THE GENETIC CONSEQUENCES OF SELF-THINNING

IN TWO POPULATIONS OF LOBLOLLY PINE (*Pinus taeda* L.)

by

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology.

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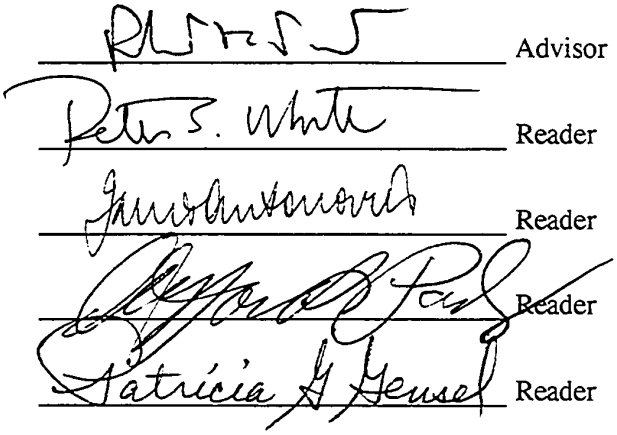
Approved by:



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Abstract

Kathleen F. Baker-Brosh. The Genetic Consequences of Self-thinning in Two Populations of Loblolly Pine (*Pinus taeda* L.)
(Under the direction of Dr. Robert K. Peet)

Although many aspects of forest stand development have been studied, little is known of genetic changes that may accompanying it. In this study, I explore the relationship between plant genotype, as assessed by genetic heterozygosity, and the intense competition trees experience during the thinning stage of forest stand development. The questions addressed by the study are 1) does density-dependent thinning act as a selective force for individuals with higher heterozygosity levels, and 2) does stand heterozygosity increase with self-thinning?

I use allozyme analysis to examine population-level changes in tree genetics during stand development in two populations. In the first, I grew loblolly pine seedlings at three density levels and recorded height measurements and mortality over a two-year span. In a second population, I examine a mature stand for which height and diameter data spanning 60 years were available.

In the seedling population, asymmetric competition for light caused the formation of a size hierarchy under high density conditions, which led to mortality of the shorter, light-suppressed individuals. Plant genotype was then related to growth and survivorship. Mean heterozygosity appeared to confer no advantage to height growth or survivorship among loblolly pine seedlings. However, single locus heterozygotes for two genotypes had higher survivorship rates than the alternate homozygous gen-

otypes. This result indicates that heterozygous individuals have a growth and survivorship advantage during stand thinning.

Among trees which survived to become the mature population, diameter was significantly correlated with mean heterozygosity during early stand growth. However, this correlation diminished after the trees were approximately 18 years old. This result indicates that heterozygosity confers a growth advantage to individuals, but is less influential once competitive interactions between trees becomes intense. Although the differences were not statistically significant, mean heterozygosity was highest in mature stands of intermediate densities which suggests that heterozygosity increases due to self-thinning at intermediate levels of intra-specific competition.

The results of this study indicate that asymmetric competition for light, the development of a size hierarchy, and differential mortality can cause changes in heterozygote frequency in plant populations during the thinning stage of forest development.

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Chapter 1

Introduction

Competition influences the growth and development of plants (Weiner 1985, Weiner and Fishman 1994), and is a structuring force for the development of many plant communities (Christensen and Peet 1984). Competitive interactions are known to shape the course of forest stand succession, affecting biological and biophysical processes such as the accumulation of biomass, nutrient cycling, and changes in species richness (Peet 1992).

The growth and survival of an individual plant depend on its ability to acquire needed resources. Many factors influence whether a plant will be a successful competitor, such as microsite quality, neighborhood density, disturbance, and plant genotype (Harper 1977). Genotype has been considered important to a plant's success, but few studies have looked at the interaction between genotype and competition, especially in forest trees. Secondary forest succession is driven by the differential success of some individuals and the death of others. This is a system based on natural selection, and it would be valuable to know to what degree genetic differences between individuals influence the outcome of competition during forest stand development. The differential survival associated with such competition for resources has possible consequences for genetic change in populations. However, as Endler (1986) points out, there is a lack of studies exploring evolutionary consequences of intraspecific competition.

In this thesis, I seek evidence for genetic changes that occur during the highly competitive self-thinning process in forest stand development. I measure genetic

change during the thinning stage, and examine whether genetic differences between plants influence the outcome of competition. This chapter is an overview of the literature on forest dynamics and genetic studies of forest trees. First I describe the process of forest development, taking into consideration the ecological variables and processes that affect and are affected by stand development. Special emphasis is placed on the Piedmont region of North Carolina. Next, I focus on what is known about the relationship between ecology and genetics, especially in relation to trees. Lastly, I describe genetic implications of successional processes, with an emphasis on important topics that need further attention, and, specifically, the topics covered by this research.

Forest Stand Development

Many changes during succession, including level and type of competition, allocation of biomass, and nutrient cycling are similar across numerous successional systems. In general, stand development can be divided into four stages (Oliver 1981, Peet 1992) with properties independent of species and consistent across many different ecosystems.

The first stage of forest development is the "stand initiation" or "establishment" stage. This stage starts with abandoned agricultural land or burned-over forest. The seeds of many species of herbs and trees, either present in the soil or dispersed there soon after a disturbance event, begin to germinate and grow. At this time, biomass is low (Odum 1969), but accumulation increases as establishment progresses. This stage is characterized by open space, high levels of light, and low competition for resources (Peet and Christensen 1987). Due to low intensity of competition, the small size of the plants, and the high number of stems per unit area at the beginning of the establishment stage, species richness may be quite high (P.S. White, unpublished). Many different herbs and woody seedlings are able to become established, with dis-

persal to the site being the limiting factor. After catastrophic disturbance events, soil nutrient levels are often high and therefore not limiting to vegetative growth (Peet 1992), although soil nutrients can be significantly depleted by a history of agriculture. In the Piedmont region of North Carolina, this stage is dominated by a variety of herbs, including crabgrass, horseweed, ragweed, aster, and broomsedge (Keever 1950).

After several years of domination by herbaceous species, one or more tree species begins to overtop the herbs, generating an increase in stand biomass (Odum 1969) with a concomitant increase in the amount of nutrients withdrawn from the soil and held in the biomass (Gorham et. al 1979). The crowns of young trees begin touching and competition grows intense as light and soil resources become increasingly scarce. At ground level, light is effectively unavailable, thereby excluding new seedling recruits from entering the stand. This stage is known as the "stem exclusion" or "thinning stage" and is characterized by density-dependent mortality. During this phase of stand development, intense competition causes the formation of a size hierarchy and an unequal concentration of biomass in the stand with the greatest amount of biomass in a few large individuals (Peet and Christensen 1987). Most evidence suggests that asymmetric competition for light, in which larger individuals obtain a disproportionate share of light at the expense of shorter neighbors, drives this process of density-dependent thinning (Weiner and Thomas 1992). Mortality continues in the stand until a group of survivors reaches reproductive maturity and canopy height, usually after 20 to 50 years. In the Piedmont region of North Carolina, this stage is often dominated by loblolly pine.

The "understory reinitiation" or "transition" stage occurs as the overstory ages. Undergrowth, including herbaceous species and trees, begins to appear. Competition slackens during the transition stage as gaps created by the death of the original canopy trees create patches of accessible light and soil resources (Christensen

and Peet 1981). In the North Carolina Piedmont, hardwoods growing up in the shadow of the pine canopy realize canopy positions as the loblolly pines of the original canopy begin to senesce or succumb to various destructive forces such as windthrow and bark beetles. Eventually, all the pines are replaced by hardwoods, ushering in the final stage of forest stand development.

The "old growth" or "steady-state" stage is characterized by canopy trees dying and creating gaps which are filled by the same or other canopy tree species. The amount of biomass in the forest remains stable and nutrient inputs to the system are equivalent to outputs (Vitousek and Reiners 1975). In the North Carolina Piedmont, early successional species such as loblolly pine are unable to invade, except in the largest gaps.

This general pattern of forest succession is known to characterize many plant communities. The intensity of competition serves as a structuring force behind successional patterns; predictability of species composition, species richness, accumulation of biomass, amount of production, and tightness of nutrient cycling are dependent on the intensity of competition (Peet 1992). We might expect genetic changes to be similarly predictable under the structuring force of competition, yet we know little of genetic processes that accompany stand development.

Competition During the Thinning Stage

The thinning stage of forest development has been studied extensively and can be described by a mathematical relationship between stand biomass and stand density over time. Once self-thinning commences, the relationship between biomass and density is described in the -3/2 Thinning Law (Clark 1990, Weller 1989, Yoda et al. 1963), depicted in Figure 1-1. As total stand biomass increases, density steadily decreases along a line with negative slope. Stands usually start out below the self-thinning line, and through time move toward the line. Westoby (1984) indicated that

plant populations converge along the self-thinning line starting from different initial densities. Once thinning begins, density decreases and biomass increases as stands move along the line toward the upper left of the graph until maximum plant size is reached. The -3/2 relationship is thought to be constant through time (Clark 1990), until plants approach maximum size, after which a stand can be represented by a stable point on the graph.

The thinning stage begins as a cohort of pines, usually loblolly pine in the North Carolina Piedmont, forms a closed canopy. In high density populations this may happen within the first few years, but where seedling density is low, actual thinning may not start for five to ten or more years. Most stands, depending on the proximity of the seed trees, are patchy. Some patches within a population enter into density-dependent thinning before others, as trees respond to other plants in their immediate neighborhood, rather than to the population as a whole (Harper 1977). Once the closed canopy forms, seedlings compete with each other for resources. In forest trees, diameter is measurably more sensitive to density than height (Lanner 1985), evidenced by the fact that trees in dense stands may be as tall but have more slender stems than trees in sparse stands. However, trees within a stand with a height advantage are generally more successful than shorter trees within the stand. Height, compared to height of neighbors, appears to be the most important characteristic of a plant influencing its future success in a stand (Weiner and Fishman 1994). As trees get larger and require more resources, density-dependent thinning occurs, and primarily it is the shorter individuals which are suppressed and eventually die. As the thinning stage progresses, the number of trees in the stand decreases and the concentration of biomass within a small fraction of the population increases (Peet and Christensen 1987).

Loblolly pine is extremely shade-intolerant, and during the thinning stage, taller trees appear to exclude shorter individuals, primarily by depriving them of light.

This type of resource competition is known as one-sided (Cannell et al. 1984), or asymmetric (Ewgon 1984) competition, in which larger individuals obtain a disproportionate share of a resource relative to their smaller neighbors (Weiner and Thomas 1986). Initial size differences and differences in growth rates are magnified through time as plants compete asymmetrically for light (Weiner and Thomas 1986). Stands undergoing competition tend to form size hierarchies in which a few large individuals dominate many smaller individuals (Koyama and Kira 1956). Most evidence points to size hierarchies forming due to asymmetric competition, although Bonan (1988) has found evidence for the formation of size hierarchies due to symmetric competition (where individuals compete equally for a resource). Size hierarchies are effectively characterized by measures of size inequality such as the Gini coefficient and the Coefficient of Variation (Knox et al. 1989, Weiner and Solbrig 1984), and become more pronounced as density and competition increase (Weiner 1985). Size inequalities may begin as differences in growth rates due to genetic differences, differences in age, or heterogeneity of soil resources (Weiner 1985, Bonan 1988). Knox et al. (1989) found that size inequality was greater at higher initial densities and that size inequality increased prior to self-thinning, then decreased after the onset of self-thinning. Once self-thinning commences, size inequalities decrease as more and more individuals are lost from the stand (Weiner and Thomas 1992), creating a stand where trees are more alike in height. Big trees still get bigger, but the loss of small trees balances increases in the large ones.

Succession and Genetic Change

Very little evidence exists for genetic change as a direct result of plant community succession. Most studies on this subject used populations of herbaceous species of varying ages, and even though genetic differentiation between populations is prevalent, it is impossible to determine if differences are a direct result of succes-

sional pressures or other selective or random forces (Gray 1987). Although Gray presents changes in plant density as a confounding factor for successional studies of genetics, thinning is a direct successional force that has the potential to drive selection. Perhaps the first study to look at the interaction between plant genotype and density relationships, aside from agricultural research, was Antonovics' (1978) study with *Plantago lanceolata*. In this study, it was determined that genetic change in populations during self-thinning was due to differential mortality of genotypes. This is evidence that intra-specific competition and the resulting mortality associated with it can be implicated in microevolutionary change.

The level of competition in successional stands varies, however, depending on stand density. It is well known that plants competing for resources during stand development have altered growth rates and morphology as a result of that competition (Geber 1983). What role might density, and the resulting coincidental competition, play in determining the success of certain genotypes? Thomas and Bazzaz (1993) tested the effects of different levels of competition, in terms of population density, on plant growth using cloned varieties of the herb *Polygonum pensylvanicum*. Several plants selected in the field were cloned to produce many individuals of the selected genotypes, and these individuals were then grown in experimental plots of varying densities. When the plants were not competing, genotype explained much more variance in size and reproductive output than in the presence of intense competition. Their findings show that competition does affect genotype performance, and demonstrate that plant genotype is more influential under conditions of low competition.

The studies of Antonovics (1978) and Thomas and Bazzaz (1993) suggest that competition can influence genetic-based growth differences in plants as the population matures. Studies of successional genetic change in forests, however, are nonexistent, yet the thinning stage provides an opportunity to look at long-term trends in genetic change during succession.

Competition and Stand Genetic Structure

The level of competition a stand experiences during the thinning stage affects both plant morphology and stand structure. Competition, as assessed by density of the local neighborhood patch, stochastic processes such as whether or not the seed landed in a safe site and microsite disturbance, have enormous effects on the ability of a seedling to survive the thinning stage. However, the question arises as to what role the genetics of a plant plays in its ability to compete for a canopy position. The differential growth and survival during the thinning stage set a framework for natural selection that is at least partially based on competitive, and presumably to some degree on genetics. However, the degree to which genetics and stochasticity interact during the formation of a mature stand is unclear. There may be genetic trends underlying the formation of size hierarchies in forest stands caused by varying competitive responses and resulting in differential survival of genotypes. By comparing growth relationships and mortality under conditions of different densities, we can assess the extent to which genetic constraints among individuals affect tree population size structure and forest neighborhood relationships.

The importance of stochastic and genetic effects on a population may be constrained by the amount of competition it experiences. If competition has a density-dependent influence on genotypic performance, seedling density during the thinning stage of forest development may be a meaningful determinant of the future genetic structure of the stand. Competition and the resultant mortality during the thinning stage may prompt a directional change in the genetic makeup of the population. If allele frequencies of reproductive individuals are different from the starting population, evolutionary change will occur (Weiner 1985). Stand density, then, may be linked to microevolutionary processes of genotype and allele frequency changes due to natural selection.

The Role of Enzyme Heterozygosity in Forest Stand Development

Background of Heterozygosity

Heterozygosity has long been an issue of interest to population geneticists. Many studies have found correlations between heterozygosity at allozyme loci and various fitness traits in forest trees, such as survivorship, growth, reproduction, and size (for review, see Mitton 1987). Such correlations make heterozygosity a prime target for research on competitive pressures associated with genetic change in forest trees. This study focuses primarily on observed differences in growth rate and stand structure that are correlated with enzyme heterozygosity during stand thinning. Before more detailed information about heterozygosity in trees is presented and related to stand thinning, it is important to trace the history of the topic of heterozygosity.

An ongoing debate exists concerning the cause of heterozygote superiority. The dominance hypothesis (Keeble and Pellow 1910; Bruce 1910) resulted from information from centuries of inbreeding studies on animals and plants, and ascribes inbreeding depression to the additive effects of homozygosity at many deleterious alleles. This genome-wide hypothesis states that lethal and harmful recessive or incompletely dominant alleles are common throughout the genome and constitute a negative effect in homozygous form so that an individual homozygous at many loci will perform poorly compared to one less homozygous. When two unrelated individuals are crossed, the 'hybrid vigor' that results is due to the low amount of homozygosity for deleterious alleles in the genome. Related to the dominance hypothesis is the complementary gene hypothesis (Powers 1944), which emphasizes the complementary effects of genes and not necessarily the dominance of the favorable ones. In terms of allozymes, heterozygosity detected at representative loci are assumed to be indicative of genome-wide heterozygosity.

