}	0.0350			

10 Loci, embryos not included

Diversity Parameter	OVERALL (10 loci)	MATURE	JUVENILE
No. of Trees	74	34	40
Total no. of alleles over all loci	89	61	77
Mean no. alleles per locus*	7.10	6.100	7.100
Mean effective no. alleles per locus*	4.373	3.977	4.288
Mean heterozygosity (observed)*	0.762 (0.112)	0.750 (0.144)	0.765 (0.170)
Mean heterozygosity (expected)*	0.730 (0.110)	0.713 (0.111)	0.717 (0.157)
Mean heterozygosity (Nei's expected)*	0.705 (0.109)	0.702 (0.109)	0.705 (0.118)
Mean F_{IS}	-0.0745	-0.1332	-0.1099
Mean F_{IT}	-0.0492		-
Mean F_{ST}	0.0235		

*SE in parentheses

Table 2.6: Diversity parameters for population and generation groups. Only 8 loci (above) amplified reliably in embryos, so separate comparisons are shown for the two subsets of loci. Differences in diversity parameters were not significant, however, so further comparisons were made using the 10 loci subset for mature and juvenile groups, and the 8 loci subset for embryos.

8 loci	Total Alleles in sample	Percent alleles represented	Range of # alleles/locus	Mean alleles/locus	Loci in HWE
Embryo	60	96.7%	3-13	7.38	3
Juvenile	57	76.0%	3-14	7.13	4
Mature	49	65.3%	3-14	6.13	2
Total Alleles	62				

10 loci	Total Alleles in sample	Percent alleles represented	Range of # alleles/locus	Mean alleles/locus	Loci in HWE
Juvenile	71	86.5%	3-14	7.10	5
Mature	61	68.5%	3-14	6.10	4
Total Alleles	76				

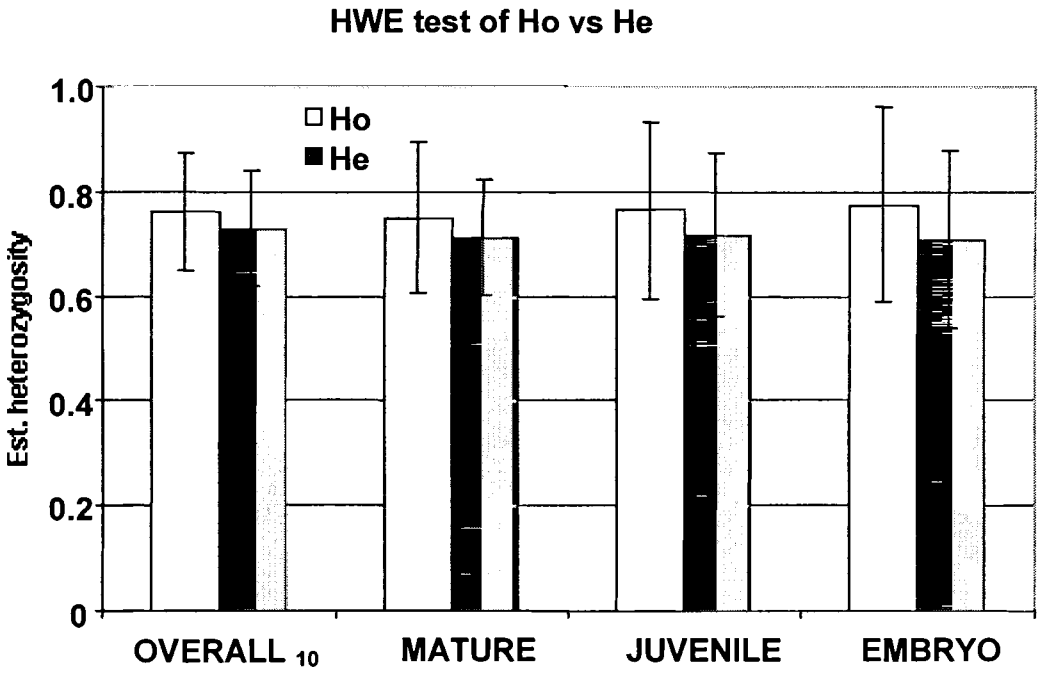
Table 2.7: Alleles found in generation groups for 8 loci and 10 loci subsets.

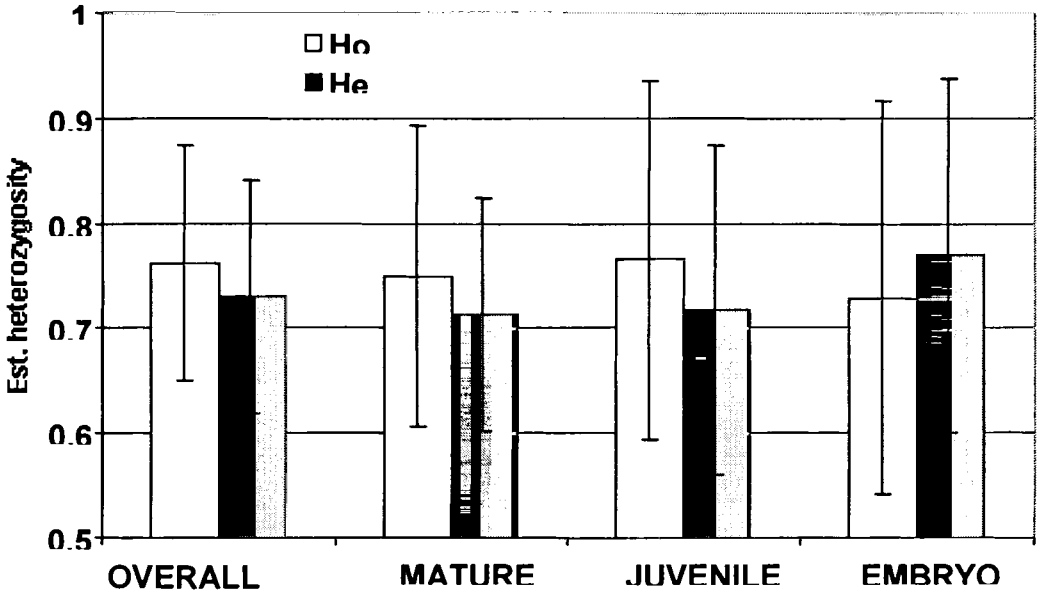
8 Loci	Mature	Juvenile	Embryo
Mature	****	0.8839	0.8773
Juvenile	0.1234	****	0.8771
Embryo	0.1309	0.1311	****

10 Loci	Mature	Juvenile
Mature	****	0.9004
Juvenile	0.1049	****

Table 2.8: Nei's genetic identities and distances. Genetic identities (above diagonal) and genetic distances (below diagonal) show that all groups are genetically undifferentiated.

equilibrium expectations (differences were based on comparing bounds of confidence intervals at the 95% significance level) (Figure 2.5). Individually, most loci produced significant departures from Hardy-Weinberg expectations in at least one of the generation groups (see Appendix C for frequency data). Using 8 loci, the average expected heterozygosity (genetic diversity) within the total population was 0.752. The embryo group had the highest H_e (0.769), while the juvenile group had the lowest (0.708). Using 10 loci, the average $H_e = 0.730$, with the mature group lower than the juvenile group (0.713 and 0.717, respectively). Chi-squared tests ($P<0.05$) for mean heterozygosity show no significant differences between mature, juvenile, and embryo groups. In a comparison of mature and juvenile groups, 4 of 10 loci show significant differences in heterozygosity, but genetic identities are very high (0.9004) between these groups. Significant differences in heterozygosity were also found in individual loci when the three age groups were compared, with 7 of 8 loci showing significant differences of heterozygosity estimates between all three groups. Mature and embryo age groups showed heterozygosity differences at 6 of 8 loci. Only 4 of 8 loci displayed significant differences between juvenile and embryo groups. Nei's unbiased genetic identities (1978) are high (>0.877) between all groups, indicating a close genetic relationship with little differentiation (Table 2.8). Mature and juvenile groups shared closer identity than either of those groups does with embryos, but the differences between the distances were not significant. Generally, these data are not supportive of specific relationships among generation groups.





Ho =	0.762	0.750	0.765	0.729
He =	0.730	0.713	0.717	0.769

Figure 2.5: Bar charts of heterozygosity levels. Comparisons between observed and expected heterozygosities with 95% confidence intervals shows no significant departures from Hardy-Weinberg equilibrium in each group (above). With a smaller y-axis range, it is evident that no significant differences between groups were found (below).

Overall Allele Frequency : 8 loci								
Allele/ Locus	**rps2**	**rps6**	**rps20**	**rps39**	**rps50**	**rps60**	**rps90**	**rps127**
A	0.1853	0.2682	0.0973	0.0609	0.0302	0.0302	0.2252	0.2885
B	0.4741	0.4045	0.1106	0.3478	0.0302	0.0560	0.0450	0.5096
C	0.2974	0.3000	0.0708	0.3217	0.0431	0.0819	0.0495	0.2019
D	0.0302	0.0273	0.1195	0.2652	0.1336	0.0647	0.0495	
E	**0.0086**		0.3053	**0.0043**	0.0560	0.0259	0.0405	
F	**0.0043**		0.0841		0.1810	0.0474	0.0450	
G			0.0310		0.1164	0.1983	0.4189	
H			0.052		0.2069	0.1767	0.1261	
I			0.0354		0.1724	0.0603		
J			0.0398		0.302	0.0690		
K			**0.0177**			0.0690		
L			**0.0133**			0.0302		
M						0.0560		
N						0.0345		

Overall Allele Frequency : 10 loci - juvenile and mature groups only										
Allele Locus	**rps2**	**rps6**	**rps20**	**rps39**	**rps50**	**rps60**	**rps90**	**rps127**	**rps25b**	**rps84**
A	0.1486	0.2746	0.1197	0.0274	0.0473	0.0338	0.2917	0.2803	0.0299	0.0417
B	0.5541	0.4155	0.1127	0.3562	0.0405	0.0338	0.0486	0.5758	0.1642	0.1597
C	0.2770	0.2676	0.0986	0.3699	0.0608	0.0676	0.0347	0.1439	0.0672	0.0764
D	0.0203	0.0423	0.1408	0.2466	0.1284	0.0878	0.0486		0.2761	0.5208
E			0.4225		0.0338	0.0405	0.0208		0.2388	0.2014
F			0.0423		0.1216	0.0608	0.0417		0.1045	
G			0.0141		0.1351	0.2162	0.3819		0.0672	
H			0.0141		0.2432	0.1486	0.1319		0.0448	
I			0.0211		0.1622	0.0608			0.0075	
J			0.0141		0.0270	0.0608				
K						0.0811				
L						0.0338				
M						0.0405				
N						0.0338				

Table 2.9: Allele frequencies. Overall allele frequencies at 8 loci (above) for all three generation groups -- embryo, juvenile, and mature, and allele frequencies at 10 loci (below) which excludes embryos. Alleles exclusive to embryos (private alleles) are shown in bold above; these are also the most rare alleles ($P<0.01$). All other alleles are shared among generation groups; alleles of rps25b and rps84 are all shared among juvenile and mature groups except for one private allele found in the juvenile group. No common alleles ($P>0.75$) were present in this overall group for 10 loci.