With the overdominance hypothesis, it is assumed that heterozygosity at specific loci is responsible for detectable differences in fitness traits. The overdominance

hypothesis came about due to the observation that hybrids can be significantly larger than the non-inbred lines from which they descended. This could indicate a more complicated interaction than just having fewer homozygous loci. Shull (1910) asserted that hybrid vigor was due to an interaction of the alleles at the heterozygous loci. Having two different alleles at a locus could be beneficial for a plant or animal. Lerner (1954) expanded this hypothesis by suggesting that having two different forms of the same enzyme could allow a plant or animal to respond better to variable environmental conditions. Using a mathematical model, Ginzburg (1979) showed that heterozygote superiority due to overdominance is caused by natural selection on a subset of alleles such that the heterozygote is not always superior to both homozygotes, but is superior to at least one, so that both polymorphisms at a locus and heterozygosity result from natural selection.

A more recent hypothesis is the cytoplasmic hypothesis of Michaelis (1951), which suggests that heterosis may arise due to interactions between nuclear and cytoplasmic genes.

At present, most investigators believe that none of these four hypotheses is fully correct, but the most plausible explanation for why heterozygotes are often superior is some combination of them.

The Role of Heterozygosity in Stand Thinning

Regardless of why heterozygosity is often correlated with fitness characteristics, many studies have indeed shown such correlations. Several investigators have shown that in forest trees, mean heterozygosity increases from young to old age classes (Hamrick, Platt, and Hessing 1993; Brotschol et al. 1986; Farris and Mitton 1984). In young stands, there is often an excess of homozygous individuals, probably due to selfing, especially in wind pollinated species (J. Hamrick, personal communication). In mature populations of the same species, Hardy-Weinberg expectations are more

closely met (Mitton 1989). This suggests that inbred individuals are being selected against. Parks et. al (1983) found such results in experimental crosses of *Liriodendron*, as did Bush (1988) for loblolly pine, where inbred individuals had lower survivorship than outcrossed individuals. In some old-growth stands of longleaf pine, there is a tendency toward an excess of heterozygotes in older trees (Hamrick, Platt, and Hessing 1993), suggesting that fitness increases with heterozygosity. This trend has been found by other investigators for other species as well (Linhart et al. 1981; review by Mitton 1989). The loss of homozygotes throughout the thinning stage would result in the more heterozygous individuals dominating the stand.

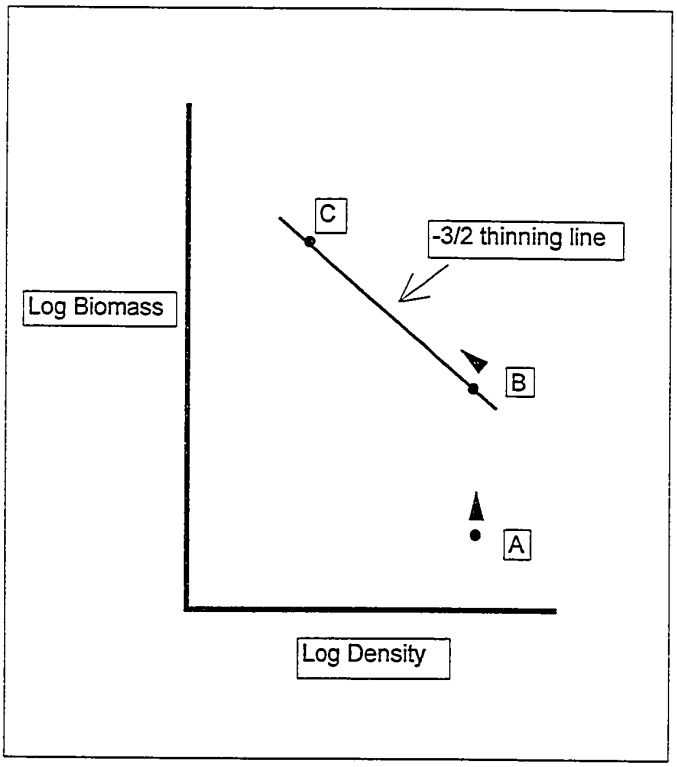
An increase in heterozygous individuals in a stand throughout thinning could result from higher growth rates among heterozygous individuals. Growth rates of many different organisms have been shown to increase with heterozygosity at enzyme loci, but studies of forest trees have yielded mixed results. Several studies showed a positive correlation between growth rate and heterozygosity (Mitton and Grant 1980; Ledig et al. 1983; Strauss 1986), although Bush et al. (1987), in reanalyzing the data of Ledig et al. (1983), found their reported correlation was nonsignificant. Bush and Smouse (1991) found a positive correlation between heterozygosity and height at one polymorphic locus among outcrossed loblolly pine individuals. Other studies that have looked for a positive relationship have reported negative (Knowles and Mitton 1980) or unrelated results including correlations between heterozygosity and variance in growth rate (Knowles and Grant 1981; Linhart and Mitton 1989). Mitton (1983) suggested correlations between heterozygosity and growth rate may be age-dependent and more apparent in young trees when energy is primarily invested in growth.

Genetic Questions for Succession

Genetic change during succession is difficult to study due to our inability to account for and control for variables which may affect it. However, the exploration

of genetic change and how it fits in to the framework of other forest changes during succession will improve our understanding of plant interactions during succession. Answers to several questions should provide a broad framework from which to approach this issue. First, can non-random genetic change during succession be observed? Second, how might genetic changes in a population affect the ecology of a population as it grows, and the future generations which arise from it? And third, could genetic change retard or promote succession? The scope of this thesis is limited to the first and most basic question. This study attempts to tie the competition and mortality present during the self-thinning stage to genetic changes observed by other investigators and examines the possibility that ecological mechanisms set the circumstances for the superiority of heterozygotes. Two questions are explored: 1) does density-dependent thinning act as a selective force favoring individuals with higher heterozygosity levels, and 2) does stand heterozygosity increase with self-thinning? Chapter 3 is an analysis of the effect of density on seedling growth and early stand development of seedlings of known genotype. Plant growth and survivorship are examined in terms of their genotype and whether they experience asymmetric competition, symmetric competition, or no competition. In Chapter 4, a mature stand is used to compare genotype and growth of surviving trees during early, mid, and late thinning stage. By investigating genetic change in stands, we can attain a better understanding of forest stand development and how a population and the individuals within a population respond to their environment.

Figure 1-1. Relationship between log of total stand biomass and log stand density. A young stand prior to competition, stand A will increase in biomass moving toward the -3/2 thinning line at point B. From point B, stand density decreases as biomass increases along the line toward point C, which represents a stand near maximum biomass.


C
-3/2 thinning line
Log Biomass
B
A
Log Density

Chapter 2

Laboratory Methods

This study uses allozyme analysis to examine genetic structure in populations of loblolly pine (*Pinus taeda*) seedlings and mature trees. Many studies have focussed attention on the genetic analysis of forest trees using allozyme analysis. RFLPs, RAPDs, and other molecular approaches are becoming more common, but a broad base of literature has grown from allozyme research in forest trees. Included in these findings are studies of correlations between heterozygosity at enzyme loci and fitness characteristics (Mitton 1989, for review), spatial patterns in alleles assumed to be due to environmental heterogeneity (Loveless and Hamrick 1984; Furnier and Adams 1986), and demographic patterns in genetic variation (Schaal 1985, Fore et al. 1992).

Sample Preparation

Loblolly pine needle samples were prepared for gel electrophoresis by grinding in a reducing buffer. Grinding buffer (Mitton et. al 1977) was prepared fresh each day and stored in an ice bucket until ready for use. Mortars and pestles were stored overnight in a refrigerator until ready for use and were kept on ice in trays during the grinding process. 0.15g of pine needle tissue was clipped into a waiting mortar. A small amount of PVPP (Poly-vinyl polypropanol, a reducing agent) and a small amount of sand (fine-grained, grinding agent) were added to each mortar. 1.0 ml of grinding buffer was added to each mortar and each sample was ground until a smooth watery paste was achieved. A 2cm x 2cm square of Kim-Wipe paper was placed on top of the pool of liquid to filter out larger plant tissue particles. Eight 3mm x 8mm

wicks were placed on the Kim-Wipe to soak up the slurry.

Sample Storage

When the wicks fully absorbed the slurry, they were placed in eppendorf tubes labeled with the plot and ID numbers of the individual. The tubes were stored in boxes at -80° until ready for gel electrophoresis. The frozen samples produced readily decipherable gels. Samples were stored for up to 36 months without deterioration, although most were run within 12 months after grinding.

Gel Preparation

Procedures, gel buffer solutions, and stain recipes follow Parks et. al (1990), unless otherwise noted. Three electrophoresis buffer systems were used: LBTC, HC, and TC. All electrophoresis buffers were prepared within 2 weeks of use and HC was routinely prepared within one week of use. Gels were run either during the day at 14.5 watts (for 6 1/2 hours) or overnight at 3.0 watts (for 14 1/2 hours).

Gels were made with gel buffer, Connaught starch, and sucrose (Parks et. al 1990). Gel trays were prepared by sealing the open ends of each tray with labeling tape and rinsing the inside surface with a 1:200 dilution of Photoflo (Kodak Corporation) and water to prevent the gel from sticking to the surface. Starch and sucrose were weighed and put into a 500ml volumetric flask. While 200 ml of gel buffer was heated to boiling in a microwave oven, 125 ml of cold gel buffer was swirled with the starch and sucrose in the volumetric flask. The boiling buffer was then added to the cold solution and vigorously swirled. The solution was then alternately heated in the microwave and swirled until uniformly hot and gently boiling. The solution was degassed by aspiration and immediately poured into the waiting gel tray. Small bubbles were removed with a scoopula. Once cooled, the gels were covered with Saran plastic wrap until ready for use.

Gel Loading and Running

Gels were cooled in a refrigerator for at least 30 minutes before loading and were kept on ice packs during loading. The plastic wrap was removed from the surface of a gel and the gel mass was separated from the sides of the tray by sliding a thin blade in between the gel and the sides. A vertical slit to serve as the origin was cut into the gel 2.2 cm from the anodal end, and the gel was separated at the origin by gently sliding it apart. Previously prepared wicks were loaded vertically into the origin with tweezers, approximately 2-3 mm apart. The order of the wicks in each gel was recorded on a gel data sheet marked with the date and samples used. When loading was complete, the two sides of the gel were gently pushed together at the origin. The masking tape on the legs of the gel tray was removed and the plastic wrap was replaced on top of the gel. Gels were then placed in electrode buffer trays (Figure 2-1) in a refrigerator and subjected to electrophoresis at the wattage referred to above. After 15 minutes of running, the wicks were removed. Six spacers, thin rectangles of plastic (2cm x 6cm x 0.5 cm), were placed between the gel and the front and back walls of the tray to keep the origin tightly sealed during electrophoresis. At this point the gels were replaced in the buffer trays and electrophoresis continued.

Gel Slicing and Staining

After electrophoresis, gels were removed from the refrigerator and sliced into 7 slices using a wire tool fashioned from a hacksaw handle with a piano wire. Each slice was placed in a 10cm x 8cm x 3cm lidded clear plastic gel box and stained for a different enzyme (Parks et. al 1990). Although 19 different polymorphic enzyme loci had reactions strong enough to be seen, only 15 of these were reliably interpretable in the Botany Pond study, and 10 in the Duke Forest study (Table 2-1). The enzymes stained were PGI (Phosphoglucose isomerase, 2 loci), GDH (Gluteraldehye dehydrogenase, 1 locus), DIA (Diaphorase, 1 locus), 6-PGD (6-Phosphoglucose dehydroge-

nase, 1 locus), PGM (Phosphoglucose mutase, 2 loci), TPI (Triosephosphate isomerase, 2 loci), SAD (Shikimic acid dehydrogenase, 1 locus), MDH (Malate dehydrogenase, 2 loci), FE (Fluorescent esterase, 1 locus), IDH (Isocitrate dehydrogenase, 1 locus), GOT (Gluteraldehyde oxalate transferase, 1 locus). Several of these enzymes, the two PGM loci and the two TPI loci and to some extent the DIA locus, were inconsistent in their performance, but were easily interpretable when present and so were used when present. An extra band, interpreted as a breakdown product, appeared in IDH gels above the two alleles only in dying seedlings.

The stains contained substrates for the specific enzymatic reaction. Stains for FE, TPI, and IDH were mixed and poured over the gels as agar overlays. All other liquid stains were mixed and poured over the gel slice in the box, and then agitated gently until the gel was free from the bottom of the box.

Scoring Gels

The gel slices were incubated in the boxes at 37° until bands were readable. The slices were then scored by reading the bands on the gels as alleles of the enzyme.

Gel Storage

After the gels were scored, they were soaked in fixative (Appendix A) in the gel boxes for one day. Fixed gels were then removed from the boxes, blotted to remove excess moisture, and wrapped in plastic wrap. The fixed gels were stored at 4°.

Gel Interpretation

Alleles were named numerically from the anode end of the gel: alleles running furthest from the origin had lowest numbers, except for one case in locus PGI2 where an allele was discovered between alleles 4 and 5. This allele was named 8. Likewise

the fastest loci were named the lowest numbers. All loci scored ran toward the anode end of the gel. Table 1 lists the enzymes analyzed and characteristics of each. Pictoral representations of alleles for each enzyme are presented in Figures 2-2 through 2-13.

Table 2-1.
Characteristics and Nomenclature of Enzymes.
E.C. refers to the enzyme commission official identification number for the enzyme. 'X' indicates whether an enzyme was used in the Duke Forest (DF) study and/or the Botany Pond (BP) study.

Code	Name	E.C.	# Subunits	# Alleles	DF	BP
DIA1	Diaphorase	1.6.99.-	monomer	3	X	X
FE	Fluorescent esterase	3.1.1.-	dimer	2	X	X
GDH	Glutamate dehydrogenase	1.4.1.2	hexamer	2	X	X
GOT2	Glutamate oxaloacetate transaminase	2.6.1.1	dimer	2	X	X
IDH	Isocitrate dehydrogenase	1.1.1.41	dimer	2	X	X
MDH1	Malate dehydrogenase	1.1.1.37	dimer	4		X
MDH2	Malate dehydrogenase	1.1.1.37	dimer	6	X	X
PGI1	Phosphoglucoisomerase	5.3.1.9	dimer	3	X	X
PGI2	Phosphoglucoisomerase	5.3.1.9	dimer	6	X	X
6PGD	6-Phosphogluconate dehydrogenase	1.1.1.44	dimer	4	X	X
PGM1	Phosphoglucomutase	5.4.2.2	monomer	3		X
PGM2	Phosphoglucomutase	5.4.2.2	monomer	2		X
SAD	Shikimic acid dehydrogenase	1.1.1.25	monomer	4	X	X
TPI1	Triosphosphate isomerase	5.3.1.1	dimer	2		X
TPI2	Triosphosphate isomerase	5.3.1.1	dimer	2		X

Figure 2-1. Gel tray containing gel, placed in electrode buffer tray.