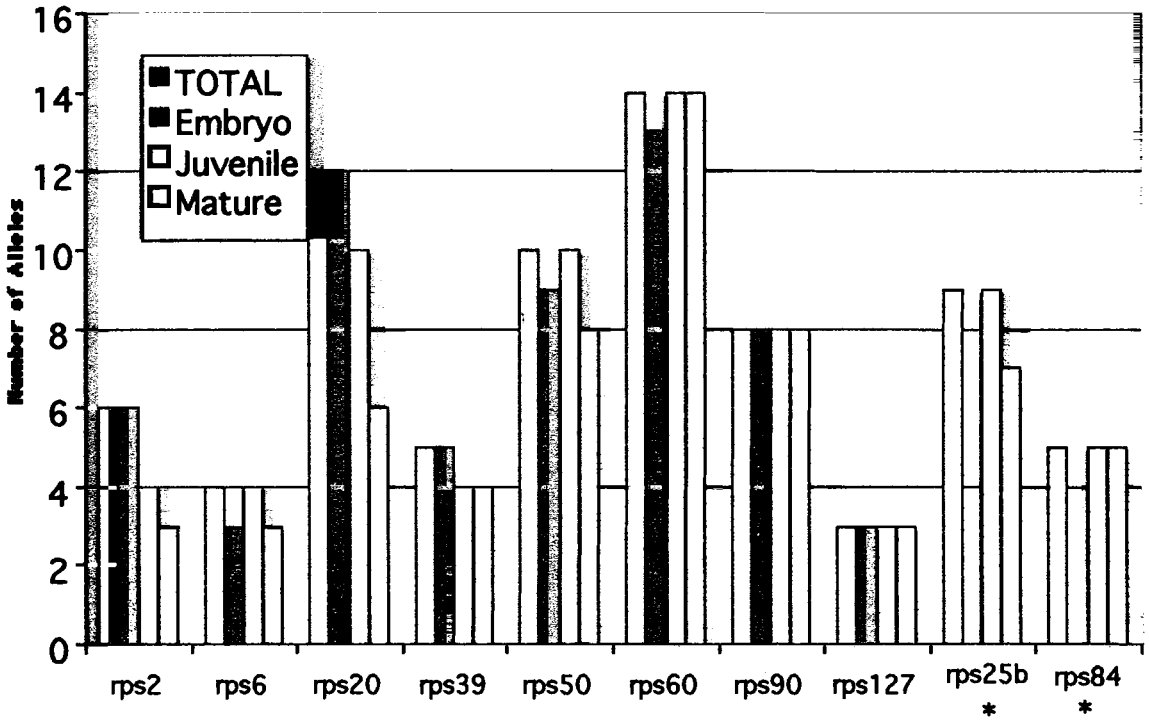


Figure 2.6: Allele numbers for each locus in each generation group. The mature group generally has fewer alleles represented than the juvenile and embryo group. * Embryos not genotyped at these loci.

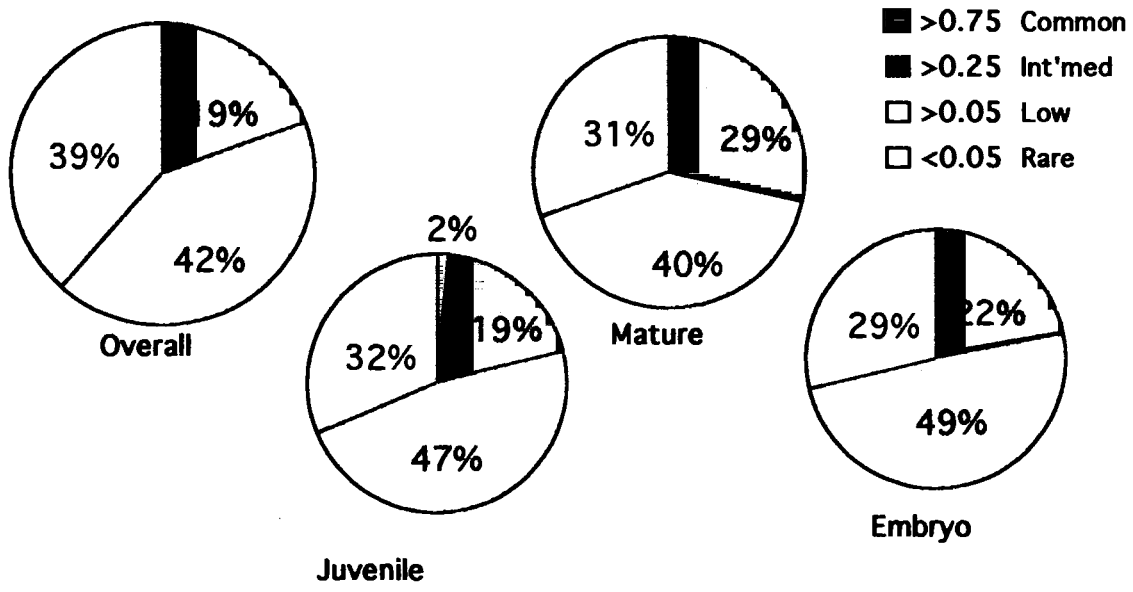


Figure 2.7: Allele frequencies grouped into frequency classes. Frequencies show proportions of common, intermediate, low, and rare alleles in each generation group. The greatest proportion of alleles are low frequency; common alleles are found only within the juvenile group.

Numbers of alleles represented in each group generally show more alleles present in embryos than in mature trees (Figure 2.6). Overall allele frequencies show that the majority of alleles are low frequency (P=0.05 - 0.24) (Table 2.9). In overall data, no common alleles (P>0.75) were found. Several loci are clearly more variable than others, and therefore, some loci are more informative than others. For example, locus rps127 with only three alleles offers heterozygosity estimates, but is limited as use for allele frequency comparisons. Loci with few alleles do not often display rare or private alleles, although they seem to have a tendency toward homozygosity, as in rps127.

Allele frequencies can be used to describe genetic variation within and among groups. Nei's G_{ST} uses allele frequencies to determine proportions of genetic variation. Yeh (2000) explains that G_{ST} values range from 0 to 1.0, with values less than 0.05 indicating low levels of differentiation, and higher values reflecting higher differentiation. In this analysis, $G_{ST} = 0.0350$ indicates that 3.5% of the genic variation was due to among-population (or group) genetic differentiation, or gene frequency differences. Thus, the majority of variation, 96.5%, was maintained within-population. G_{ST} values support the lack of differentiation also demonstrated in heterozygosity comparisons. Allele frequency comparisons can also be used to reveal distinct distribution differences that do not necessarily show in heterozygosity comparisons, particularly as all generation groups contain high diversity. Figure 2.7 shows comparison charts of allele frequencies in overall, mature, juvenile, and embryo groups. Clearly, embryos contain a higher percentage of rare and low frequency alleles than either of the older groups. The juvenile group does not have any rare frequency alleles, but does have common and intermediate alleles that do not exist in mature groups. Five private alleles

are very specifically contained in the embryo groups for 3 of 8 loci, although there is one private allele in the juvenile group.

Wright's F-statistics reveal some trends, but have large standard errors because of small population sizes (Table 2.10). The overall F_{ST} value of 0.0.035 indicates only 3.5% among-group variation, and very low levels of genetic differentiation. The individual F_{ST} values also suggest that differentiation was not strongly observed between any of the groups. Heterozygote excess is evident at many loci throughout all the age groups, and this affects mean F-statistics. Nei's (1978) genetic diversity statistic G_{ST} was very low (0.0350), although only slightly lower than the average value of 0.068 observed in gymnosperms (Hamrick and Godt 1990); Young *et al.* (2000) explains that G_{ST} values range from 0 to 1.0, with values less than 0.05 indicating low levels of differentiation. Comparable low levels of differentiation can be found in other pine species with large, continuous distributions : lodgepole pine, $G_{ST} = 0.036$ (Wheeler and Guries 1982); jack pine, $G_{ST} = 0.030$ (Dancik and Yeh 1983); and ponderosa pine, $G_{ST} = 0.015$ (Hamrick *et al.* 1989).

Locus	Sample Size	F_{IS}	F_{IT}	F_{ST}
rps2	232	-0.2294	-0.1866	0.0348
rps6	220	-0.0605	-0.0432	0.0136
rps20	226	0.1559	0.2027	0.0555
rps39	230	-0.2186	-0.2017	0.0139
rps50	232	0.0622	0.0927	0.0326
rps60	232	-0.0319	-0.0186	0.0129
rps90	222	-0.0409	-0.0144	0.0254
rps127	208	-0.1748	-0.0596	0.0981
Mean	**225**	**-0.0544**	**-0.0175**	**0.0350**

Table 2.10: Wright's (1951) F-statistics shown for 8 loci. F_{IS} = -0.0544 indicates a 5.44% heterozygote excess within groups relative to Hardy-Weinberg expectations. F_{IT} = -0.0175 indicates a 1.75% heterozygote excess of the population in relation to Hardy-Weinberg expectations. F_{ST} = 0.035 is the correlation of two random gametes, indicating3.5% among-population genetic differentiation.

DISCUSSION

The results from this study suggest a high heterozygosity value for *Pinus strobus*, which is in agreement with other reports for microsatellite markers (Echt *et al.* 1996, Rajora *et al.* 2000) and for other markers (Buchert *et al.* 1997, Rajora *et al.*. 1998) (Table 2.11). With all age groups pooled, heterozygosity values for the entire population were considerably higher than other reported values for white pine; however isozyme values are typically quite low. The very high values reported in this study may simply reflect the mutability and hyper-variation associated with microsatellite markers, but may also be a result of the subset of markers used. Echt *et al.* (1996) reports heterozygosity ranges from 0.125 to 0.812 for the characterized markers, indicating that heterozygosity estimates may be affected by the subset of markers used. This is particularly important when considering marker subsets that contain monomorphic loci, as reported by Rajora *et al.* (2000). For the values reported by Echt *et al.* (1996) when characterizing the rps markers, excluding two monomorphic markers (with an inherent $H_o = 0$), the mean H_o values for 18 SSR markers change from 0.458 to 0.515. In the same example, removing the five least heterozygous loci from the group, the mean H_o over 11 markers is 0.744. The multiple age groups represented here may also be a factor in the high heterozygosity values. The two previous studies using these SSR markers were restricted to either a small sample size of only N = 16 trees (Echt *et al.* 1996), or only old growth, mature trees (Rajora *et al.* 2000). The standard errors in this study are higher than those reported by Rajora *et al.* (2000), which may be an effect of smaller population size, as well as a range of age groups. Although homozygosity is expected to be higher in

embryos and seedlings because they have undergone less natural selection (Ledig 1986), there is also a possibility that a larger array of alleles may be represented as rare or uncommon alleles because of the inclusion of embryos. This larger array of alleles could lead to the high heterozygosity values observed in this study.

Method	N trees	N loci	A	P	H_o	Reference
Isozymes	300	12	1.96	50.6%	0.176	Beaulieu and Simon 1994
Isozymes	95	54	2.37	75.9%	0.126	Buchert *et al.* 1997
Isozymes	102	20	1.75	47.8%	0.215	Rajora *et al.* 1998
SSRs	16	16	5.4	91.0%	0.515	Echt *et al.* 1996
SSRs	102	13	9.43	92.3%	0.522	Rajora *et al.* 2000
SSRs	115	10	7.1	100%	0.762	Sokol, 2001

Table 2.11: Comparisons of genetic diversity of white pine. A = Alleles per locus, P = Percent polymorphic loci, H_o = Mean observed heterozygosity

Several possible outcomes can be predicted for potential differences among age groups. One possibility is that because only well-adapted individuals survive to successfully reproduce, and their offspring will most likely inhabit a similar ecosystem, successful progeny will be genetically uniform and no significant genetic differences will be detected. Over the course of several generations, this could lead to increased homozygosity (see Ledig 1986). In white pine this scenario is unlikely for several reasons: mutation rates are moderate to high (Echt *et al.* 1996), wind distributes pollen over a large area(Wendel and Smith 1990), inbreeding depression selects against deleterious recessives (Epperson and Chung 2001), and the long life span of these trees dictates adaptation to frequently changing environmental conditions from generation to generation. Even after inbreeding depression and natural selection have reduced the number of genotypes in a new cohort of seeds, the

sheer numbers of seeds and successive seedlings suggests that many more genotypes would be represented in these younger groups. Conversely, significant genetic differences in age groups could be attributable to very high heterozygosity levels combined with small sample sizes; with extremely high amounts of genetic variation present, sampling effects could result in many rare or unique alleles that create significant differences among any two sets of samples. However, with high levels of rare alleles comes the potential for deleterious recessive alleles. These alleles could be selected against, causing older trees to have higher heterozygosity levels than younger trees which have not yet been affected. This would be an effect of overdominance, heterozygosity conferring increased fitness in individuals; however, the validity of this effect has been in contention and is not necessarily supported by these results. Rare alleles may also be advantageous, which sparks the argument that significant genetic differences between age groups may be an indication of selection for well-adapted, new alleles with age; or indeed, selection against more common good alleles, or gene complexes, as in high-grade harvesting. No significant differences were found in heterozygosity measures, indicating that all three age groups contain comparably high genetic diversity. This is a significant finding, as it suggests that the variation existing in white pine over 200 years ago is well-represented in recent cohorts.

Although overall heterozygosity estimates were high, Wright's F-statistics for this study show some heterozygote deficiencies at individual loci in all age groups, with heterozygote excess increasingly apparent in juvenile and mature groups. This observation may be a factor of the distribution of allele frequencies, discussed below.

F-statistics demonstrate a trend with more heterozygote deficiencies (or more homozygosity) in embryos than in mature trees. Slight heterozygote excess is evident in both mature and juvenile groups; allele frequency analysis indicates that juvenile trees may harbor more intermediately common alleles than either embryos or mature trees, and this may act to mask homozygotes. Slight deficiencies of heterozygotes have been previously reported among embryos, often with heterozygosity increasing with tree age (Ledig 1986). Similar results were reported for 110-year old white pine stands and their direct progeny: adult populations showed a slight excess and filial populations showed a slight deficiency of heterozygotes (Beaulieu and Simon 1995), which suggests selection against homozygotes during the life cycle. This pattern may be quite common for conifers (Cheliak *et al.* 1985, El-Kassaby *et al.* 1987, Knowles *et al.* 1987). A low F_{ST} value indicates very little genetic differentiation between any of the groups. The low G_{ST} value, nearly equivalent to F_{ST}, indicates little population differentiation which is relatively common for conifers with large, continuous ranges (Wheeler and Guries 1982, Dancik and Yeh 1983, Hamrick *et al.* 1989). This may be an indication that anthropogenic forces have not effectively fragmented these populations, creating a continuous regional corridor of gene flow.

The most compelling results from these data are found in allele frequency trends. Private alleles, those found in one group or population but not shared by any other group, and rare alleles, those found throughout the samples but in very low frequencies ($P<0.01$) are often useful for analysis. In this case, if private alleles were apparent in the mature trees but not the juvenile or seed groups, a loss of genetic variation becomes clear and quantitative; that allele has been lost from the

reproduction. Of course, this may be simply an effect of small sample size (the allele was present in other groups but missed in the sampling) or of changing adaptational requirements (that allele conferred fitness in another environment and is not successful in a new environment). Conversely, if a private allele were found in the juvenile or embryo group, it could be an indication of gene flow from surrounding stands in which the allele is more common. In addition, the presence of many rare alleles may indicate high mutation rates or high gene flow. Common alleles, those shared by all the groups and found in high frequencies ($P>0.75$) are also indicative, representing a group of shared genetic traits that persist in large numbers within and among populations. Theoretically, these may be linked to well-adapted genes, but even when considered as neutral, they can be an indication of low mutation rates or low gene flow, as many trees share the same genetic make-up with little infusion of new genes. The lack of private alleles in the mature trees suggests that these alleles either do not significantly influence success, or that trees in the stand containing these alleles have been harvested; in either case, the presence of 3 private alleles in embryos reveals that nearby stands harbor these alleles. A significant factor in this observation, however, is the relatively small sample size of older trees. The fact that the majority of alleles occur at intermediate or low frequencies suggests that maintaining diversity is an important part of long-term species success.

Although effects of along history of high-grading eastern white pine throughout its natural range cannot be quantified using these data, some key issues arise when considering the results of this study. Special considerations must be made for the tendency of white pine populations to maintain most genetic variation within-

populations, and for the patterns of within-stand spatial genetic structure (Beaulieu and Simon 1994, Ryu and Eckert 1983, Brym and Eckert 1983, Epperson and Chung 2001). Local diversity, consisting of gene complexes well-adapted to local sites as well as locally deleterious gene complexes, can be an important adaptational resource. Beaulieu and Simon (1994) report several local, rare alleles found only in a small subset of white pine populations in Quebec. Ledig (1994) and Buchert (1994) have theoretically analyzed potential effects of selective harvesting on eastern white pine populations; they both conclude that despite the current lack of experimental evidence, selective harvesting of white pine will change mating system dynamics, reduce genetic variation, and degrade local genetic resource, leading to stands with lower future value. Applied research on the genetic impacts of harvesting reveals a pattern of rare and low frequency allele loss with no changes in overall genetic diversity. Although Neale (1985) and Neale and Adams (1985) reported no loss of low frequency isozyme alleles after a shelterwood harvest of Douglas-fir, Adams *et al.*'s(1998) similar study, Adams *et al.* (1998) documented a slight loss of low frequency isozyme alleles in residual trees after a similar Douglas-fir shelterwood harvest. The authors suggest that the lost alleles were most likely deleterious, as the harvested trees were smaller than the residual trees, but point out the potential for these alleles to have future adaptational value. Despite a loss of alleles from the stand, there were no significant differences in pre- and post-harvesting heterozygosity values, particularly when natural regeneration was present. Beaulieu and Simon (1994) report on a population of genetically isolated white pine in the St.Lawrence region that are genetically less diverse than other Quebec populations. Based on

historical information for the region and the species, refugia effects were rejected as a possible cause, but the effects of long-term harvesting creating a small genetic bottleneck did apply to the situation. Additionally, these data show that white pine populations can be genetically depauperate despite high gene flow. Rajora *et al.* (2000) and Buchert *et al.* reported a loss of 40% of low frequency alleles and an 80% loss of rare alleles following harvests in old growth white pine stands; however, diversity levels did not significantly change after harvest and genetic composition of regeneration was not assayed so it is difficult to make inferences on long-term effects. In a contrasting study of diameter-limit harvests of eastern hemlock in Maine, rare alleles actually increased in strongly selected stands (Hawley, DeHayes and Brissette, 1990). Like Adams *et al.* (1998), the authors suggest that these rare alleles represent deleterious genotypes that remained in the stand after the best genotypes had been removed.