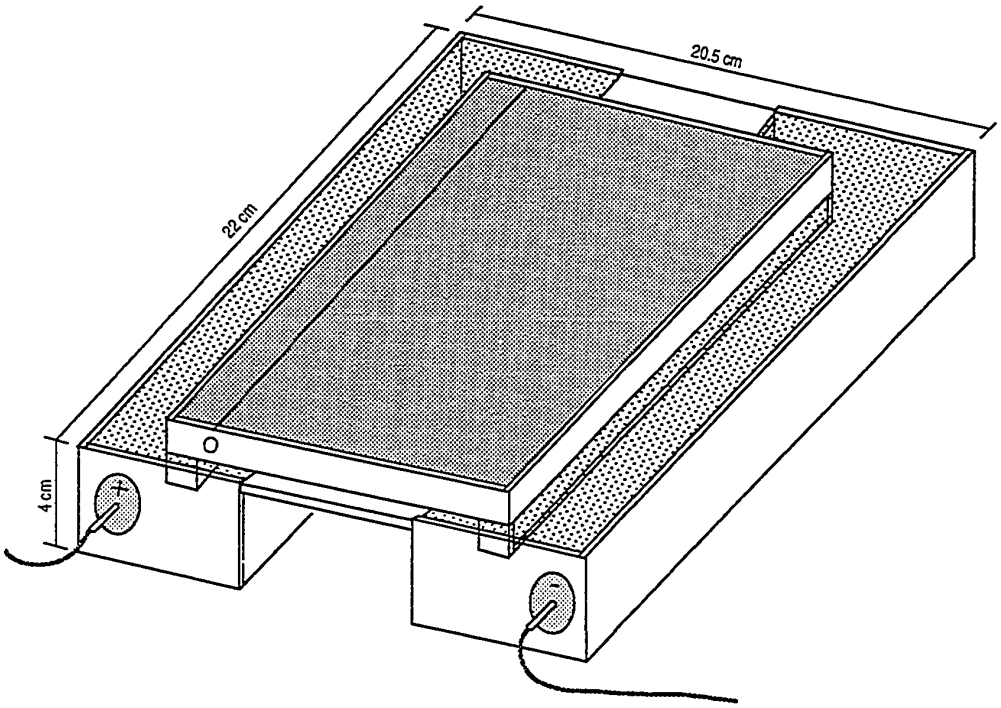

20.5 cm
22 cm
4 cm
+
-

Figure 2-2. SAD: Shikimic acid dehydrogenase.

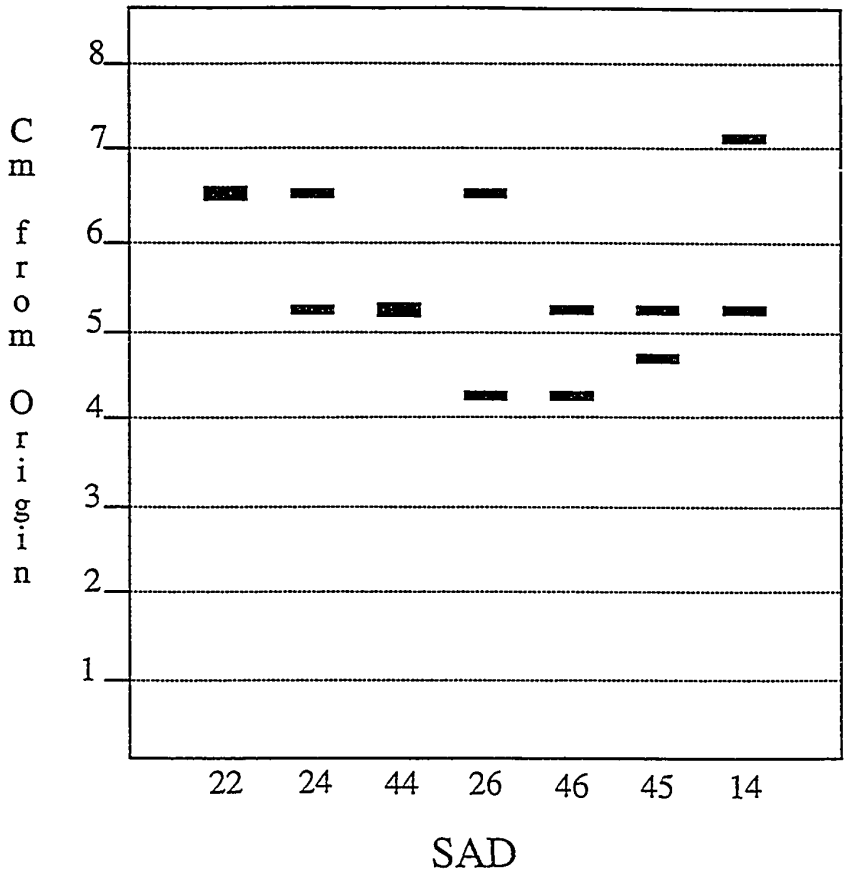

Cm from Origin
8
7
6
5
4
3
2
1
22
24
44
26
46
45
14
SAD

Figure 2-3. GOT2: Glutamate oxaloacetate transaminase.

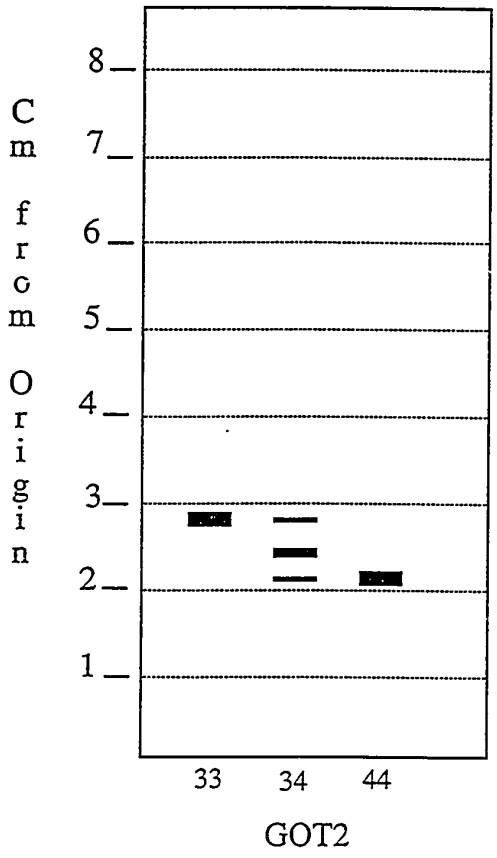

Cm from Origin
8
7
6
5
4
3
2
1
33
34
44
GOT2

Figure 2-4. PGM1 and PGM2: Phosphoglucomutase.

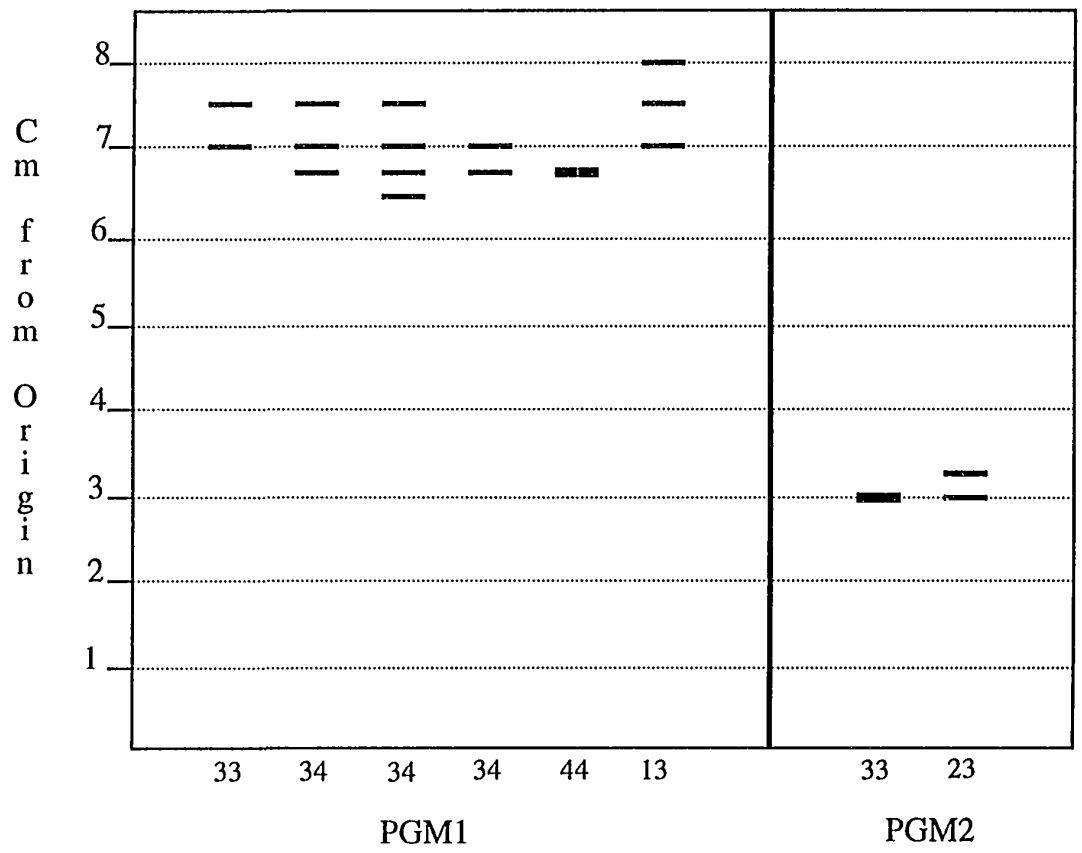

Cm from Origin
8
7
6
5
4
3
2
1
33
34
34
34
44
13
33
23
PGM1
PGM2

Figure 2-5. 6-PGD: 6-Phosphogluconate dehydrogenase.

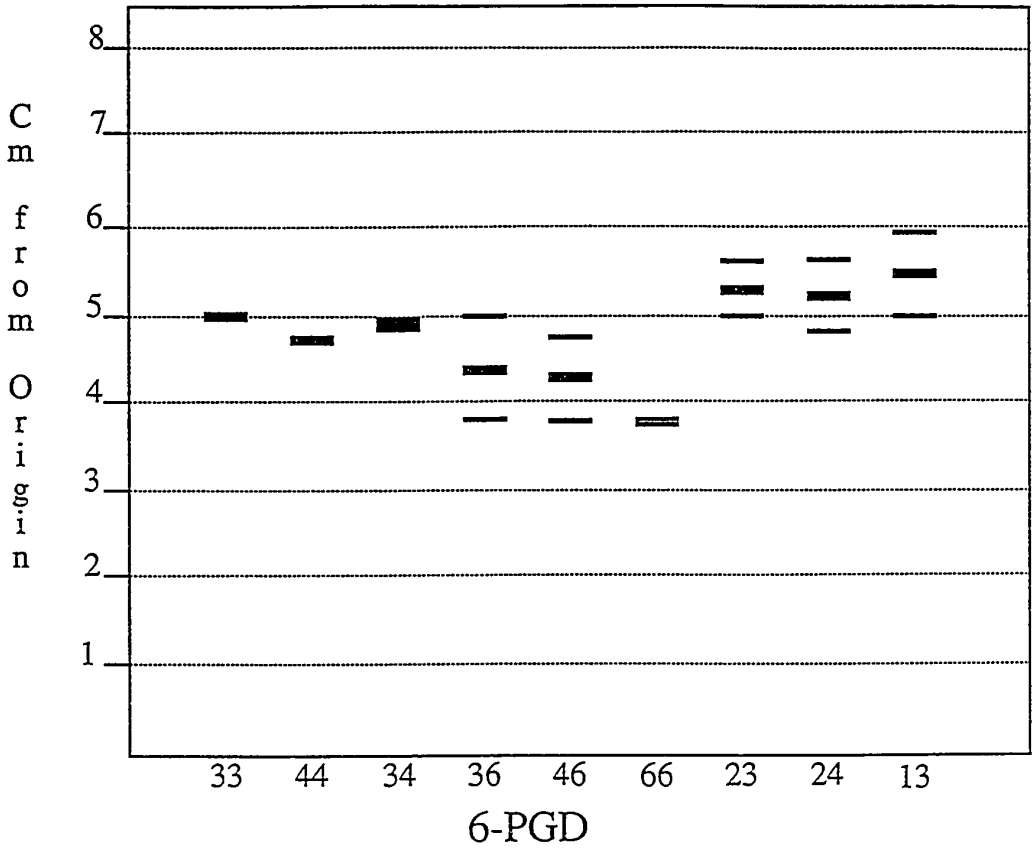

Cm from Origin
8
7
6
5
4
3
2
1
33
44
34
36
46
66
23
24
13
6-PGD

Figure 2-6. IDH: Isocitrate dehydrogenase.

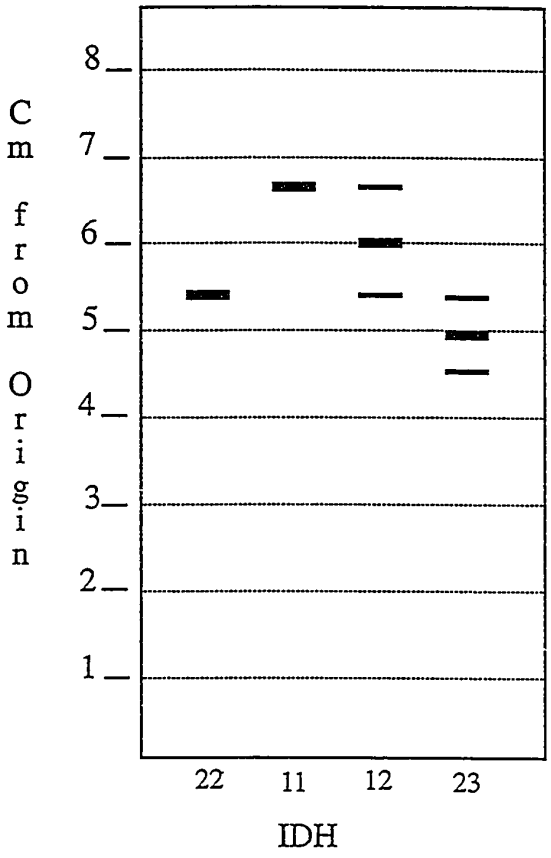

Cm from Origin
8
7
6
5
4
3
2
1
22
11
12
23
IDH

Figure 2-7. FE: Fluorescent esterase.

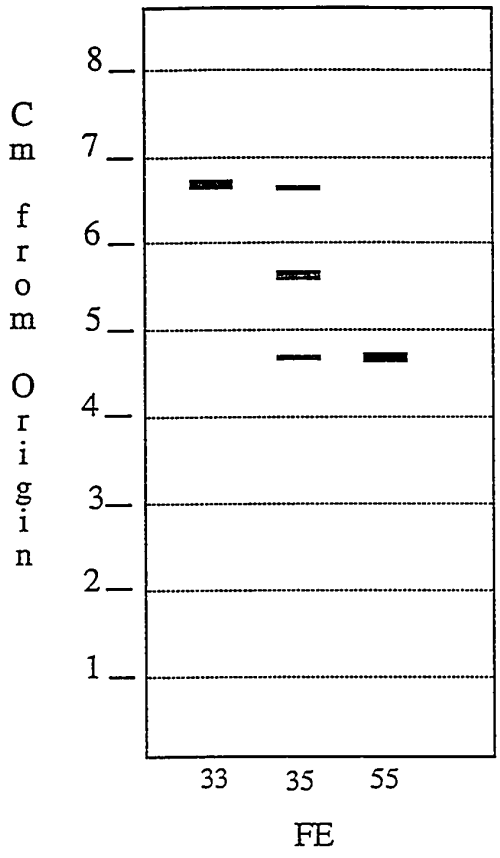

Cm from Origin
8
7
6
5
4
3
2
1
33
35
55
FE

Figure 2-8. DIA1: Diaphorase.

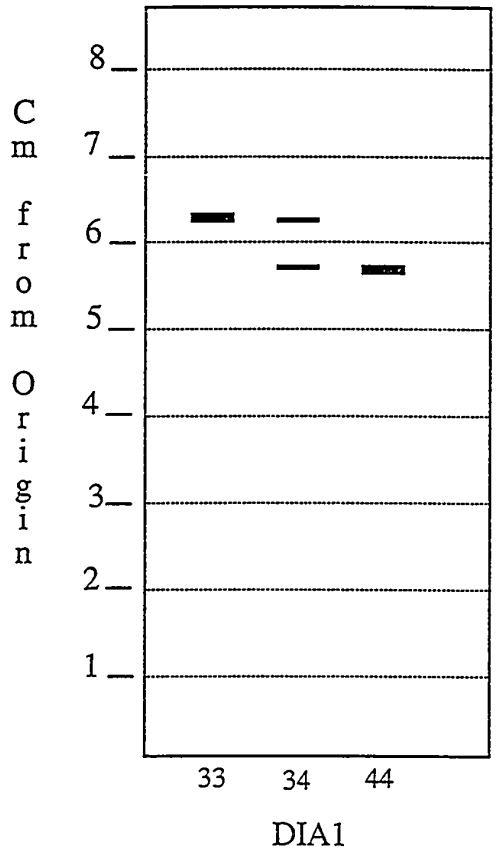

Cm from Origin
8
7
6
5
4
3
2
1
33
34
44
DIA1

Figure 2-9. TPI1 and TPI2: Triosphosphate isomerase.