These results lead to two possible conclusions regarding the implications of this study: either selective harvesting (positive or negative) will lead to a loss of rare and low-frequency alleles in the mature residual populations; or negative selective harvesting will specifically leave rare, deleterious alleles as heterozygotes in the residual stands. My data show that most alleles of white pine microsatellite markers fall into the low and rare frequency classes, with few intermediate, and very few common or private alleles. This may be an effect of past losses of rare, private alleles, or of deleterious alleles being lost to natural selection. Although there are more rare and private alleles in embryos and juvenile trees than in mature trees, it is not apparent that diameter-limit harvesting has led to an increase of rare alleles in the

population, as the embryos and regeneration are expected to contain more low frequency alleles as a result of higher sample sizes and less natural selection over time.

The low sample sizes of individual age groups and particularly of individual age groups within the 2 stands create an incomplete picture of allele frequencies, so these data cannot be used independently to support conclusions about the structure or dynamics of genetic diversity. However, heterozygosity values for pooled data are useful for building a foundation of knowledge about the high genetic variability of white pine in Maine. In addition, F-statistics and allele frequencies do offer a good perspective of the potential directions and pitfalls that may be encountered on further investigation.

Beyond a small sample size, other weaknesses of these data include the assumptions of sequence identity for the amplified microsatellites and their scoring for allele length instead of repeat number. Because these PCR products were not sequenced, the sequence variation is assumed to be linearly correlated with fragment size. However, mutations in flanking sequences, interruptions, and even new microsatellite repeat motifs have been found to corrupt some loci (Richards and Sutherland 1994, Zhu *et al.* 2000). Additionally, the PAGE protocol was part skill and part art, developed as the investigation progressed, and therefore was not meticulously consistent throughout. In addition, visualization of products varied dramatically for each gel, although utmost care was taken to correctly measure allele sizes.

CONCLUSIONS AND IMPLICATIONS

In the spruce-fir forests of Maine, fixed diameter-limit harvesting can result in significant devaluation of the residual stand. In comparison with 5-year positive selection stands in which trees are not harvested according to diameter, residual FDL stands are composed of smaller, slower-growing trees. Changes in species composition, standing volume, and growing volume of wood lead to decreased residual stand value. If FDL harvests occur at a stand-level, degradation of the stand will result; if strong selection is used over a wider range, or in fragmented forests, a long-term decrease in forest productivity could result, leading to a loss of ecological and economic resources. Theoretically, this practice could also lead to range-wide genetic degradation of the species. In the case of red spruce, these data support the hypothesis that range-wide, long-term selective harvesting may have depreciated the genetic quality of red spruce stands, and that this genetic deterioration may be a contributing factor in the observed decline in softwood productivity in Maine. If the practice continues, seriously limited evolutionary potential of the species and its related ecosystems can be anticipated over the long-term.

Despite centuries of high-grade harvesting throughout its range, eastern white pine in Maine exhibit very high genetic diversity with little differentiation between generation groups. In this study, genetic diversity of 200-275 year old mature trees is well represented in both the embryos and the regeneration present at or near these sites. Private alleles are found only in the seed or juvenile regeneration, indicating that they were either introduced from outside of the stand or have been eliminated through natural selection as the stands age. All alleles in the mature trees are present in the sampled gene pool of potential regeneration, and frequencies are relatively similar. These data suggests

that in this region of the white pine range, gene flow is high enough to maintain genetic diversity between generations even after fragmentation and selective harvesting of the species. A broad, relatively continuous range and high gene flow are significant factors in ameliorating the impacts of harvesting and fragmentation for white pine; other species, however, may not enjoy the same characteristics. For species with limited natural ranges and low natural genetic diversity levels, such as red spruce, dysgenic harvesting will have more serious implications, particularly if rare and low frequency alleles are lost in harvest and not distributed or replenished by gene flow. A smaller, less continuous range makes fragmentation more acute and allows for less gene flow. Lower inherent genetic variation and loss of allelic richness could result in larger-scale disturbances associated with any fluctuations in allele frequencies, particularly if many rare or private alleles exist in the species.

Measures of genetic diversity and variation should be a primary concern in designing a forest management plan. With a more complete foundation of knowledge about genetic systems, the forestry community can implement harvesting regimes and tree improvement programs that are more specifically targeted to increasing productivity and decreasing resource degradation. Both of these goals include maintenance of a healthy and versatile genetic resource. Based on these findings, I recommend a reassessment of any harvesting prescription that includes fixed diameter-limit removals, particularly for species that have low natural genetic diversity levels or a limited natural range, such as red spruce. Maintenance of a healthy genetic reserve can avoid effects of dysgenic harvesting.

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APPENDIX A:
Red Spruce Radial Increment Statistics

	S05_20	FDL_20	S05_40	FDL_40	S05_60	FDL_60
N of cases	53	60	53	60	53	60
Minimum	0.5	0.5	6	6	12.5	12
Maximum	56.65	28.5	102.35	64	153.44	83.5
Range	56.15	28	96.35	58	140.94	71.5
Mean	23.255	12.122	41.553	23.508	62.043	36.542
Std. Error	1.717	0.904	2.983	1.734	4.225	2.299
Std. Dev	12.503	7.001	21.718	13.433	30.759	17.811
Variance	156.316	49.014	471.682	180.445	946.141	317.214

	S05_80	FDL_80	S05_100	FDL_100
N of cases	53	60	53	60
Minimum	18.92	16	24.41	29.5
Maximum	199.33	119	235.67	162
Range	180.41	103	211.26	132.5
Mean	88.352	55.078	113.271	79.813
Std. Error	5.625	3.159	6.605	4.276
Std. Dev	40.951	24.467	48.087	33.124
Variance	1676.99	598.612	2312.318	1097.175

Table A1: General statistics for cumulative radial growth data.
GP = Growth Period
GP_20 = 0 - 20 years
GP_40 = 20 - 40 years
GP_60 = 40 - 60 years
GP_80 = 60 - 80 years
GP_100 = 80 - 100 years

	S05_20 vs FDL_20	S05_40 vs FDL_40	S05_60 vs FDL_60	S05_80 vs FDL_80	S05_100 vs FDL_100
Mean S05	23.255	41.553	62.043	88.352	113.271
Mean FDL	11.742	23.217	35.708	53.815	78.242
Mean Difference	11.514	18.336	26.335	34.537	35.029
95.00% CI	7.350 to 15.678	10.930 to 25.741	16.272 to 36.399	21.291 to 47.783	18.253 to 51.805
SD Difference	15.107	26.867	36.510	48.056	60.863
t	5.548	4.968	5.251	5.232	4.190
df	52	52	52	52	52
Prob	0.000	0.000	0.000	0.000	0.000

Table A2: Paired t-tests for cumulative radial growth.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
GP	441742.952	4	110435.738	145.378	0.000
HVST_TRT	82963.914	1	82963.914	109.214	0.000
HVST_TRT*GP	10646.449	4	2661.612	3.504	0.008
Error	421604.428	555	759.648		

Table A3: ANOVA model for cumulative radial growth.
HVST_TRT = Treatment categories (S05 and FDL)
S05 = 5-year selection stands
FDL = Fixed diameter-limit stands

	S05_20	FDL_20	S05_40	FDL_40	S05_60	FDL_60	S05_80	FDL_80	S05_100	FDL_100
N of cases	53	60	53	60	53	60	53	60	53	60
Minimum	0.5	0.5	3	2	3.62	3.5	1.81	3.5	5.4	6.5
Maximum	56.65	28.5	55.81	39	59.94	34	63.24	53	63.91	79.8
Mean	23.255	12.122	18.298	11.387	20.49	13.033	26.309	18.537	24.919	24.735
Std. Error	1.717	0.904	1.497	1.001	1.755	1.002	1.91	1.474	1.791	2.044
Std. Dev	12.503	7.001	10.895	7.752	12.78	7.761	13.904	11.416	13.039	15.833

Table A4: General statistics for non-cumulative radial growth data.

	S05_20 vs FDL_20		S05_40 vs FDL_40		S05_60 vs FDL_60		S05_80 vs FDL_80		S05_100 vs FDL_100	
Mean S05	23.255		18.298		20.490		26.309		24.919	
Mean FDL	11.742		11.475		12.491		18.107		24.427	
Mean Difference	11.514		6.822		7.999		8.202		0.493	
95.00% CI	7.350 to	15.678	2.943 to	10.702	4.000 to	11.999	3.356 to	13.047	-5.717 to	6.703
SD Difference	15.107		14.075		14.511		17.579		22.530	
t =	5.548		3.529		4.013		3.397		0.159	
df	52		52		52		52		52	
Prob	0.000		0.001		0.000		0.001		0.874	

Table A5: Paired t-tests for non-cumulative radial growth.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
GP	7791.082	4	1947.771	14.577	0.000
HVST_TRT	6300.406	1	6300.406	47.153	0.000
HVST_TRT*GP	1979.663	4	449.416	3.363	0.010
Error	74157.659	555	133.617		

Table A6: ANOVA model for non-cumulative radial growth.