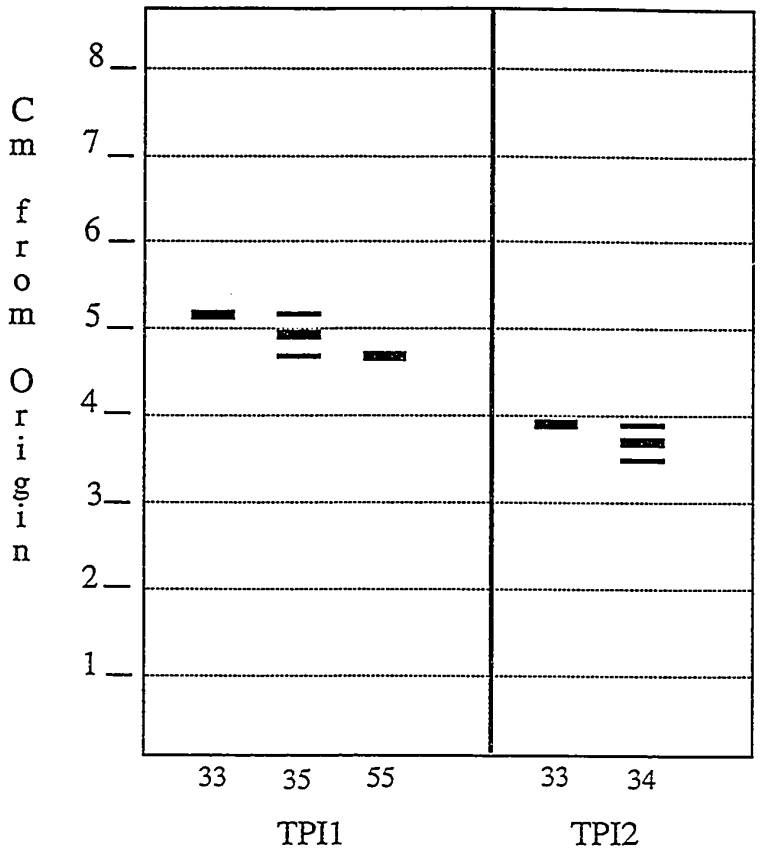

Cm from Origin
8
7
6
5
4
3
2
1
33
35
55
33
34
TPI1
TPI2

Figure 2-10. GDH: Glutamate dehydrogenase.

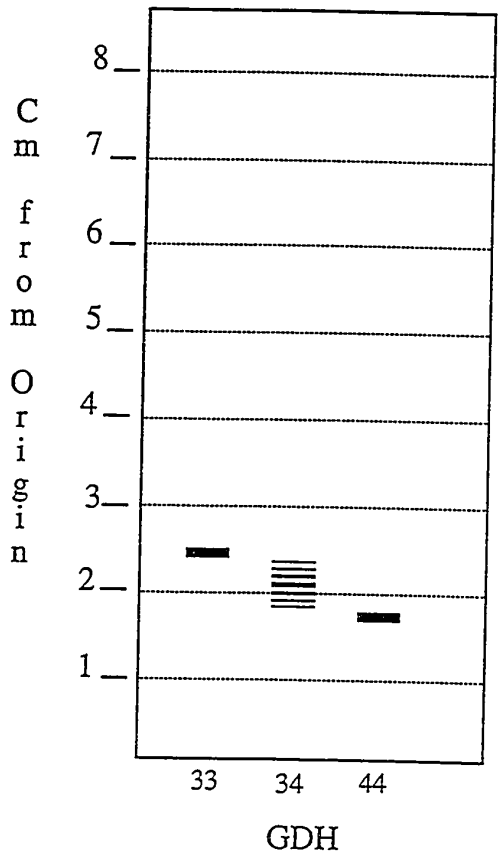
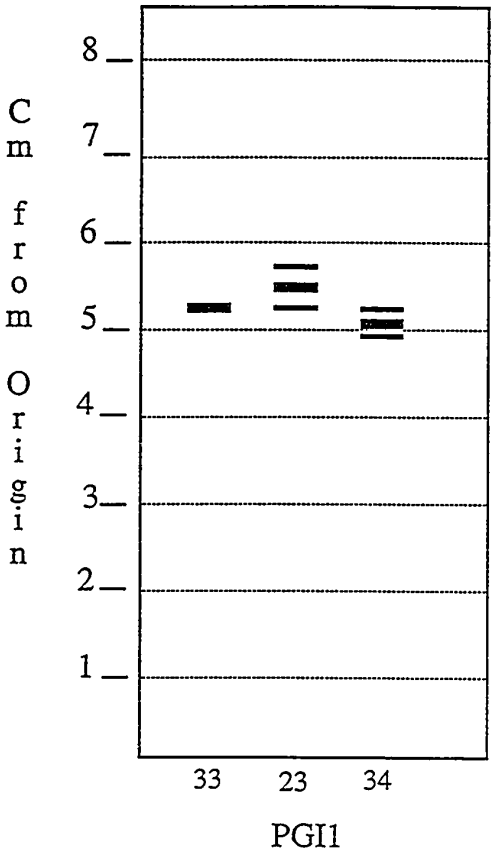
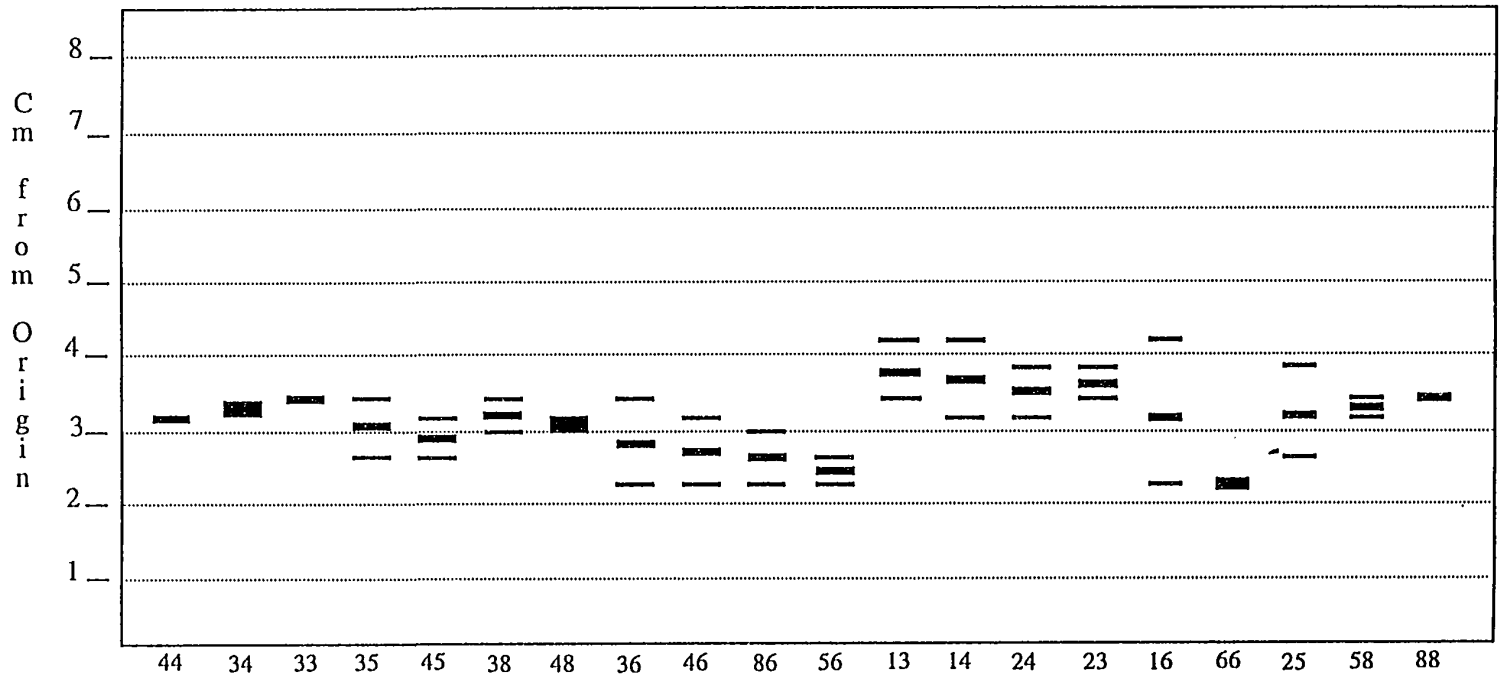

Cm from Origin
8
7
6
5
4
3
2
1
33
34
44
GDH

Figure 2-11. PGI1: Phosphoglucoisomerase, locus 1.



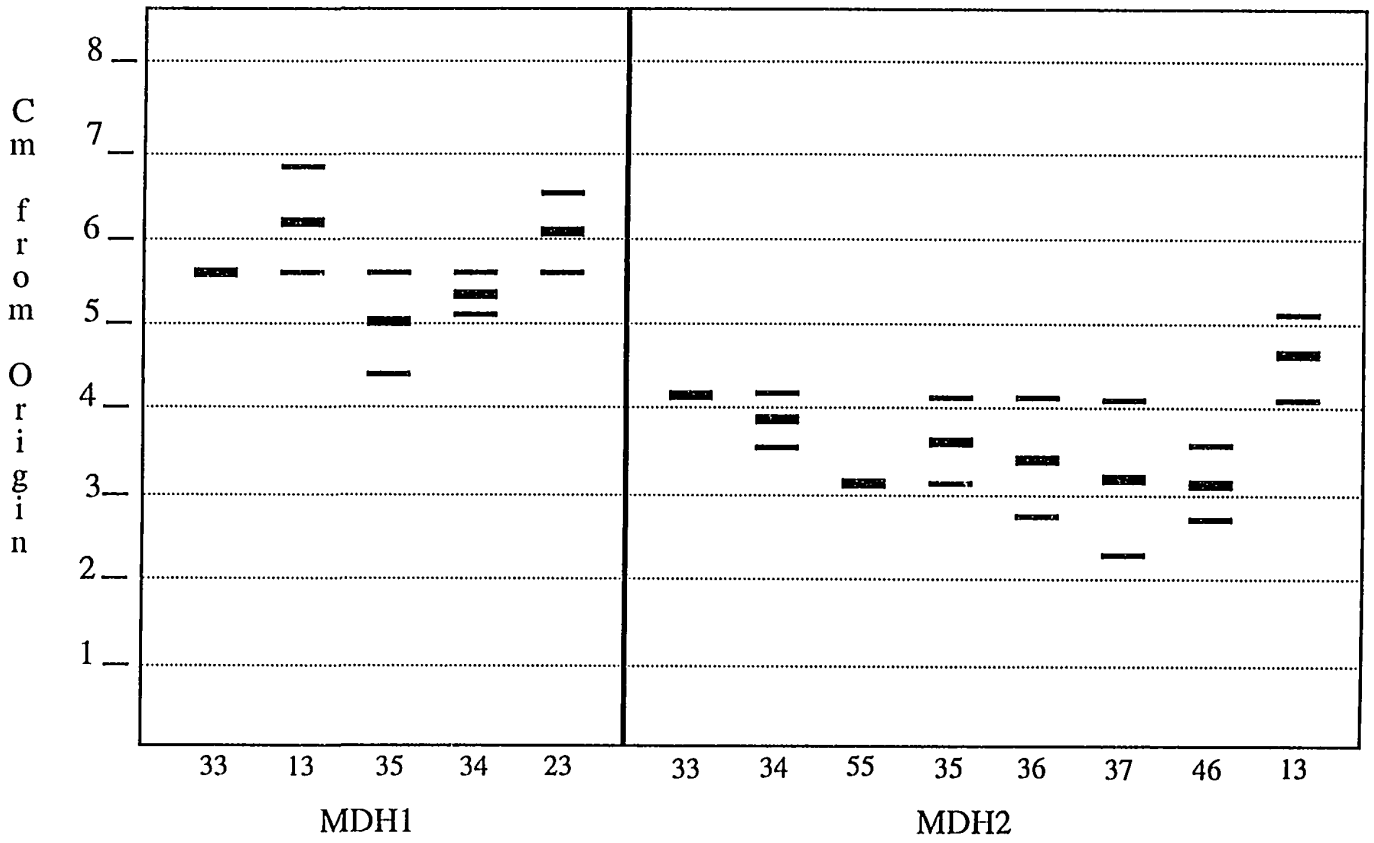
PGI1

Figure 2-12. PGI2: Phosphoglucoisomerase, locus 2.


Cm from Origin
8
7
6
5
4
3
2
1
44
34
33
35
45
38
48
36
46
86
56
13
14
24
23
16
66
25
58
88

PGI2

Figure 2-13. MDH1 and MDH2: Malate dehydrogenase.


Cm from Origin
8
7
6
5
4
3
2
1
33
13
35
34
23
MDH1
33
34
55
35
36
37
46
13
MDH2

Chapter 3

Competition and Survival in Seedling Stage Loblolly Pine: The Botany Pond Study

Although the thinning stage of forest stand development has been the subject of numerous studies (Peet 1992, for review), little is known about genetic changes that occur during stand thinning. The purpose of this study is to look at the effect of plant genotype, as assessed by allozyme analysis, on individual growth and stand development in the context of the competitive pressures occurring during the thinning stage in stands of loblolly pine seedlings.

The thinning stage is characterized by intense competition for resources, especially light, among individual trees. Most evidence suggests that asymmetric competition for light, in which larger individuals obtain a disproportionate share of light at the expense of shorter neighbors, drives the thinning process (Weiner and Thomas 1992). Early mortality is usually concentrated among the shorter, light-suppressed trees. Intense competition also causes the development of size hierarchies (Weiner 1985, Knox et. al 1989), where a few large trees dominate the stand and, by depriving them of light, suppress the growth of the many shorter trees.

In addition to competition, stochastic factors such as microsite disturbance and whether or not the seed landed in a safe site have large effects on the ability of a seedling to survive the thinning stage. The question arises, however, as to what role the genetics of a plant plays in its ability to compete for a canopy position. Differential growth and survival during the thinning stage provides a framework for natural selection that includes the influences of competition and stochastic processes such as

time of germination, relative sizes of individuals, and heterogeneity of soil resources. Plant genotype is also assumed to influence individual plant success, but it is unkown how much plant genotype affects plant growth in the presence of competition and stochastic effects as a stand matures.

Even though researchers (Hamrick, Platt, and Hessing 1992; Brotschol et al. 1986; Farris and Mitton 1984) have reported stands of mature trees to have higher levels of heterozygosity than nearby juvenile stands, the process of stand genetic development has not been studied and is little understood. One logical way to examine this problem is to follow the development of a stand over time, observing and comparing density and genetic changes throughout stand development. The Botany Pond seedling study was designed to observe population and individual-level changes within juvenile stands throughout the early part of the thinning stage of forest development. Individual and population level growth traits were examined to determine the amount and type of competition occurring in three density treatments. In this chapter, I first examine morphological data and growth rates of seedlings to determine the effect competition, as assessed by population density, on plant growth. Second, I incorporate genetic data from allozyme analysis with the morphological and growth data to determine the effect of genotype on plant growth.