APPENDIX B:
Scoring Microsatellite Alleles from PAGE Gels

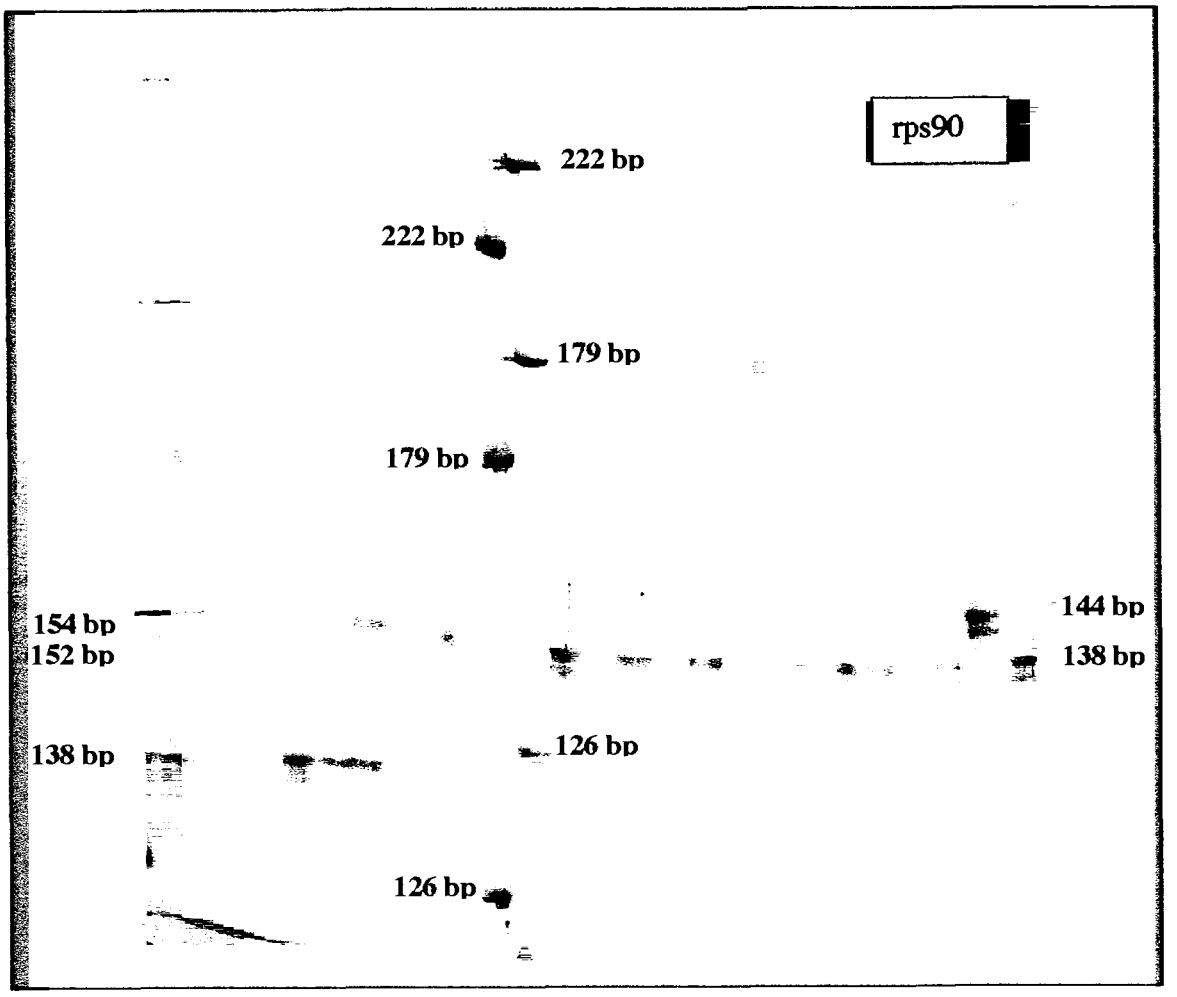

Figure B1: General allele sizing example. Middle lanes show size ladders. The first half of the gel was loaded, then run -10 minutes to reduce well leakage. The second half (at the red arrow) was then loaded and run. Each run is given independent reference ladders. The differences in reference ladder sizes show the difference in between run times. Once reference size fragments are identified, based on supplied banding patterns, three to five reference bands (not all are shown to save space) are entered into the imaging program. Then alleles can be selected, and based on references, sized according to base pairs. For consistency, the lower threshold of each band is used. Stutter bands occur at 2bp intervals and can easily be identified. Artifact bands also occur with some markers, but are generally weak and appear at random sizes.

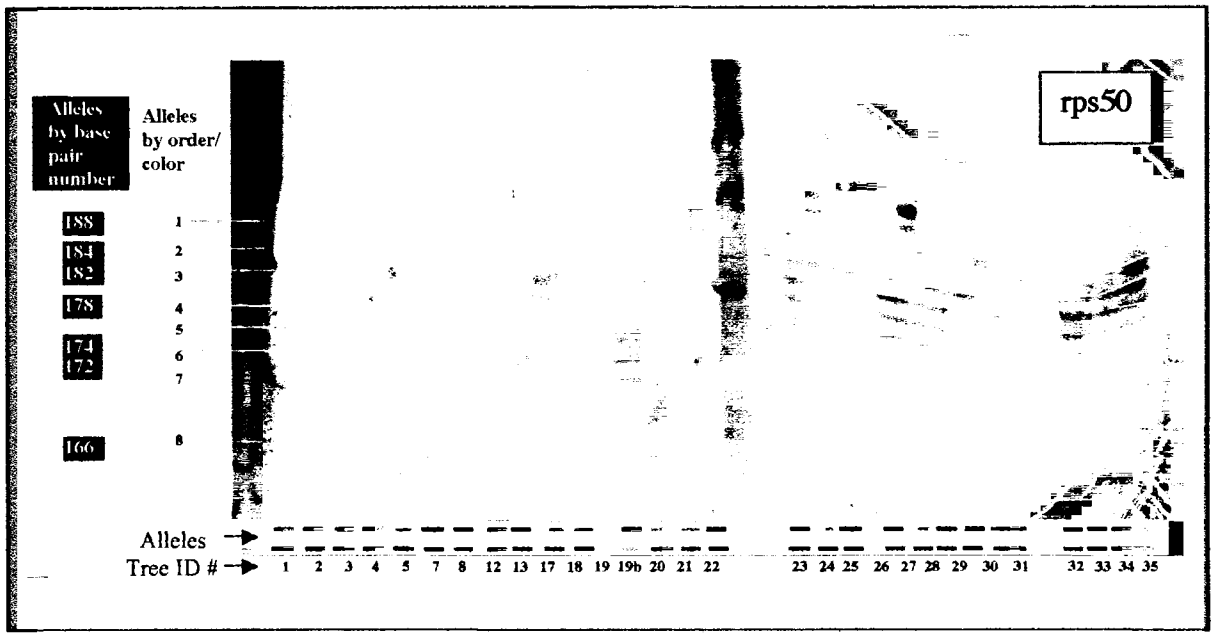

Figure B2: Genotyping example An example of how alleles were scored. The size standard was run on both end lanes and in the middle lanes - in the full gel, 5 standard lengths could be scored, and from them, the alleles can be scored base on length. Each fragment length is one allele. After scanning in digital gel images, it was easiest to color-code alleles for genotyping with corresponding allele sizes and numbers shown here at left. Allele numbers were changed to letters for genotype analysis.

* Tree #3, #12 either heterozygous or null allele. Four megagametophytes from Tree ID#3 indicate homozygosity (run separately)

* Trees #4 & #8 are scored for both alleles 6 and 7 b/c the strength of the second band and the number of stutters beneath indicate the presence of allele 7. Compared with Tree #13 (homozygous for 6), there is one extra stutter band, and stutters are stronger.

Tree #31 spans 2 lanes. For scoring Trees #32-34, based on stutter band strength and number of stutter bands, these samples are homozygous.

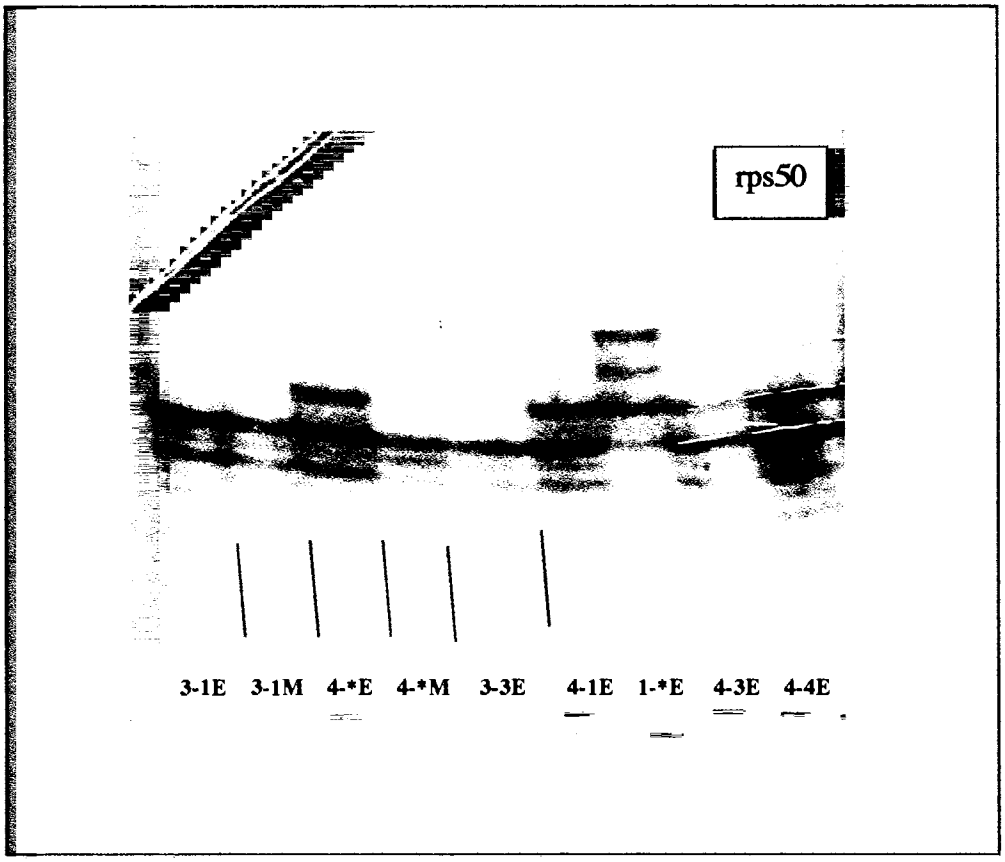

Figure B3: Embryo and megagametophyte comparisons.
Notation : E = embryo M = megagametophyte 3-1E indicates the embryo from the 1st seed of a cone from Tree #3.
A comparison of the mature tree ID (if known) and the megagametophyte and embryos could be made to ensure correct allele and genotype scoring. In this example, the same marker (rps50) is genotyped for trees #3 and #4 seen in the previous Figure A2. Because they all share the same maternal allele (the orange allele in tree 3 and the orange or green allele in tree 4), genotypes can be relatively certain. Samples 3-1E and 3-1M could indicate a null allele – more megagametophytes should be examined to be sure, however, null alleles are considered very rare (Ciofi *et al.* 1998), whereas homozygotes would be less rare. Unfortunately, most seeds were very degraded and did not produce much DNA.