Study Site and Methods

Experimental Design

This study was designed to provide experimental density treatments to test growth and survivorship under varying levels of competition. Replicate study samples provided more robust results, and controlled conditions reduced the influence of variables other than genotype or density. Three density classes were chosen: a low-density class where competition for light was not expected to occur during the experiment, a medium-density class where competition but not mortality was expected,

and a high-density class designed to force density-dependent mortality. Four replicate blocks were established to allow the experiment to be statistically treated in a split plot design with replicates. Variables controlled for included soil (importing a homogeneous soil mixture), light (randomizing the placement of each module), moisture (daily uniform watering across plot), and soil nutrients (uniform fertilization).

Plot Establishment

Ten pounds of loblolly pine seed were acquired from the North Carolina Forest Service in Raleigh, NC. The seed was collected from two counties in the Piedmont area of North Carolina in the fall of 1984. Both sites were naturally seeded stands being cut for harvest and reforestation. No data are available concerning the genetic makeup of the parent trees.

The seeds were stratified in January, 1993. Seeds were soaked overnight in a cold room environment (4°C) in beakers of water. The beakers were stirred every 6-8 hours for 24 hours. Floating seeds were assumed to be aborted and were discarded. The seeds were then drained and placed in a plastic bag in a cold room for 30 days.

Tree tube trays (#84; 1.5" x 1.5" x 3" deep) were obtained from Tray Masters of Lithia, Florida. These trays were filled with a mixture of 50% screened peat moss/50% vermiculite. Stratified seeds were planted in the trays, two seeds per tube, in February, 1993. The trays were watered lightly twice daily to stay moist.

Pine seedlings began to appear ten days after planting. In the event that two seedlings germinated in a tube, the second seedling to emerge was discarded. The trays were maintained in a greenhouse for the remainder of the spring.

In May 1993, a gravel bed at the Mason Farm Biological Reserve with 2 1/2 foot high cement block walls was prepared for the seedlings. The bed measured approximately 16 x 2 meters. The bed had adequate drainage through the bottom via the gravel and a drainage pipe system. 200 yards of soil mixture, 50% finely ground

pine bark/50% sand, was delivered to the gravel bed by Sands and Soils of Durham, NC. The soil was spread evenly throughout the bed using shovels and rakes. Soil depth was measured in the bed at 35 cm. Twenty lb. of pelleted gypsum and 10 lb. of Peter's 20-20-20 fertilizer was scattered evenly over the bed. This was lightly worked into the top layer of soil using a rake.

Standard 3 inch garden edging was used to delineate the modules within the bed. One 16 meter piece was inserted down the center line of the bed, dividing the bed into two 1 x 16 meter sections. Thirty two 1 x 1 meter modules were created by inserting sixteen 2 meter lengths of the edging perpendicular to the long axis of the bed at 1 meter intervals. From this time forward, standing and walking on the plot was avoided by using heavy 2 x 8 ft boards placed across the walls of the bed and working in the modules from atop the boards. In this way, soil compaction was minimized.

Seedlings were delivered to Mason Farm Biological Reserve and stored outdoors for one week before being planted into the bed. For the low-density treatment, each module was measured and marked off at 0.15, 0.45, and 0.75 meter intervals on two perpendicular sides. Where each set of lines crossed, a narrow hole 20 cm deep was dug. Into this hole, one seedling was placed, and the soil was drawn around it and patted down. A total of 9 seedlings (3 rows x 3 columns) were planted in each of the 24 low-density modules. In the medium-density modules, seedlings were planted at the following intervals: 0.0375, 0.1125, 0.1875, 0.2625, 0.3375, 0.4125, 0.4875, 0.5625, 0.6375, 0.7125, 0.7875, and 0.8625 meters making a total of 144 seedlings (12 rows x 12 columns) per each of the four medium-density modules. The four high-density modules each had approximately 1,200 seedlings per m^2. These seedlings filled the high-density modules completely with the seedling plugs abutting against each other.

The raised bed was divided into 4 blocks along the long axis of the plot. Each

block consisted of one high-density module, one medium-density module, and six low-density modules. The planting design for the Botany Pond study is illustrated in Figure 3-1. Within the low-density modules, each of the nine seedlings was tagged for study. The central 64 seedlings in each medium-density module were tagged leaving a buffer of two rows of seedlings surrounding the experimental individuals. In the high-density modules, 200 centrally located seedlings were tagged for study leaving a wide buffer of seedlings surrounding the experimental seedlings.

After planting in the summer of 1993, the plot was watered once per day, soaking the soil well. Once every two weeks, the plot was fertilized with Peter's Pete Lite Special (20-19-18 + micronutrients) using a Hozon 15-1 system. 5.63 grams of fertilizer was evenly applied to the plot at each fertilizing using this system. Watering was discontinued in November of 1993. The same watering and fertilizing schedule was maintained throughout the summer of 1994 from April until November. No watering or fertilizing was done during the summer of 1995.

Several pest problems surfaced during the study. During the winters of 1993-94 and 1994-95, bird netting was placed over the seedlings in the plot to protect from deer browsing. Substantial damage was done by deer, especially to the low-density modules, early in the first winter before the problem was recognized. The deer tended to bite off the apical meristems of the low-density seedlings. In the height data set, it is apparent which seedlings were affected because growth rate is negative for that period. Seedlings affected by deer browsing recovered quickly and suffered no mortality. As this disturbance tended to be fairly uniform within each module in which it occurred and occurred while the seedlings were still quite small, deer herbivory was not factored in to the statistical analysis. The netting adequately deterred further damage from the deer. The second pest problem occurred when a species of moth larva invaded the apical meristems of many of the growing seedlings during the summers of 1993 and 1994. Most affected were the seedlings in the low and medium-

density modules. The effect of the invasion was the mortality of the apical meristem followed by the appearance of a new apical meristem arising from an axillary meristem near the dead one. This often caused a negative or neutral growth increment for a seedling which would otherwise have shown growth. Nearly all seedlings in the low and medium treatment were affected by the moth at one time or another, so although such insect invasion was noted on data sheets, it was not incorporated into data analysis. Spraying with insecticide did not deter the insects. The third pest problem to surface was a rabbit that invaded one of the high-density modules. Before being detected, the rabbit had bitten off one-third of the study seedlings, and reduced the density sufficiently to substantially influence the results. For this reason, high-density module #23 was abandoned and not used in statistical analysis.

Seedling Measurement

Seedlings were measured every six weeks during the summers of 1993 and 1994, and once in the spring of 1995. Seedling height was measured in centimeters from the base of the seedling (ground level) to the highest point reached by the needles. This was done for two reasons: 1) height of apical meristem vs. total height is difficult to detect due to the way the needles cling together at the top of the very young seedling, and 2) because the study was designed to look at competition for light, total photosynthetic height presumably is the most important quality of a seedling. After the third measurement period, both total height and height of the bud were recorded. In the fall of 1994, seedling diameter at the base and total length of side branches was also recorded. This was used as a surrogate variable for biomass without sacrificing the individuals.

Harvest Procedure

Pine needle tissue was harvested from the seedlings during the summer of

1993. Three to four needles were removed from each seedling for analysis. In the high-density modules, some of the seedlings were already dying by the end of the summer. To get sufficient material for analysis, the remainder of the living part of the seedling was sometimes removed, resulting in the death of the seedling. In about 8% of the high-density seedlings, mortality due to density-dependent thinning had already occurred before tissue was harvested. Harvested needles were placed in a ziploc bag with the module number and tree number of the seedling. Samples were kept in a cooler until they could be taken back to the laboratory where they were stored in a cold room (4° C) until ready for processing.

Allozyme Analysis

Nineteen loci polymorphic in loblolly pine were resolved, although two of these were monomorphic, and two had complex patterns of overlapping, polymeric loci to reliably score in this population. The fifteen polymorphic loci used in the analysis were PGD, PGI1, PGI2, GDH, IDH, DIA1, FE, MDH1, MDH2, GOT2, PGM1, PGM2, TPI1, TPI2, and SAD. Studies to establish that loci were not linked were not possible, due to the absence of controlled crosses. Because, for most of the loci studied, previous work suggests the loci are not linked (Bush and Smouse 1991, Roberds and Conkle 1984, Conkle 1981, and Adams and Joly 1980), we assumed none of the loci were linked.

Analysis of Individual and Population-level Effects of Density

Methods

To determine the effects of density on individual plant response and population structure in each density treatment, comparisons between treatments were made and several statistical techniques were employed. First, simple comparisons of plant size and morphology were made between seedlings in each density treatment. Sizes and

growth characteristics of seedlings in the three density treatments were compared at each of the nine measurement times using height and diameter measurements and the number of side branches.

Coefficients of variation were calculated for each treatment to infer the type and level of competition occurring. Measures of inequality, such as the coefficient of variation, are suitable ways to explore competitive interactions in plants (Weiner and Solbrig 1984, Knox et al 1989). Coefficients of variation can point out the intensities and different types of competition occurring in a population. Plant size distributions were measured within each block*density combination using the Gini coefficient and the coefficient of variation (CV). The gini coefficient and the coefficient of variation gave results which were highly correlated (Table 3-4). Because calculation of the coefficient of variation was computationally simpler, CV was employed for analysis. The coefficient of variation was calculated by dividing the standard deviation of plant height by the mean. Coefficients of variation in plant height were used to compare the variance in height between the three density treatments over time, both block-by-block and by pooling the blocks. An analysis of variance was used to look for density effects on inequality (SAS Proc GLM):

$$CV = B\ D$$

where CV is coefficient of variation in a Block*Density combination and where block and density are both class variables and fixed effects. Type III sums of squares were used due to the unbalanced data set. Initially, one ANOVA was done to detect three way differences in the experimental design, then two factor ANOVAs were used to compare low and medium, medium and high, and low and high treatments.

To determine whether growth rate and height are good predictors of future growth in this species, Spearman rank correlations were used. Actual growth rate and relative growth rate for height were calculated for all plants in the experiment:

$$RGR=(H_2-H_1)/H_1$$

$$AGR = H_2 - H_1$$

where RGR is relative growth rate, AGR is actual growth rate, H is height and (1,2) refer to beginning and ending time intervals for determining the rate. Height was used as the indicator variable for asymmetric competition because it is highly correlated with success in this shade intolerant species. RGR and AGR were calculated using initial height in July of 1993 as H_1 and ending height in May of 1995 as H_2.

Lastly, to determine the effect of initial height on the ultimate success of a plant, mortality status (whether the seedling lived or died) was examined in the high-density treatment. Mortality status at the end of the experiment (May 1995) as a factor of initial height was tested using a two-factor T test. Spearman rank correlations for initial height vs. height at May 1995 were also calculated to determine differences in the endurance of size rankings over time between density treatments.

Results

Comparisons of mean heights and morphologies among density treatments showed shifts due to density from the beginning to the end of the study. During the growing season of 1993 and April of 1994, the high-density seedlings had the highest mean height, the medium-density seedlings were next tallest, and those at low-density were shortest (Table 3-1). In May of 1994, however, the medium and low-density seedlings overtook the high-density modules in height and in July of 1994 the low-density seedlings were on average taller than the medium and high-density treatments. By the end of the study, low-density seedlings were tallest, had the largest stem diameters (Table 3-2), and had the largest number of side branches while high-density seedlings were shortest, had the smallest diameters, and had few if any side branches (Table 3-3).

Coefficients of variation for the density treatments over time were compared to each other. Coefficients of variation represent size inequalities, with greater differ-

ences in seedling sizes within a treatment represented by a higher CV. The CV for each density treatment averaged for the 4 blocks are graphed in Figure 3-2. In general, a cyclical pattern was apparent in high-density, where CV fluctuated through time. A decrease in CV early on in medium-density eventually leveled off, resulting in depressed CV values for much of the study, followed by an increase in May 1995. Low-density showed an initial decrease in the summer of 1993 followed by an increase in April 1994, with a decrease from July 1994 to May 1995. Separate figures of the density treatments in each of the blocks show similar, although not identical, trends; in all but block 3, the low-density CV was consistently much higher than the medium-density CV (Figure 3-3, a-d).

Analysis of variance was used to detect differences in the components of variance between the density treatments. Throughout the first year, beginning in October, the analysis of variance model yielded significant or nearly significant results for density (Table 3-5). Differences between low and medium densities were most pronounced as shown by the separate analysis of variance for these two plots in Table 3-5. The differences between plots are apparent in Figure 3- 1 where the medium-density CV decreased in August 1993 while the low-density CV decreased but stayed higher than the medium-density CV. As the summer of 1994 progressed, the CV's became more significantly different. Near the end of the study, however, low-density CV decreased while medium-density CV rose, causing the ANOVA to yield nonsignificant results.

There were no detectable differences between low and high densities using the ANOVA model, probably due to the high values for low-density and the cyclical nature of CV in the high-density modules. However, the ANOVA model showed significant differences between medium and high especially during the summer of 1994 when the high-density treatment showed high size size inequality. These results indicate that density has a significant effect on the formation of a size hierarchy, as

predicted by Weiner (1985).

Comparisons of initial height and measures of growth yielded different results between the three density treatments, indicating that the effect of initial height is density-specific (Table 3-6). Actual growth rate (AGR) was strongly correlated with initial height in the high-density modules, less so in the medium-density modules, and not significant at low densities (Figure 3-4, a-d). Relative growth rate (RGR) showed the converse pattern; it was highly correlated with initial height at low densities, less so at medium densities, and not significantly so at high densities (Figure 3-5, a-d).

The importance of initial height to plant success was also examined using mortality rates and comparisons of final height. Mortality in the high-density modules was severe, ranging from 82.5% (block 1) to 74% (block 4) by the end of the study. The results of the t test showed that initial height in July 1993 was highly negatively correlated with mortality status at May 1995 ($p < .0001$, Table 3-7). Negligible mortality occurred in the low and medium-density modules and was random with respect to plant size. Two individuals which died in a medium-density module over the winter of 1994-95. Spearman rank correlations for initial height vs. height in May of 1995 showed slightly different results between density treatments. The correlation was very strong for high-density seedlings; and although it was significant for low-density seedlings, the correlation was weaker (Figure 3-6, a-c).

Discussion

Morphological data show direct evidence for differences in the amount of competition occurring in the three density treatments. High-density seedlings were tall and invested relatively more energy in height growth and less in stem diameter than the medium and low-density seedlings, as predicted by Weiner and Thomas (1992) for plants experiencing intense competition. Low-density seedlings appeared stunted and stressed during the summer of 1993 due to harsh conditions, although

alternatively the shortness could be the result of a larger investment in diameter or root growth because they weren't competing for light. Low-density seedlings, because of the distance between plants, may have experienced different conditions than high-density seedlings such as more rapid evaporation of water from the soil due to higher sun radiance and reflectance. By the end of the study, however, low-density seedlings were taller than those in the medium-density treatment, which were taller than the high-density seedlings. In addition, lower diameters in the high-density indicate intense intr-specific competition. High competition reduced the growth of individuals in the high-density treatment.

Morphological differences in branch number and branch length were also measurable among density classes. The low-density seedlings attained the highest number of stem branches, and these branches were on average longer than the branches of seedlings in the other density treatments. This supports Geber's (1989) findings that as competitive effects between individual plants increase, shape differences become more pronounced leaving individuals in intensely competitive conditions with fewer and shorter branches.

It is apparent from these data that the morphological differences between seedling densities were due to competition, with the high-density seedlings experiencing the highest intensity of competition and the low-density seedlings experiencing the least competitive pressure. To interpret the type of competition occurring in the density treatments, other measures had to be used. There are three possible competitive situations that could occur in the treatments: asymmetric competition, symmetric competition, or no competition.

The question of whether populations experienced asymmetric or symmetric competition or no competition was examined using coefficients of variation and growth rate data. The coefficient of variation is an indication of the development of a size hierarchy. A high CV can indicate the presence of asymmetric competition for

light; taller individuals take greater advantage of the resource and grow faster, meanwhile suppressing shorter individuals by preventing their access to light (Weiner and Thomas 1986) and causing greater differences in height between seedlings. Alternatively, a high CV can be indicative of a release of competition as each individual grows at its own inherent growth rate (Turner and Rabinowitz 1983). A relatively low CV can be a manifestation of below-ground or symmetric competition where all individuals are suppressed to the same degree (Turner and Rabinowitz 1983), leading to a less pronounced size hierarchy.

In the high-density treatment, the coefficient of variation shows a cyclical pattern, replicated in all three blocks. This cyclical pattern is related to the mortality events occurring in the high-density modules; asymmetric competition created a size hierarchy which was then destroyed as the short suppressed seedlings died off. The high values for CV occur both times in the spring, perhaps partially due to the spring growth flush. Swift growth is not solely responsible for the trend because neither the low nor medium-density treatments show this pattern. The increase in the coefficient of variation at the beginning of the study is evidence for asymmetric competition, which exaggerates the size differences among individuals in a stand (Weiner and Thomas (1986). It appears that the cyclical pattern arises from the interaction between the spring growth flush and competition for light, creating the dramatic size inequality. Thinning then began in the high-density treatment in October of 1993 and inequality decreased due to the loss of the smaller seedlings, resulting in a stand of seedlings of more similar size. After April 1994, however, inequality again increased in high-density modules indicating the formation of a size hierarchy which was then destroyed by mortality events in the summer and fall, continuing the seasonal, cyclical pattern.

The coefficient of variation for height in the high-density modules shows an unprecedented cyclical trend not before reported in other studies. This cyclical trend,

occurring in each of the three replicate populations, is contrary to other findings which report a continuing decrease in inequality or skewness as self-thinning proceeds (Knox et al. 1989, Weiner and Thomas 1986, Mohler 1978). The concurrence of this pattern in all three replicates suggests a consistent mechanism is responsible. This periodic pattern appears to be due to local interactions between seedlings caused by the collaborative effects of mortality and growth. The low measures of inequality consistently occur after a large mortality event followed by a low amount of absolute growth, as those individuals near newly created gaps are relieved of suppression. The high inequality values occur after low mortality and rapid growth during spring, as taller individuals again begin to attain dominance. This only occurs in the high-density treatment where asymmetric competition for light is intense. This phenomenon may not be evident under heterogeneous field conditions and in situations where the trees are of different ages. This study, which minimizes the effects of soil heterogeneity and tree age, is perhaps better able to detect this population-level trend.

In the medium-density modules, the seedlings experienced a quick decrease in CV, probably due to the excessive summer heat and lack of shade between seedlings. Alternatively, a decrease in CV of height may be due to the greater investment in diameter growth than the high-density seedlings. This low CV continued into the following summer, unlike the high and low-density treatments, indicating symmetric competition for water or other below-ground resources. Koyama and Kira (1956) predicted decreased levels of size inequality under conditions of competition for resources, based upon the idea that each individual has access to its proportional share of the resources. Turner and Rabinowitz (1983) fashioned their "resource-depletion" model for the reduced growth rates all individuals suffer when resources are scarce. Competition for soil resources occurred before competition for light in medium-density modules. In May 1995 for all replicates but block 4, competition for light became important as is evident by the increasing CV, apparently due to the formation of a size

hierarchy, in these blocks. At this point, asymmetric competition for light began to exaggerate the growth rates of individuals and taller seedlings began to overtake and suppress their neighbors.

Size inequalities in the low-density modules were less predictable. In general, the trend was an initial decrease in size inequality followed by a stable high in the summer of 1994. The initial decrease is a sign of the lack of competitive interactions as seedlings invested more energy into diameter and branch growth. Alternatively, this may be due to the excessive heat and sun suppressing these vulnerable and more widely spaced seedlings during their first summer. On average, the higher inequality in the low-density modules after the summer of 1993 is probably due to a lack of intraspecific competition, where each individual grew at its own inherent growth rate, unencumbered by competition for soil resources (Koyama and Kira 1956, Turner and Rabinowitz 1983). At the end of the growing season of 1994 and especially in May of 1995, the inequality of low-density stands decreased. During this time, the trees in the low-density modules were probably large enough to have significant root interactions causing competition for below-ground resources, thus reflecting the "resource-depletion" effect (Turner and Rabinowitz 1983) of lowered growth rates in all individuals. As no more watering or fertilization of the modules occurred after May 1995, this effect may become even more pronounced in the near future.

Additional evidence for asymmetric competition was obtained through analysis of actual and relative growth rates. The correlation between actual growth rate and initial height in the high-density modules is evidence that asymmetric competition for light occurred throughout most of the study. This correlation results from the suppression of the short seedlings by the tall ones, because the taller seedlings capture the sunlight, thereby reducing the amount the shorter seedlings receive and thus reducing the increment gained by the shorter seedlings. The lack of a correlation between relative growth rate and initial height reflects the fact that small seedlings put on small

height increments while large seedlings put on large increments. We might expect the relationship between relative growth rate and initial height to be a positive one in high-density, as predicted by Geber (1989) for high-density, non-thinning populations, but mortality events occurring among the shortest individuals alter this trend. The loss of the shortest individuals is indicated in Figure 3-3 a where the left side of the X axis is truncated compared to the medium and low-density modules (Figures 3-3, b and c).

A correlation between actual growth rate and initial height was not found in the low-density modules. At low-density, each seedling put on approximately the same increment irregardless of initial height, indicating a lack of competition for light. The correlation between initial height and actual growth rate is significant in the medium-density treatment, indicating that competition for light was present during the study, although the correlation coefficient shows that less variance is explained compared to the high-density treatment. Competition for light probably occurred only at the end of the study in the medium-density treatment as indicated by the rise in the coefficient of variation.

In the low and medium-density modules, relative growth rate was significantly negatively correlated with initial height. In these treatments, each seedling was able to grow at its inherent growth rate, with small seedlings putting on a similar increment as the large seedlings, but a relatively greater amount according to their size. The taller individuals did not suppress the shorter ones in the low-density modules, and for most of the study in the medium-density modules as well.

Correlations between initial height and final height, and initial height and mortality indicate the importance of plant height to future success in the presence and absence of competition. For plants in high-density situations, Weiner and Fishman (1994) reported that height was the most important aspect of size influencing future growth. Small individuals in general have a low probability of surviving density-

dependent thinning (Weiner 1985, Peet and Christensen 1987). For loblolly pine seedlings, we found that height has a large influence on later success of seedlings at high densities, as mortality was highly correlated with initial height. However, initial height had less of an effect at low densities. The significant correlation between initial height and height in May 1995 in the low-density modules, shows that even at low densities, tall seedlings tend to keep their size rankings, even without the influence of asymmetric competition.

Conclusions

Density has an effect on both the size structure of seedling stands and on the competitive responses of individuals. These analyses enabled me to determine the relative amount and type of competition occurring in each treatment over time. Medium-density modules experienced symmetric competition (for below-ground resources) for the majority of the study. Low-density modules experienced no intra-specific competition for most of the study, then experienced competition for below-ground resources at the end. High-density modules, however, experienced intense asymmetric competition throughout the study. In the next part of this chapter, this information is used to compare individual plants for height and growth in the context of genotype.

Analysis of Genetic Trends Under the Influence of Density

For this portion of the study, seedlings of known genotype were used to detect an influence density on plant growth. One objective was to determine the effect of the self-thinning stage on the genetic development of a juvenile loblolly pine stand (ie., whether or not the thinning stage exerts a selective force on plant genotype). The second objective was to determine how competition might influence the growth of

plants of different genotypes and heterozygosity levels.

Methods

An analysis of variance model is used to interpret the effects of density and heterozygosity on seedling growth. The experiment is a two-way factorial design where main effects are density and number of heterozygous loci. Both the number of heterozygous loci and density are considered fixed effects. Along the main axis of the plot (Figure 3-1) there appeared to be a gradient where seedlings at the east end grew taller than those toward the west end. To control for this effect, blocking was employed from east to west, and block is treated as a fixed effect. The effect of module is also considered in the model because the low-density modules were replicated within each block. The experiment is a split plot design with replication (SAS Proc GLM, due to the unbalanced data set). Class variables in the model are block, density, module, and number of heterozygous loci (hetscor). Interaction terms for block*density, density*hetscor, and block*hetscor are included in the model. All terms are tested over type III sums of squares due to unequal sample sizes within the treatments. At each new time measurement, dead individuals were removed from the analysis so that height at time T is not affected by scores of 0 for an individual and the number of high-density individuals in each module decreased over time. The full model is:

$$S_T = B\ D\ L\ D{*}L\ B{*}L\ M(B{*}D)$$

where S is height at time T, B is block, D is density, L is number of heterozygous loci, and M is module.

Spearman rank correlations were calculated to observe correlations between height and number of heterozygous loci in the treatments, and to compare the three densities throughout the length of the experiment. The correlations were partial correlations to factor out the effect of location along the long axis of the plot.

Population-level heterozygosity was calculated to determine whether increases in this statistic occurred over time due to mortality in the high-density treatment. The correlation between mean population-level heterozygosity and height in loblolly pine was analyzed using the heterozygosity (H) calculation from Zaykin and Pudovkin's (1993) CHIHW program. The change in average heterozygosity due to mortality in the high-density modules was examined throughout the span of the experiment and compared to the mean H in the low and medium-density modules. The program CHIHW calculated H for each enzyme at times T=1,3,6, and 9 for each block*density combination. Mean H for each block*density combination was then calculated and a repeated measures model was constructed (SAS Proc GLM) with mean H at times 1,3,6, and 9 as the dependent variables and block and density as independent variables. Not all individuals were included in the analysis; some enzyme genotypes are missing for some individuals. H values were calculated for each block*density combination using individuals with known genotypes at each enzyme. Therefore, the number of individuals used to determine the H value varies. However, missing values are random with respect to individual and genotype because missing values indicate a gel which did not resolve well. The program CHIHW will not calculate statistics of a monomorphic locus. Therefore, H for enzymes monomorphic in block*density combinations were manually set to zero before the ANOVA was run. This ensured that mean H was calculated using 15 loci for each block*density combination. The full model was:

$$H_1 H_3 H_6 H_9 = B D$$

where H is population-level heterozygosity, B is block, D is density, and 1,3,6,and 9 indicate measurement times.

To determine whether there was differential mortality between genotypes within an enzyme in the high-density treatment, Chi square statistics were computed to diagnose deviations from Hardy-Weinberg expectations for each enzyme. Ob-

served and expected Hardy-Weinberg ratios were determined for all seedlings in the experiment, and then high-density modules were examined intensively; at the time during the experiment when approximately half the seedlings were dead in the high-density modules, Hardy-Weinberg and heterozygosity statistics were compared for each enzyme for the dead population and the live population. Analysis of variance was used to determine if differences in genotype frequencies between dead and suviving populations were significant.

Results

The full analysis of variance model was used to determine the variance in seedling height explained by density and by number of heterozygous loci. Table 3-8 shows the full results of the model. Using the direct count heterozygosity variable and density, the model yielded significant results only for density. The pattern for density shows significant influence for most of the study. The nonsignificant result for density in May of 1994 is due to the fact that during this time the medium and low-density treatments caught up to the high-density seedlings in height. No significant results were apparent between heterozygosity and height, indicating that direct-count heterozygosity does not explain differences in height for loblolly pine seedlings under different levels of competition.

Spearman rank correlations were used to further explore a relationship between heterozygosity and height under different levels of competition (Spearman rank correlations between heterozygosity and height are shown for the three separate density treatments in Table 3-9). Although none of the three treatments have significant correlations at any of the measurement times, a pattern between negative and positive values is apparent. In the medium and high-density treatments, nearly all correlation coefficients are negative, while in the low-density treatment, nearly all are positive.

Population-level heterozygosity (H) yielded no evidence for heterozygote

advantage. The results are shown in Table 3-10. Average heterozygosity fluctuated in the high-density modules after each mortality event occurred, although the change was not significantly different from the medium and low-density modules. This result, again, clarifies the lack of an effect of general heterozygosity on height and height growth rate.

A summary table for Hardy-Weinberg analysis for all enzymes in the population is shown in Table 3-11. Enzymes deviating significantly from Hardy-Weinberg expectations included GOT2, GDH, PGI1 and IDH. IDH is probably a special case, however, because allele 1 is likely a breakdown product in as much as it is only found in high-density modules in dying seedlings. PGI1 deviates from Hardy-Weinberg expectations because of the presence of one rare individual. GOT2 and GDH both deviate from Hardy-Weinberg expectations due to an excess of individuals in the homozygous classes in the total population and so represent justifiable deviations from Hardy-Weinberg expectations. When only the high-density modules are considered, Hardy-Weinberg expectations are more closely met (Table 3-12, a and b).

After thinning events occurred in the high-density treatment, and when approximately half the seedlings died, mortality was concentrated among the homozygous individuals at these two loci (Table 3-12, a and b). Graphs of the mortality patterns for GOT2 and GDH are in Figure 3-7, a-c and Figure 3-8 a-c, respectively. For the enzyme GOT2, the Chi square test shows a significant deviation from Hardy-Weinberg expectations due to higher than expectd mortality in the homozygous classes. This pattern is also present for GDH, although the Chi square test value is not significant. After these mortality events, the surviving population for GOT2 and GDH show Chi square values very close to Hardy-Weinberg expectations. In addition, analysis of variance shows that the frequencies of individuals in the dead vs. surviving populations for GOT2 genotypes are significantly different from each other ($p < 0.05$).

Spearman rank matrices were also used to look for correlations between specific enzyme genotypes and height for each density treatment. These were partial correlations to factor out the plot gradient effect. Spearman rank correlation matrices for height over time showed that during the early part of the study, the heterozygous genotypes of GOT2 and GDH were correlated with height (Table 3-13). This correlation diminished as the thinning stage progressed. In medium-density treatments, one genotype, SAD44, was significantly correlated with height throughout the study. This was investigated further by dividing the data set into 2 halves, testing blocks 1 & 2 together and also testing blocks 3 and 4 together. Although correlation coefficients decreased, positive correlations were still apparent in both halves of the data set (Table 3-14).

Discussion

Although a number of studies found significant links between the number of heterozygous loci and fitness traits in various organisms (Schaal and Levin 1976; Singh and Zouros 1978; Zouros, Singh, and Miles 1980; Pierce, Mitton, and Rose 1982; and review by Mitton for forest trees, Mitton 1989), neither the full analysis of variance model for direct-count heterozygosity nor the model using mean population-level heterozygosity (H) show such a correlation between growth and survival for loblolly pine seedlings. In short, this study failed to reveal any advantage to having generally high levels of heterozygosity. Individuals within a density class had similar heights whether, they had zero heterozygous loci or six heterozygous loci.

Results of Spearman rank correlations between number of heterozygous loci and height for the three density treatments, while not significant, show mostly positive coefficients at low densities and mostly negative coefficients in the medium and high-density modules. This implies that at low densities, heterozygosity is related to height growth rate. Such a relationship may become more substantial over time when

competition is not present, especially as the trees grow without hindrance from neighbors. In the study by Bush (1988) where a positive correlation was found between height growth and heterozygosity in outcrossed trees, the loblolly trees were planted in an array spaced 6 feet apart and would not have competed for light until they were in at least the pole stage.

If heterozygosity does confer a faster growth rate, how ever slight it may be, by the time the trees reached the pole stage (in Bush's study) the more heterozygous ones may have had a height advantage as competition for light commenced. Early growth without asymmetric competition in the low-density treatment of the present study may allow the more heterozygous seedlings to gain an advantage over other seedlings, as indicated by the positive correlation coefficients. When asymmetric competition for light begins, the taller seedlings in the stand will be the more heterozygous ones, giving them a better chance for survival as stand thinning progresses. In short, a period of no competition for light followed by intense thinning is needed for heterozygous individuals to gain an advantage. This phenomenon may account for the higher levels of heterozygosity found in old vs. young tree stands (Hamrick et al 1992, Brotschol et al. 1986, Farris and Mitton 1984). However, the random spatial distribution of seedlings in a field is also influential. Seedlings tend to grow in patchy conditions, not the uniform modules we present here. If we were to imagine all of block 1 as a patchy field of seedlings, it is obvious that none of the high-density individuals could compete with the low-density seedlings as time progresses, no matter how well they do within their own density class.