APPENDIX C:
Eastern White Pine Microsatellite Genotypes and Loci Statistics Eight and Ten Loci Subsets

	rps2	rps6	rps20	rps39	rps50	rps60	rps90	rps127
Chi-square	31.585648	2.332413	104.446862	4.843596	71.359210	70.663818	35.114782	0.332514
df	15	3	66	10	36	78	28	3
Probability	0.007328	0.506340	0.001803	0.901378	0.000406	0.709876	0.166551	0.953802
G-square	28.890892	2.291170	91.475690	5.468583	61.707853	60.514585	30.729490	0.337333
df	15	3	66	10	36	78	28	3
Probability	0.016613	0.514215	0.020708	0.857763	0.004850	0.928722	0.329223	0.952861

Table C1: Chi-square and likelihood ratio tests for Hardy-Weinberg equilibrium, embryo samples, 8 loci.

Allele Frequency of embryos :

Allele	rps2	rps6	rps20	rps39	rps50	rps60	rps90	rps127
A	0.2500	0.2564	0.0595	0.1190		0.0238	0.1026	0.3026
B	0.3333	0.3846	0.1071	0.3333	0.0119	0.0952	0.0385	0.3947
C	0.3333	0.3590	0.0238	0.2381	0.0119	0.1071	0.0769	0.3026
D	0.0476		0.0833	0.2976	0.1429	0.0238	0.0513	
E	0.0238		0.1071	0.0119	0.0952		0.0769	
F	0.0119		0.1548		0.2857	0.0238	0.0513	
G			0.0595		0.0833	0.1667	0.4872	
H			0.1786		0.1429	0.2262	0.1154	
I			0.0595		0.1905	0.0595		
J			0.0833		0.0357	0.0833		
K			0.0476			0.0476		
L			0.0357			0.0238		
M						0.0833		
N						0.0357		

Table C2: Allele frequencies for embryo samples, 8 loci.

Locus	Sample Size	na*	ne*	I*
rps2	84	6.0000	3.4759	1.3657
rps6	78	3.0000	2.9194	1.0842
rps20	84	12.0000	9.3087	2.3460
rps39	84	5.0000	3.6942	1.3747
rps50	84	9.0000	5.6720	1.8853
rps60	84	13.0000	8.1478	2.3001
rps90	78	8.0000	3.5746	1.6577
rps127	76	3.0000	2.9499	1.0904
Mean	82	7.3750	4.9678	1.6380
St. Dev		3.8149	2.4931	0.5002

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table C3: Observed and effective numbers of alleles, embryos, 8 loci.

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
rps2	84	0.1190	0.8810	0.2791	0.7209	0.7123	0.6220
rps6	78	0.4359	0.5641	0.3340	0.6660	0.6575	0.6597
rps20	84	0.2857	0.7143	0.0967	0.9033	0.8926	0.7964
rps39	84	0.2857	0.7143	0.2619	0.7381	0.7293	0.6892
rps50	84	0.3095	0.6905	0.1664	0.8336	0.8237	0.8266
rps60	84	0.2143	0.7857	0.1122	0.8878	0.8773	0.8823
rps90	78	0.2308	0.7692	0.2704	0.7296	0.7202	0.7286
rps127	76	0.2895	0.7105	0.3302	0.6698	0.6610	0.5505
Mean	82	0.2713	0.7287	0.2314	0.7686	0.7592	0.7194
St. Dev		0.0905	0.0905	0.0938	0.0938	0.0930	0.1112

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

Table C4: Heterozygosity estimates for embryo samples, 8 loci.

Allele \ Locus	rps2	rps6	rps20	rps39	rps50	rps60	rps90	rps127
Allele A	-0.3333	0.0586	0.3620	-0.1351	****	-0.0244	-0.1143	-0.0599
Allele B	-0.1786	0.1333	0.3778	0.0357	-0.0120	0.1711	-0.0400	-0.1014
Allele C	-0.3929	0.2200	-0.0244	-0.0500	-0.0120	0.1289	0.2778	-0.0599
Allele D	0.4750	****	0.2208	0.1458	0.0278	-0.0244	-0.0541	****
Allele E	-0.0244	****	0.3778	-0.0120	0.1711	****	-0.0833	****
Allele F	-0.0120	****	0.3629	****	0.1833	-0.0244	-0.0541	****
Allele G	****	****	-0.0633	****	0.5325	0.1429	-0.1289	****
Allele H	****	****	0.2696	****	0.0278	0.2518	-0.1304	****
Allele I	****	****	-0.0633	****	0.2279	-0.0633	****	****
Allele J	****	****	-0.0909	****	-0.0370	0.2208	****	****
Allele K	****	****	-0.0500	****	****	-0.0500	****	****
Allele L	****	****	-0.0370	****	****	-0.0244	****	****
Allele M	****	****	****	****	****	-0.0909	****	****
Allele N	****	****	****	****	****	-0.0370	****	****
Total	-0.2368	0.1420	0.1997	0.0206	0.1617	0.1044	-0.0680	-0.0749

Table C5: Wright's (1978) fixation index (F_{is}), embryo samples, 8 loci.

Locus	Sample Size	na*	ne*	I*
rps2	80	4.0000	2.8597	1.1570
rps6	80	4.0000	3.4152	1.2794
rps20	80	10.0000	3.7123	1.7378
rps39	78	4.0000	3.1987	1.2148
rps50	80	10.0000	7.3903	2.1174
rps60	80	14.0000	7.9602	2.3394
rps90	78	8.0000	3.8950	1.6495
rps127	64	3.0000	1.5026	0.5919
Mean	78	7.1250	4.2417	1.5109
St. Dev		3.9799	2.2464	0.5652

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table C6: Observed and effective numbers of alleles, juvenile, 8 loci.

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
rps2	80	0.1750	0.8250	0.3415	0.6585	0.6503	0.6220
rps6	80	0.1750	0.8250	0.2839	0.7161	0.7072	0.6597
rps20	80	0.3750	0.6250	0.2601	0.7399	0.7306	0.7964
rps39	78	0.0769	0.9231	0.3037	0.6963	0.6874	0.6892
rps50	80	0.1000	0.9000	0.1244	0.8756	0.8647	0.8266
rps60	80	0.0250	0.9750	0.1146	0.8854	0.8744	0.8823
rps90	78	0.2821	0.7179	0.2471	0.7529	0.7433	0.7286
rps127	64	0.5938	0.4062	0.6602	0.3398	0.3345	0.5505
Mean	78	0.2253	0.7747	0.2919	0.7081	0.6990	0.7194
St. Dev		0.1868	0.1868	0.1694	0.1694	0.1676	0.1112

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

Table C7: Heterozygosity estimates for juvenile samples, 8 loci.

Allele \ Locus	rps2	rps6	rps20	rps39	rps50	rps60	rps90	rps127
Allele A	-0.2500	-0.1285	0.3143	-0.0400	-0.0127	-0.0256	-0.1556	-0.2075
Allele B	-0.3033	-0.1740	-0.0256	-0.2257	-0.0811	-0.0390	-0.0263	-0.2549
Allele C	-0.2815	-0.2218	-0.0959	-0.4717	-0.0811	-0.0667	-0.0541	-0.0323
Allele D	-0.0390	-0.0811	-0.1268	-0.3929	-0.1268	-0.1765	-0.0833	****
Allele E	****	****	0.3985	****	-0.0667	-0.0667	-0.0263	****
Allele F	****	****	-0.0667	****	-0.1765	-0.0526	-0.0685	****
Allele G	****	****	-0.0256	****	-0.2698	-0.2000	0.2121	****
Allele H	****	****	-0.0256	****	0.4805	-0.1594	0.1643	****
Allele I	****	****	-0.0390	****	-0.0959	-0.0390	****	****
Allele J	****	****	-0.0256	****	-0.0390	-0.0526	****	****
Allele K	****	****	****	****	****	-0.0526	****	****
Allele L	****	****	****	****	****	-0.0127	****	****
Allele M	****	****	****	****	****	-0.0390	****	****
Allele N	****	****	****	****	****	-0.0390	****	****
Total	-0.2686	-0.1666	0.1446	-0.3429	-0.0408	-0.1151	0.0341	-0.2146

Table C8: Wright's (1978) fixation index (F_{is}), juvenile samples, 8 loci.

Locus	Sample Size	na*	ne*	I*
rps2	68	3.0000	2.0139	0.8477
rps6	62	3.0000	2.5938	1.0237
rps20	62	6.0000	4.2711	1.5696
rps39	68	4.0000	2.8649	1.1185
rps50	68	8.0000	4.7967	1.7594
rps60	68	14.0000	9.5537	2.4161
rps90	66	8.0000	3.6000	1.4821
rps127	68	3.0000	2.9082	1.0821
Mean	66	6.1250	4.0753	1.4124
St. Dev		3.8336	2.3925	0.5103

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table C9: Observed and effective numbers of alleles, mature, 8 loci.

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
rps2	68	0.4118	0.5882	0.4890	0.5110	0.5035	0.6220
rps6	62	0.2903	0.7097	0.3755	0.6245	0.6145	0.6597
rps20	62	0.3226	0.6774	0.2216	0.7784	0.7659	0.7964
rps39	68	0.1176	0.8824	0.3393	0.6607	0.6510	0.6892
rps50	68	0.2647	0.7353	0.1967	0.8033	0.7915	0.8266
rps60	68	0.0294	0.9706	0.0913	0.9087	0.8953	0.8823
rps90	66	0.2121	0.7879	0.2667	0.7333	0.7222	0.7286
rps127	68	0.1765	0.8235	0.3341	0.6659	0.6561	0.5505
Mean	66	0.2281	0.7719	0.2893	0.7107	0.7000	0.7194
St. Dev		0.1210	0.1210	0.1223	0.1223	0.1205	0.1112

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

Table C10: Heterozygosity estimates for mature samples, 8 loci.