The differential survivorship among GOT2 and GDH genotypes resulting in higher mortality in the homozygous classes provides evidence that heterozygosity at these loci is related to higher success during the thinning stage. The early correlation between height and heterozygosity at these genotypes is evidence for size hierarchy formation, where individuals possessing these genotypes generally dominated their

neighbors in height. Asymmetric competition for light resulted in the higher survival for seedlings with these genotypes, while alternate gentoypes had lower survivorship. This is evidence that asymmetric competition, the formation of a size hierarchy, and the resulting differential mortality can create changes in genotype frequencies during stand development. Such differential mortality, if additive over the life of the stand, may account for the higher levels of heterozygosity other researchers have observed at certain loci in older stands (Bush 1988, Hamrick et al. 1992, Brotschol et al. 1986, Farris and Mitton 1984).

Correlations between enzyme genotype and height yielded an interesting trend for the SAD enzyme in medium-density modules. The fact that the SAD 44 genotype showed a significant correlation with height throughout the study, even when the data were divided in half, indicates a meaningful pattern. Two possible scenarios are hypothesized to account for this pattern. First, shikimic acid dehydrogenase is an enzyme involved in the biosynthesis of certain amino acids such as tyrosine, and while this does not directly link it to respiration or photosynthesis, the 44 genotype may confer a physiological advantage to seedlings. A second possibility is that the SAD gene is linked to a gene or set of genes correlated with height in loblolly pine, which is evident only under the conditions the medium-density treatment received. This genotype is not favored under conditions present in high and low-density modules, only in the medium-density treatment where below-ground competition was present. The field conditions the seedlings were subjected to may have favored the 44 genotype. Whether the SAD 44 genotype confers a physiological advantage or not, the correlation of this genotype with height in the medium-density treatment is significant.

Although it is not possible in this study to determine why the SAD 44 genotype is significantly correlated with height in the medium-density modules during the seedling stage, it will tend to continue as the seedlings enter asymmetric competition, where taller seedlings have a competitive advantage. As thinning occurs, seedlings

with this genotype will have a higher rate of survival than the other genotypes, resulting in a population with a higher frequency both of the genotype and of the 4 allele than the starting population had.

Table 3-1.
Mean Heights (cm) in the Three Density Treatments

Date	Low Density		Medium Density		High Density	
	Mean	SD	Mean	SD	Mean	SD
7/93	13.33	2.92	15.45	3.06	15.51	3.21
8/93	20.57	3.76	23.18	3.72	26.27	5.67
10/93	25.62	4.16	27.42	4.20	29.79	5.94
4/94	25.46	4.85	28.57	4.31	31.95	4.94
5/94	40.89	8.67	44.64	7.43	39.80	9.14
7/94	57.23	11.80	55.21	8.88	46.37	10.18
8/94	67.18	13.74	61.45	10.01	52.06	9.42
10/94	73.24	14.94	66.16	10.71	56.72	9.30
5/95	98.47	18.91	79.55	15.46	64.41	12.25

Table 3-2.
Seedling Diameters (mm), May 1995

Density Class	Diameter Mean	Diameter S.D.	Number Seedlings Remaining	% of Initial
Low	16.58	3.96	216	100
Medium	7.82	1.73	251	98
High	6.07	1.40	135	22.5

Table 3-3.
Side Branch Statistics, October 1994

Variable	Low Density	Medium Density	High Density
Total number of Branches			
MEAN	11.91	3.98	0.22
S.D.	3.89	2.18	0.76
Cumulative branch lgth.			
MEAN (cm)	168.75	37.22	2.00
S.D.	70.03	26.06	7.07
Average branch lgth.			
MEAN (cm)	14.02	9.05	1.07
S.D.	3.81	4.88	3.31
Number of seedlings remaining	218.0	254.0	228.0
Percentage of initial seedlings	100.0	99.2	38.0

Table 3-4.
Correlation Analysis for Gini Statistic and Coefficient of Variation

Statistic	N	Mean	S.D.
Gini	99	0.10	0.02
CV	99	17.27	3.38

Spearman Correlation Coefficient: 0.98; $p < 0.0001$

Table 3-5. Analysis of Variance for CV

Low, Medium, and High Density Treatments

Time	p	R^2	Type III SS p Block	Density
7/93	0.54	0.47	0.40	0.66
8/93	0.38	0.57	0.76	0.22
10/93	0.09	0.78	0.61	0.05
4/94	0.60	0.44	0.90	0.28
5/94	0.11	0.76	0.87	0.03
7/94	0.04	0.85	0.93	0.008
8/94	0.66	0.40	0.92	0.31
10/94	0.89	0.23	0.98	0.54
5/95	0.92	0.20	0.94	0.67

Low and Medium

7/93	0.18	0.81	0.16	0.23
8/93	0.05	0.92	0.09	0.03
10/93	0.22	0.78	0.30	0.11
4/94	0.38	0.67	0.58	0.15
5/94	0.14	0.84	0.32	0.05
7/94	0.19	0.80	0.74	0.05
8/94	0.56	0.54	0.89	0.18
10/94	0.55	0.55	0.66	0.27
5/95	0.57	0.53	0.59	0.35

Low and High

7/93	0.67	0.57	0.57	0.67
8/93	0.82	0.42	0.86	0.57
10/93	0.64	0.60	0.98	0.27
4/94	0.80	0.45	0.85	0.62
5/94	0.81	0.44	0.96	0.37
7/94	0.62	0.62	0.88	0.22
8/94	0.98	0.13	0.95	0.86
10/94	0.92	0.29	0.90	0.80
5/95	0.92	0.28	0.93	0.55

Medium and High

7/93	0.80	0.45	0.74	0.94
8/93	0.52	0.69	0.78	0.27
10/93	0.13	0.93	0.39	0.06
4/94	0.63	0.60	0.58	0.75
5/94	0.17	0.91	0.81	0.06
7/94	0.02	0.99	0.28	0.007
8/94	0.24	0.87	0.29	0.17
10/94	0.88	0.34	0.84	0.77
5/95	0.90	0.31	0.83	0.84

Table 3-6.
Correlations Between Initial Height, RGR, AGR, and Height May 1995

Density/ Correlation	RGR	AGR	Ht May 1995
Low			
R	-0.71	0.08	0.24
p	0.0001	ns	0.0004
Medium			
R	-0.46	0.37	0.53
p	0.0001	0.0001	0.0001
High			
R	-0.09	0.52	0.66
p	ns	0.0001	0.0001

Partial = Block
AGR = Height May 1995 - Height July 1993
RGR = AGR / Height July 1993
ns = non-significant

Table 3-7.
Mortality Status as a Factor of Initial Height in High Density

Status	N	Mean (cm)	S.D.
Alive	136	18.11	2.63
Dead	464	14.75	2.95

F test for equal variance: $F' = 1.26$; $p = 0.11$

T test: $p < .0001$

Table 3-8.
Full Analysis of Variance Model [Height = Block, Density, Module(Block*Density), Direct-count heterozygosity, Heterozygosity*Density]

Main effects Block and Density are tested over Module(Block*Density) error term.

Date	R^2	Source	F	p
7/93	0.278	Block	7.05	0.0014
		Density	3.69	0.0395
		Module(B*D)	2.83	0.0001
		Heterozy.	0.97	0.3255
		Het*Density	0.23	0.7917
8/93	0.389	Block	0.18	0.9076
		Density	13.48	0.0001
		Module(B*D)	2.67	0.0001
		Heterozy.	0.03	0.8616
		Het*Density	0.46	0.6317
10/93	0.192	Block	0.14	0.9373
		Density	3.28	0.0543
		Module(B*D)	2.38	0.0002
		Heterozy.	0.10	0.7475
		Het*Density	0.73	0.4839
4/94	0.303	Block	0.41	0.7475
		Density	8.46	0.0016
		Module(B*D)	2.40	0.0002
		Heterozy.	0.00	0.9905
		Het*Density	0.13	0.8796
5/94	0.184	Block	4.29	0.0143
		Density	1.97	0.1612
		Module(B*D)	2.93	0.0001
		Heterozy.	1.14	0.2859
		Het*Density	0.40	0.6687
7/94	0.341	Block	3.77	0.0232
		Density	3.23	0.0563
		Module(B*D)	3.82	0.0001
		Heterozy.	1.02	0.3123
		Het*Density	2.24	0.1074
8/94	0.441	Block	4.34	0.0136
		Density	3.49	0.0461
		Module(B*D)	5.50	0.0001
		Heterozy.	1.32	0.2511
		Het*Density	1.88	0.1531
10/94	0.487	Block	6.46	0.0022
		Density	3.29	0.0541
		Module(B*D)	5.76	0.0001
		Heterozy.	1.07	0.3003
		Het*Density	1.06	0.3456
5/95	0.594	Block	6.56	0.0020
		Density	7.71	0.0025
		Module(B*D)	5.80	0.0001
		Heterozy.	0.11	0.7400
		Het*Density	0.08	0.9229

Table 3-9.
Partial Correlations for Direct-Count Heterozygosity and Height at 9 Times for Low, Medium, and High Density

Date	Low Density N = 216		Medium Density N = 251		High Density		
	Coeff	p	Coeff	p	Coeff	p	N
7/93	-0.01	0.82	-0.03	0.57	0.00	0.94	525
8/93	0.02	0.81	-0.04	0.53	0.05	0.25	525
10/93	-0.01	0.94	-0.03	0.60	0.05	0.26	514
4/94	0.07	0.31	-0.05	0.43	0.03	0.52	437
5/94	0.10	0.15	-0.02	0.68	-0.01	0.83	432
7/94	0.11	0.10	-0.02	0.65	-0.04	0.47	388
8/94	0.11	0.11	-0.03	0.58	-0.04	0.51	301
10/94	0.08	0.22	-0.02	0.69	-0.02	0.75	227
5/95	-0.00	0.99	-0.03	0.60	-0.01	0.87	135

Partial = location along long axis of plot

Table 3-10.
Analysis of Variance for H at Times 1,3,6, and 9
Type III SS

Date	R^2	Source	F	P
7/93	0.002	Density	0.16	0.85
		Block	0.05	0.99
10/93	0.003	Density	0.16	0.85
		Block	0.05	0.99
7/94	0.003	Density	0.16	0.86
		Block	0.05	0.98
5/95	0.003	Density	0.17	0.84
		Block	0.06	0.98

Table 3-11.
Hardy-Weinberg Summary Statistics for Enzymes, Full Plot

Enzyme	Chi^2	df	Chi(.05)	# Individuals
DIA1	1.27	1	3.84	930
GDH	9.327*	1	3.84	977
GOT2	6.17*	1	3.84	996
FE	0.35	1	3.84	992
IDH	320.42*	3	7.815	997
MDH1	0.017	10	18.31	998
MDH2	13.39	15	24.996	941
6-PGD	5.88	10	18.31	975
PGI1	26.55*	3	7.815	956
PGI2	16.76	21	32.67	989
PGM1	0.37	3	7.81	813
PGM2	0.06	1	3.84	814
SAD	1.62	10	18.31	996
TPI1	0.21	1	3.84	501
TPI2	0.001	1	3.84	673

Table 3-12.
Hardy-Weinberg Summary Statistics for GDH and GOT2, High Density Treatment

A. GDH

Total Seedlings N=506

Genotype	33	34	44	Total
Observed	363	126	17	
Expected	358.65	134.70	12.65	
Chi Square	0.05	0.56	1.50	2.11 (df=1)

Dead Seedlings N=216

Observed	161	47	8	
Expected	157.60	53.81	4.59	
Chi Square	0.07	0.86	2.53	3.46 (df=1)

Alive Seedlings N=290

Observed	202	79	9	
Expected	201.11	80.78	8.11	
Chi Square	0.00	0.04	0.10	0.14 (df=1)

Table 3-12.

B. GOT2

Genotype	33	34	44	Total
Total Seedlings N=525				
Observed	153	248	124	
Expected	146.1	261.7	117.1	
Chi Square	0.32	0.72	0.40	1.44 (df=1)
Dead Seedlings N=223				
Observed	75	91	57	
Expected	65.1	110.8	47.1	
Chi Square	1.05	3.53	2.07	7.11*(df=1)
Alive Seedlings N=302				
Observed	78	157	67	
Expected	81.1	150.8	70.1	
Chi Square	0.12	0.25	0.14	0.51 (df=1)

Table 3-13.
Spearman Partial Correlations for High Density Treatment

Date	% Seedlings Surviving	GDH-34 (p)	GOT2-34 (p)
Jul 93	100	0.06	0.24
Aug 93	100	0.05*	0.10
Oct 93	97	0.03*	0.04*
Apr 94	75	0.09	0.65
May 94	74	0.35	0.61
Jul 94	66	0.68	0.65
Aug 94	53	0.47	0.60
Oct 94	37	0.22	0.18
May 95	23	0.86	0.70

Table 3-14.
Partial Correlations Between Height and Shikimic Acid Dehydrogenase, Genotype 44 in Medium Density Treatment

Date	Modules 1&14 Coefficient	p	Modules 23&28 Coefficient	p
Jul 93	0.16	0.07	0.19	0.03*
Aug 93	0.16	0.07	0.15	0.07
Oct 93	0.13	0.14	0.19	0.03*
Apr 94	0.18	0.04*	0.16	0.05*
May 94	0.13	0.13	0.14	0.09
Jul 94	0.17	0.04*	0.13	0.12
Aug 94	0.18	0.03*	0.16	0.07
Oct 94	0.14	0.11	0.16	0.07
May 94	0.17	0.05*	0.15	0.09

Figure 3-1. Planting design for Botany Pond study.

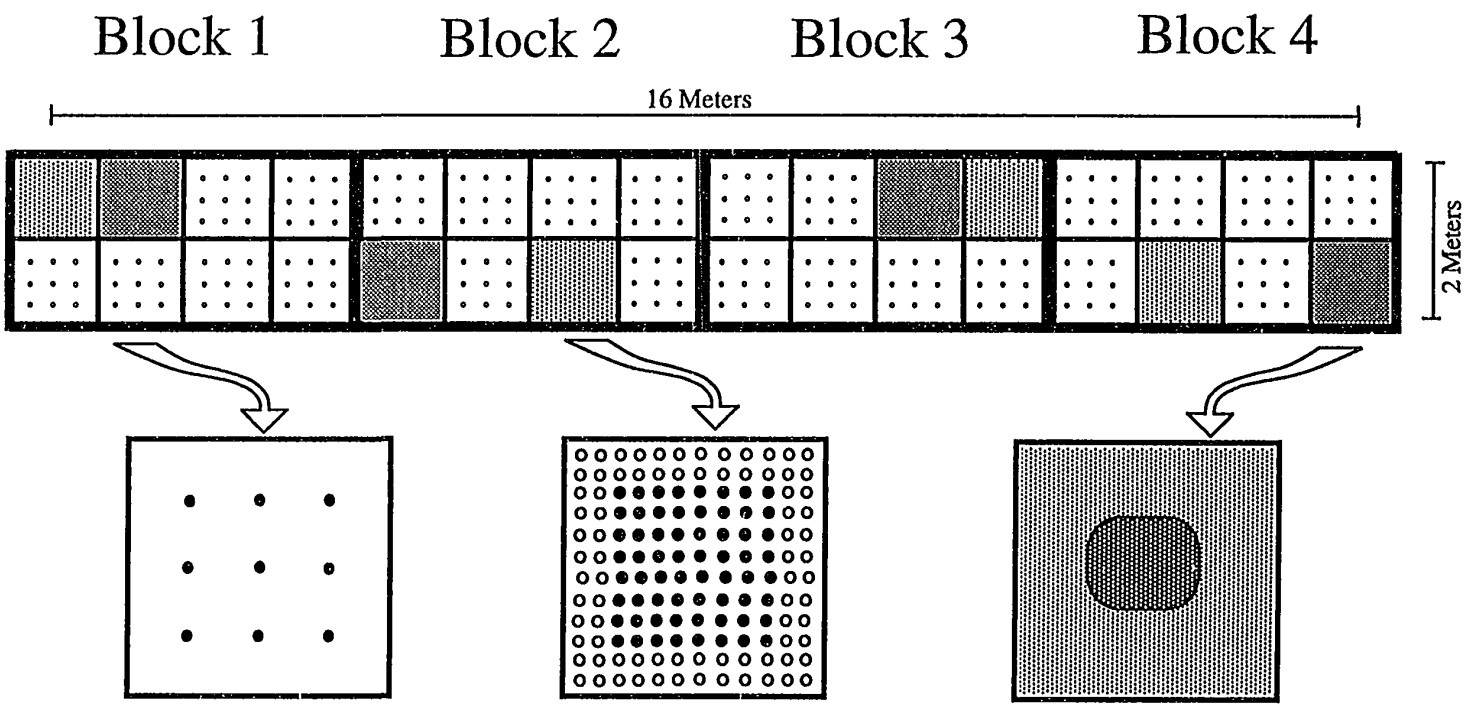

Block 1
Block 2
Block 3
Block 4
16 Meters
2 Meters
Low Density
9 plants/plot
9 Focal Plants
24 Replicates
Medium Density
144 plants/plot
64 Focal Plants
4 Replicates
High Density
1,200 plants/plot
200 Focal Plants
4 Replicates

Figure 3-2. Coefficients of variation as a function of time for the three density treatments, all blocks combined.