Allele \ Locus	rps2	rps6	rps20	rps39	rps50	rps60	rps90	rps127
Allele A	-0.0968	-0.2157	0.1948	-0.0149	-0.0968	-0.0462	0.2143	-0.2454
Allele B	-0.2879	-0.0333	-0.1071	-0.4167	****	-0.0303	-0.0820	-0.3284
Allele C	-0.0578	-0.2653	-0.1273	-0.3828	0.6513	-0.0794	-0.0154	-0.1765
Allele D	****	****	0.2264	-0.2593	-0.1724	-0.0149	-0.0154	****
Allele E	****	****	0.2955	****	****	-0.0149	-0.0154	****
Allele F	****	****	-0.0164	****	0.2688	-0.0794	-0.0154	****
Allele G	****	****	****	****	-0.0462	-0.0119	-0.3636	****
Allele H	****	****	****	****	0.1937	-0.1930	-0.2000	****
Allele I	****	****	****	****	-0.0196	-0.0968	****	****
Allele J	****	****	****	****	-0.0149	-0.0794	****	****
Allele K	****	****	****	****	****	-0.1333	****	****
Allele L	****	****	****	****	****	-0.0625	****	****
Allele M	****	****	****	****	****	-0.0462	****	****
Allele N	****	****	****	****	****	-0.0303	****	****
Total	-0.1684	-0.1550	0.1155	-0.3555	0.0710	-0.0841	-0.0909	-0.2551

Table C11: Wright's (1978) fixation index (F_{is}), mature samples, 8 loci.

	rps2	rps6	rps20	rps39	rps50	rps60	rps90	rps127
Chi-square	33.132517	2.225111	211.615654	14.308260	75.958852	114.172927	107.958815	1.524784
df	15	6	66	10	45	91	28	3
Probability	0.00450015	0.897875	0.000000	0.159390	0.002658	0.050627	0.000000	0.676562
G-square	35.249398	2.230898	146.004580	15.745451	80.584755	109.966525	69.813308	1.592684
df	15	6	66	10	45	91	28	3
Probability	0.002266	0.897285	0.000000	0.107155	0.000883	0.085774	0.000020	0.661050

Table C12: Chi-square and likelihood ratio tests for Hardy-Weinberg equilibrium, overall, 8 loci.

Allele	rps2	rps6	rps20	rps39	rps50	rps60	rps90	rps127
A	0.1853	0.2682	0.0973	0.0609	0.0302	0.0302	0.2252	0.2885
B	0.4741	0.4045	0.1106	0.3478	0.0302	0.0560	0.0450	0.5096
C	0.2974	0.3000	0.0708	0.3217	0.0431	0.0819	0.0495	0.2019
D	0.0302	0.0273	0.1195	0.2652	0.1336	0.0647	0.0495	
E	0.0086		0.3053	0.0043	0.0560	0.0259	0.0405	
F	0.0043		0.0841		0.1810	0.0474	0.0450	
G			0.0310		0.1164	0.1983	0.4189	
H			0.0752		0.2069	0.1767	0.1261	
I			0.0354		0.1724	0.0603		
J			0.0398		0.0302	0.0690		
K			0.0177			0.0690		
L			0.0133			0.0302		
M						0.0560		
N						0.0345		

Table C13: Allele frequencies, overall, 8 loci.

Locus	Sample Size	na*	ne*	I*
rps2	232	6.0000	2.8685	1.1970
rps6	220	4.0000	3.0645	1.1785
rps20	226	12.0000	6.6126	2.1597
rps39	230	5.0000	3.3494	1.2782
rps50	232	10.0000	6.9236	2.0716
rps60	232	14.0000	9.3640	2.4368
rps90	222	8.0000	3.9566	1.6684
rps127	208	3.0000	2.6063	1.0252
Mean	225	7.7500	4.8432	1.6269
St. Dev		3.9551	2.4779	0.5357

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table C14: Observed and effective numbers of alleles, overall, 8 loci.

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
rps2	232	0.2241	0.7759	0.3458	0.6542	0.6514	0.6220
rps6	220	0.3000	0.7000	0.3232	0.6768	0.6737	0.6597
rps20	226	0.3274	0.6726	0.1475	0.8525	0.8488	0.7964
rps39	230	0.1652	0.8348	0.2955	0.7045	0.7014	0.6892
rps50	232	0.2241	0.7759	0.1407	0.8593	0.8556	0.8266
rps60	232	0.0948	0.9052	0.1029	0.7506	0.7473	0.7286
rps127	208	0.3462	0.6538	0.3807	0.6193	0.6163	0.5505
Mean	225	0.2406	0.7594	0.2482	0.7518	0.7485	0.7194
St. Dev		0.0843	0.0843	0.1054	0.1054	0.1050	0.1112

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

Table C15: Heterozygosity estimates, overall, 8 loci.

Allele \ Locus	rps2	rps6	rps20	rps39	rps50	rps60	rps90	rps127
Allele A	-0.2275	-0.0885	0.2950	-0.0648	-0.0311	-0.0311	0.0707	-0.1243
Allele B	-0.1756	-0.0001	0.1455	-0.1883	-0.0311	0.1036	-0.0472	-0.0388
Allele C	-0.2583	-0.0390	-0.0762	-0.2751	0.1640	0.0255	0.1392	-0.0143
Allele D	0.2635	-0.0280	0.1167	-0.1378	-0.0798	-0.0691	-0.0521	****
Allele E	-0.0087	****	0.4367	-0.0044	0.1036	-0.0265	-0.0423	****
Allele F	-0.0043	****	0.2530	****	0.1278	-0.0498	-0.0472	****
Allele G	****	****	-0.0320	****	0.0360	-0.0304	-0.0548	****
Allele H	****	****	0.3003	****	0.2645	0.0223	-0.0626	****
Allele I	****	****	-0.0367	****	0.0938	-0.0642	****	****
Allele J	****	****	-0.0415	****	-0.0311	0.0602	****	****
Allele K	****	****	-0.0180	****	****	-0.0741	****	****
Allele L	****	****	-0.0135	****	****	-0.0311	****	****
Allele M	****	****	****	****	****	-0.0594	****	****
Allele N	****	****	****	****	****	-0.0357	****	****
Total	-0.1911	-0.0391	0.2076	-0.1901	0.0932	-0.0134	-0.0127	-0.0609

Locus	Sample Size	Fis	Fit	Fst	Nm*
rps2	232	-0.2294	-0.1866	0.0348	6.9360
rps6	220	-0.0605	-0.0432	0.0163	15.0911
rps20	226	0.1559	0.2027	0.0555	4.2567
rps39	230	-0.2186	-0.2017	0.0139	17.7689
rps50	232	0.0622	0.0927	0.0326	7.4207
rps60	232	-0.0319	-0.0186	0.0129	19.1515
rps90	222	-0.0409	-0.0144	0.0254	9.5849
rps127	208	-0.1748	-0.0596	0.0981	2.2994
Mean	225	-0.0544	-0.0175	0.0350	6.9026

* Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst.

Table C16: Wright's (1978) fixation index (F_{is}), overall, 8 loci.

pop ID	embryo	juvenile	mature
embryo	****	0.8771	0.8773
juvenile	0.1311	****	0.8839
mature	0.1309	0.1234	****

Figure C1: Nei's genetic identities and genetic distances, overall, 8 loci. Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Eastern White Pine Microsatellite Genotypes
and Loci Statistics
Ten Loci

	rps2	rps6	rps20	rps39	rps50	rps60	rps90	rps127
Chi-square	9.354554	3.405200	73.882103	13.826667	68.627851	88.701017	48.668083	1.896471
df	6	6	45	6	45	91	28	3
Probability	0.154598	0.756537	0.004263	0.031633	0.013179	0.548678	0.009071	0.594170
G-square	12.444193	3.783658	51.119955	20.856546	61.504163	66.180587	36.501284	3.095182
df	6	6	45	6	45	91	28	3
Probability	0.052762	0.705926	0.245864	0.001947	0.051358	0.976667	0.130272	0.377182

	rps25b	rps84
Chi-square	43.857417	41.301469
df	36	10
Probability	0.172730	0.000010
G-square	40.022900	30.423425
df	36	10
Probability	0.296159	0.000730

Table C17: Chi-square and likelihood ratio tests for Hardy-Weinberg equilibrium, juvenile samples, 10 loci.

Allele Frequency

Allele \ Locus	rps2	rps6	rps20	rps39	rps50	rps60	rps90	rps127	rps25b	rps84
Allele A	0.2000	0.2750	0.1250	0.0385	0.0125	0.0250	0.2308	0.1719	0.0250	0.0625
Allele B	0.4750	0.3375	0.0250	0.3590	0.0750	0.0375	0.0256	0.7969	0.1250	0.1250
Allele C	0.2875	0.3125	0.0875	0.3205	0.0750	0.0625	0.0513	0.0312	0.0625	0.0875
Allele D	0.0375	0.0750	0.1125	0.2821	0.1125	0.1500	0.0769		0.2625	0.5375
Allele E			0.4750		0.0625	0.0625	0.0256		0.2000	0.1875
Allele F			0.0625		0.1500	0.0500	0.0641		0.1375	
Allele G			0.0250		0.2125	0.2500	0.4231		0.1000	
Allele H			0.0250		0.1750	0.1375	0.1026		0.0750	
Allele I			0.0375		0.0875	0.0375			0.0125	
Allele J			0.0250		0.0375	0.0500				
Allele K						0.0500				
Allele L						0.0125				
Allele M						0.0375				
Allele N						0.0375				

Table C18: Allele frequencies for juvenile samples, 10 loci.

Locus	Sample Size	na*	ne*	I*
rps2	80	4.0000	2.8597	1.1570
rps6	80	4.0000	3.4152	1.2794
rps20	80	10.0000	3.7123	1.7378
rps39	78	4.0000	3.1987	1.2148
rps50	80	10.0000	7.3903	2.1174
rps60	80	14.0000	7.9602	2.3394
rps90	78	8.0000	3.8950	1.6495
rps127	64	3.0000	1.5026	0.5919
rps25b	80	9.0000	6.1069	1.9505
rps84	80	5.0000	2.8470	1.2939
Mean	78	7.1000	4.2888	1.5332
St. Dev		3.6347	2.1273	0.5240

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table C19: Observed and effective numbers of alleles, juvenile, 10 loci.

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
rps2	80	0.1750	0.8250	0.3415	0.6585	0.6503	0.5769
rps6	80	0.1750	0.8250	0.2839	0.7161	0.7072	0.6608
rps20	80	0.3750	0.6250	0.2601	0.7399	0.7306	0.7482
rps39	78	0.0769	0.9231	0.3037	0.6963	0.6874	0.6692
rps50	80	0.1000	0.9000	0.1244	0.8756	0.8647	0.8281
rps60	80	0.0250	0.9750	0.1146	0.8854	0.8744	0.8849
rps90	78	0.2821	0.7179	0.2471	0.7529	0.7433	0.7327
rps127	64	0.2000	0.8000	0.1532	0.8468	0.8362	0.8005
rps84	80	0.3500	0.6500	0.3430	0.6570	0.6488	0.6527
Mean	78	0.2353	0.7647	0.2832	0.7168	0.7077	0.7049
St. Dev		0.1698	0.1698	0.1570	0.1570	0.1554	0.1181

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

Table C20: Heterozygosity estimates for juvenile samples, 10 loci.

Allele \ Locus	rps2	rps6	rps20	rps39	rps50	rps60	rps90	rps127
Allele A	-0.2500	-0.1285	0.3143	-0.0400	-0.0127	-0.0256	-0.1556	-0.2075
Allele B	-0.3033	-0.1740	-0.0256	-0.2257	-0.0811	-0.0390	-0.0263	-0.2549
Allele C	-0.2815	-0.2218	-0.0959	-0.4717	-0.0811	-0.0667	-0.0541	-0.0323
Allele D	-0.0390	-0.0811	-0.1268	-0.3929	-0.1268	-0.1765	-0.0833	****
Allele E	****	****	0.3985	****	-0.0667	-0.0667	-0.0263	****
Allele F	****	****	-0.0667	****	-0.1765	-0.0526	-0.0685	****
Allele G	****	****	-0.0256	****	-0.2698	-0.2000	0.2121	****
Allele H	****	****	-0.0256	****	0.4805	-0.1594	0.1643	****
Allele I	****	****	-0.0390	****	-0.0959	-0.0390	****	****
Allele J	****	****	-0.0256	****	-0.0390	-0.0526	****	****
Allele K	****	****	****	****	****	-0.0526	****	****
Allele L	****	****	****	****	****	-0.0127	****	****
Allele M	****	****	****	****	****	-0.0390	****	****
Allele N	****	****	****	****	****	-0.0390	****	****
Total	-0.2686	-0.1666	0.1446	-0.3429	-0.0408	-0.1151	0.0341	-0.2146

Allele \ Locus	rps25b	rps84
Allele A	-0.0256	-0.0667
Allele B	-0.1429	0.0857
Allele C	-0.0667	-0.0959
Allele D	0.0315	0.0446
Allele E	0.3750	-0.0667
Allele F	0.0514	****
Allele G	-0.1111	****
Allele H	-0.0811	****
Allele I	-0.0127	****
Allele J	****	****
Allele K	****	****
Allele L	****	****
Allele M	****	****
Allele N	****	****
Total	0.0433	-0.0019

Table C21: Wright's (1978) fixation index (F_{is}), juvenile samples, 10 loci.

	rps2	rps6	rps20	rps39	rps50	rps60	rps90	rps127
Chi-square	3.667537	3.523965	14.865098	9.919273	50.750934	97.662614	155.396010	4.178379
df	3	3	15	6	28	91	28	3
Probability	0.299674	0.317667	0.461177	0.128093	0.005326	0.297553	0.000000	0.242836
G-square	5.349484	4.719985	14.863663	12.621954	31.394901	70.986148	47.033078	4.629413
df	3	3	15	6	28	91	28	3
Probability	0.147924	0.193488	0.461282	0.049448	0.299827	0.940430	0.013589	0.201033

	rps25b	rps84
Chi-square	35.632599	22.341269
df	21	10
Probability	0.024035	0.013457
G-square	32.930003	16.491885
df	21	10
Probability	0.046999	0.086391

Table C22: Chi-square and likelihood ratio tests for Hardy-Weinberg equilibrium, mature samples, 10 loci.

Allele Frequency:

Allele \ Locus	rps2	rps6	rps20	rps39	rps50	rps60	rps90	rps127	rps25b	rps84
Allele A	0.0882	0.2742	0.1129	0.0147	0.0882	0.0441	0.3636	0.3824	0.0370	0.0156
Allele B	0.6471	0.5161	0.2258	0.3529		0.0294	0.0758	0.3676	0.2222	0.2031
Allele C	0.2647	0.2097	0.1129	0.4265	0.0441	0.0735	0.0152	0.2500	0.0741	0.0625
Allele D			0.1774	0.2059	0.1471	0.0147	0.0152		0.2963	0.5000
Allele E			0.3548			0.0147	0.0152		0.2963	0.2188
Allele F			0.0161		0.0882	0.0735	0.0152		0.0556	
Allele G					0.0441	0.1765	0.3333		0.0185	
Allele H					0.3235	0.1618	0.1667			
Allele I					0.2500	0.0882				
Allele J					0.0147	0.0735				
Allele K						0.1176				
Allele L						0.0588				
Allele M						0.0441				
Allele N						0.0294				

Table C23: Allele frequencies for mature samples, 10 loci.

Locus	Sample Size	na*	ne*	I*
rps2	68	3.0000	2.0139	0.8477
rps6	62	3.0000	2.5938	1.0237
rps20	62	6.0000	4.2711	1.5696
rps39	68	4.0000	2.8649	1.1185
rps50	68	8.0000	4.7967	1.7594
rps60	68	14.0000	9.5537	2.4161
rps90	66	8.0000	3.6000	1.4821
rps127	68	3.0000	2.9082	1.0821
rps25b	54	7.0000	4.2507	1.6044
rps84	64	5.0000	2.9132	1.2411
Mean	65	6.1000	3.9766	1.4145
St. Dev		3.4140	2.1435	0.4581

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table C24: Observed and effective numbers of alleles, mature, 10 loci.

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
rps2	68	0.4118	0.5882	0.4890	0.5110	0.5035	0.5769
rps6	62	0.2903	0.7097	0.3755	0.6245	0.6145	0.6608
rps20	62	0.3226	0.6774	0.2216	0.7784	0.7659	0.7482
rps39	68	0.1176	0.8824	0.3393	0.6607	0.6510	0.6692
rps50	68	0.2647	0.7353	0.1967	0.8033	0.7915	0.8281
rps60	68	0.0294	0.9706	0.0913	0.9087	0.8953	0.8849
rps90	66	0.2121	0.7879	0.2667	0.7333	0.7222	0.7327
rps127	68	0.1765	0.8235	0.3341	0.6659	0.6561	0.4953
rps25b	54	0.5185	0.4815	0.2208	0.7792	0.7647	0.8005
rps84	64	0.1562	0.8438	0.3328	0.6672	0.6567	0.6527
Mean	65	0.2500	0.7500	0.2868	0.7132	0.7021	0.7049
St. Dev		0.1442	0.1442	0.1112	0.1112	0.1094	0.1181

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

Table C25: Heterozygosity estimates for mature samples, 10 loci.

Allele \ Locus	rps2	rps6	rps20	rps39	rps50	rps60	rps90	rps127
Allele A	-0.0968	-0.2157	0.1948	-0.0149	-0.0968	-0.0462	0.2143	-0.2454
Allele B	-0.2879	-0.0333	-0.1071	-0.4167	****	-0.0303	-0.0820	-0.3284
Allele C	-0.0578	-0.2653	-0.1273	-0.3828	0.6513	-0.0794	-0.0154	-0.1765
Allele D	****	****	0.2264	-0.2593	-0.1724	-0.0149	-0.0154	****
Allele E	****	****	0.2955	****	****	-0.0149	-0.0154	****
Allele F	****	****	-0.0164	****	0.2688	-0.0794	-0.0154	****
Allele G	****	****	****	****	-0.0462	-0.0119	-0.3636	****
Allele H	****	****	****	****	0.1937	-0.1930	-0.2000	****
Allele I	****	****	****	****	-0.0196	-0.0968	****	****
Allele J	****	****	****	****	-0.0149	-0.0794	****	****
Allele K	****	****	****	****	****	-0.1333	****	****
Allele L	****	****	****	****	****	-0.0625	****	****
Allele M	****	****	****	****	****	-0.0462	****	****
Allele N	****	****	****	****	****	-0.0303	****	****
Total	-0.1684	-0.1550	0.1155	-0.3555	0.0710	-0.0841	-0.0909	-0.2551

Allele \ Locus	rps25b	rps84
Allele A	-0.0385	-0.0159
Allele B	0.5714	-0.2549
Allele C	0.4600	-0.0667
Allele D	0.2895	-0.3750
Allele E	0.4671	-0.2800
Allele F	-0.0588	****
Allele G	-0.0189	****
Allele H	****	****
Allele I	****	****
Allele J	****	****
Allele K	****	****
Allele L	****	****
Allele M	****	****
Allele N	****	****
Total	0.3704	-0.2848

Table C26: Wright's (1978) fixation index (F_{is}), mature samples, 10 loci.

pop ID	juvenile	mature
juvenile	****	0.9004
mature	0.1049	****

Figure C2: Nei's genetic identities and genetic distances, 10 loci. Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

BIOGRAPHY OF THE AUTHOR

Kerry Sokol was born in Milwaukee, Wisconsin on June 4, 1974. She was raised in Wauwatosa, Wisconsin and graduated from Brookfield Central High School in 1992. She attended the University of Minnesota in the twin cities where she was awarded an Undergraduate Research Opportunities Program grant to explore the effects of windstorms on forest microsite plant regeneration. She was also awarded an independent research project to study maize genome mapping. Kerry graduated from the University of Minnesota in 1996 with a Bachelor's degree in Biology. Kerry is a member of the Phi Kappa Phi Honors Society and is a candidate for the Master of Science degree in Forestry from the University of Maine in December 2001.