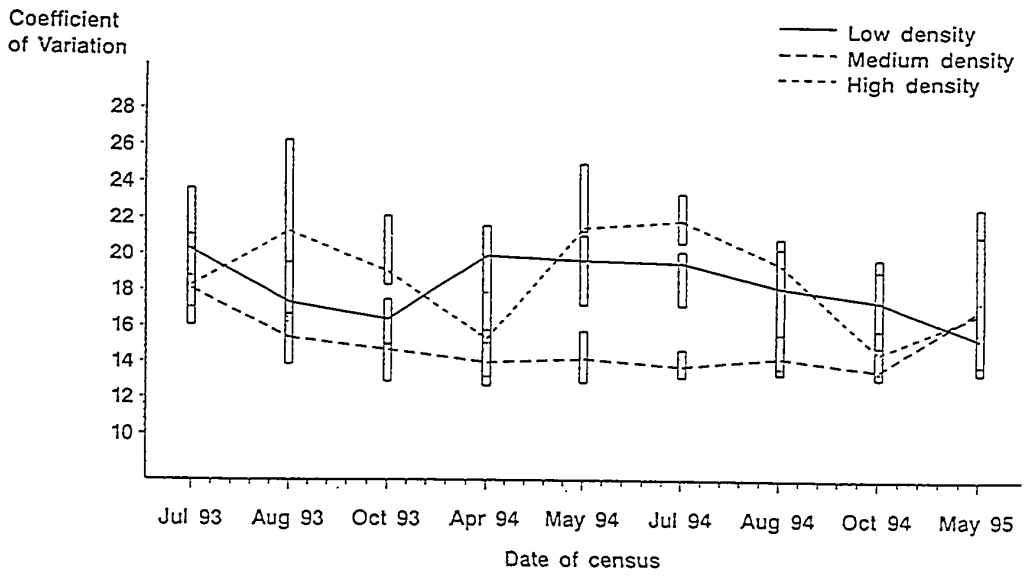
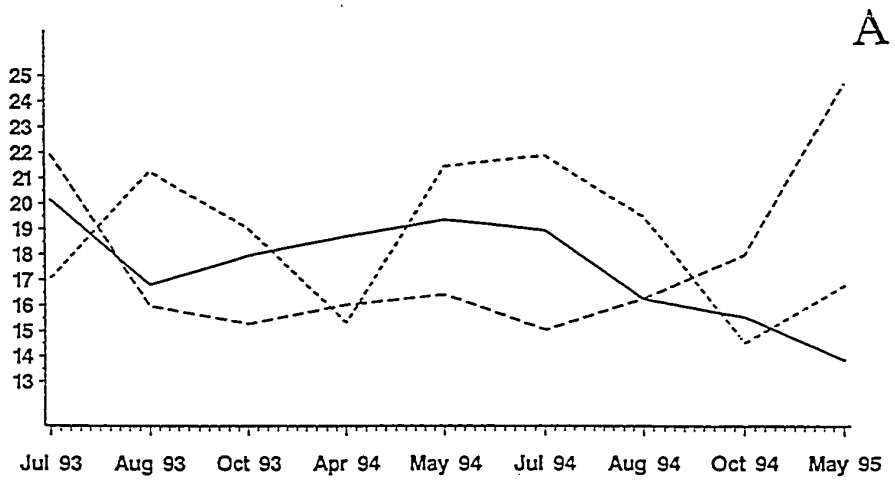
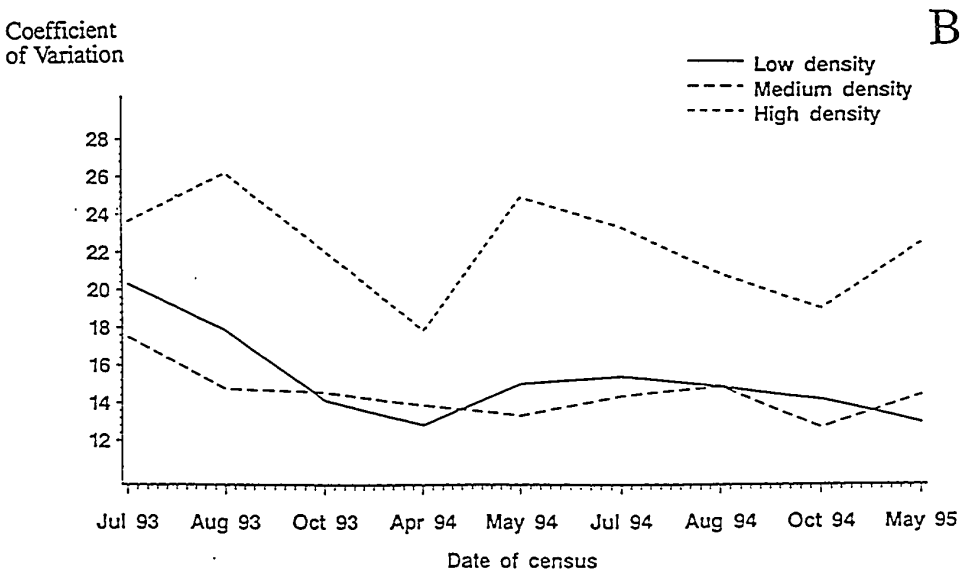
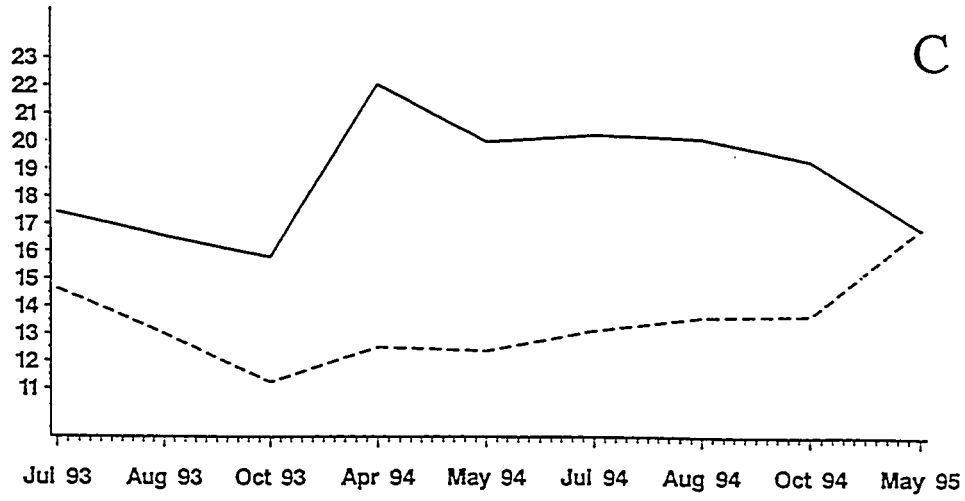

Coefficient
of Variation
Low density
Medium density
High density
28
26
24
22
20
18
16
14
12
10
Jul 93
Aug 93
Oct 93
Apr 94
May 94
Jul 94
Aug 94
Oct 94
May 95
Date of census

Figure 3-3. Coefficients of variation as a function of time for the three density treatments. A) Block 1, B) Block 2, C) Block 3, D) Block 4. Note that the high density treatment is missing from Block 3 because it was excluded from the analysis due to herbivory (see methods section).


A
25
24
23
22
21
20
19
18
17
16
15
14
13
Jul 93
Aug 93
Oct 93
Apr 94
May 94
Jul 94
Aug 94
Oct 94
May 95


B
Coefficient of Variation
Low density
Medium density
High density
28
26
24
22
20
18
16
14
12
Jul 93
Aug 93
Oct 93
Apr 94
May 94
Jul 94
Aug 94
Oct 94
May 95
Date of census


C
23
22
21
20
19
18
17
16
15
14
13
12
11
Jul 93
Aug 93
Oct 93
Apr 94
May 94
Jul 94
Aug 94
Oct 94
May 95

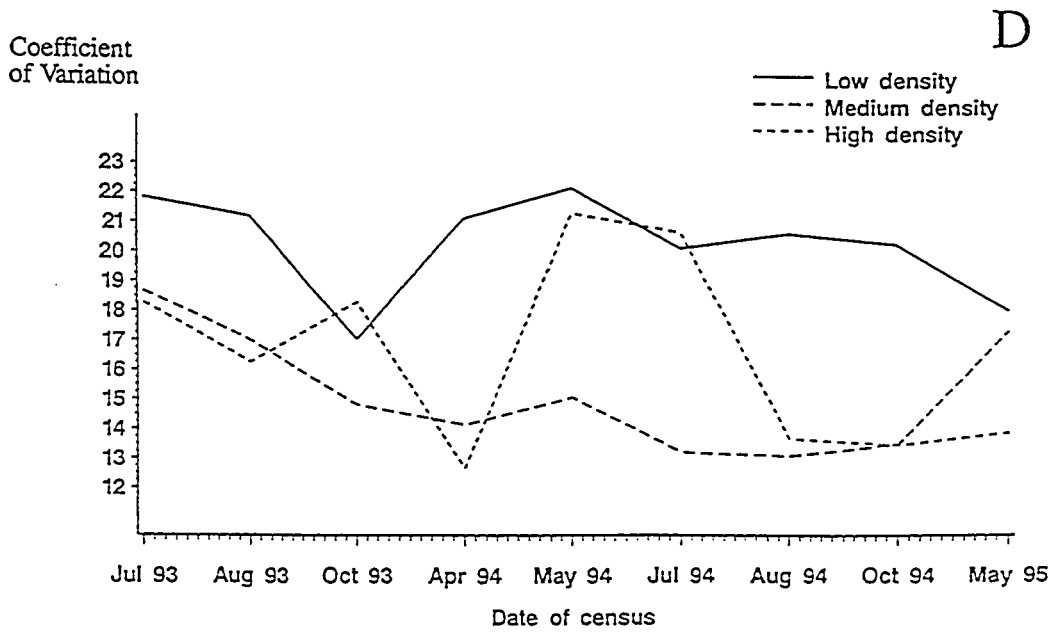

D
Coefficient
of Variation
Low density
Medium density
High density
23
22
21
20
19
18
17
16
15
14
13
12
Jul 93
Aug 93
Oct 93
Apr 94
May 94
Jul 94
Aug 94
Oct 94
May 95
Date of census

Figure 3-4. Correlation between actual growth rate (AGR) and initial height for trees surviving to May 1995 in A) high density treatment, B) medium density treatment, and C) low density treatment.

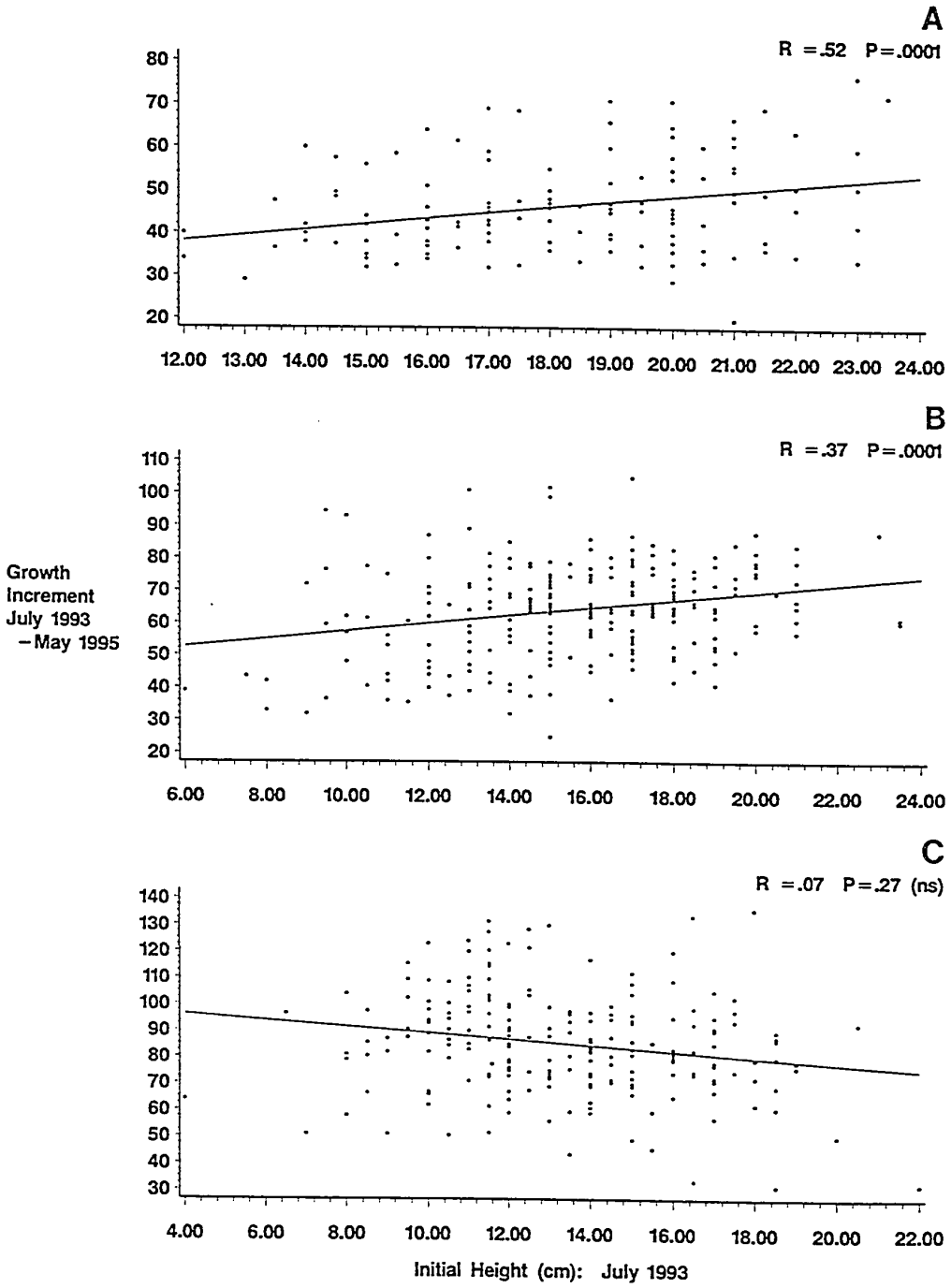

A
R =.52 P=.0001
80
70
60
50
40
30
20
12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00 22.00 23.00 24.00
B
R =.37 P=.0001
110
100
90
80
70
60
50
40
30
20
6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00
Growth Increment July 1993 –May 1995
C
R =.07 P=.27 (ns)
140
130
120
110
100
90
80
70
60
50
40
30
4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00
Initial Height (cm): July 1993

Figure 3-5. Correlation between relative growth rate (RGR) and initial height for trees surviving to May 1995 in A) high density treatment, B) medium density treatment, and C) low density treatment.

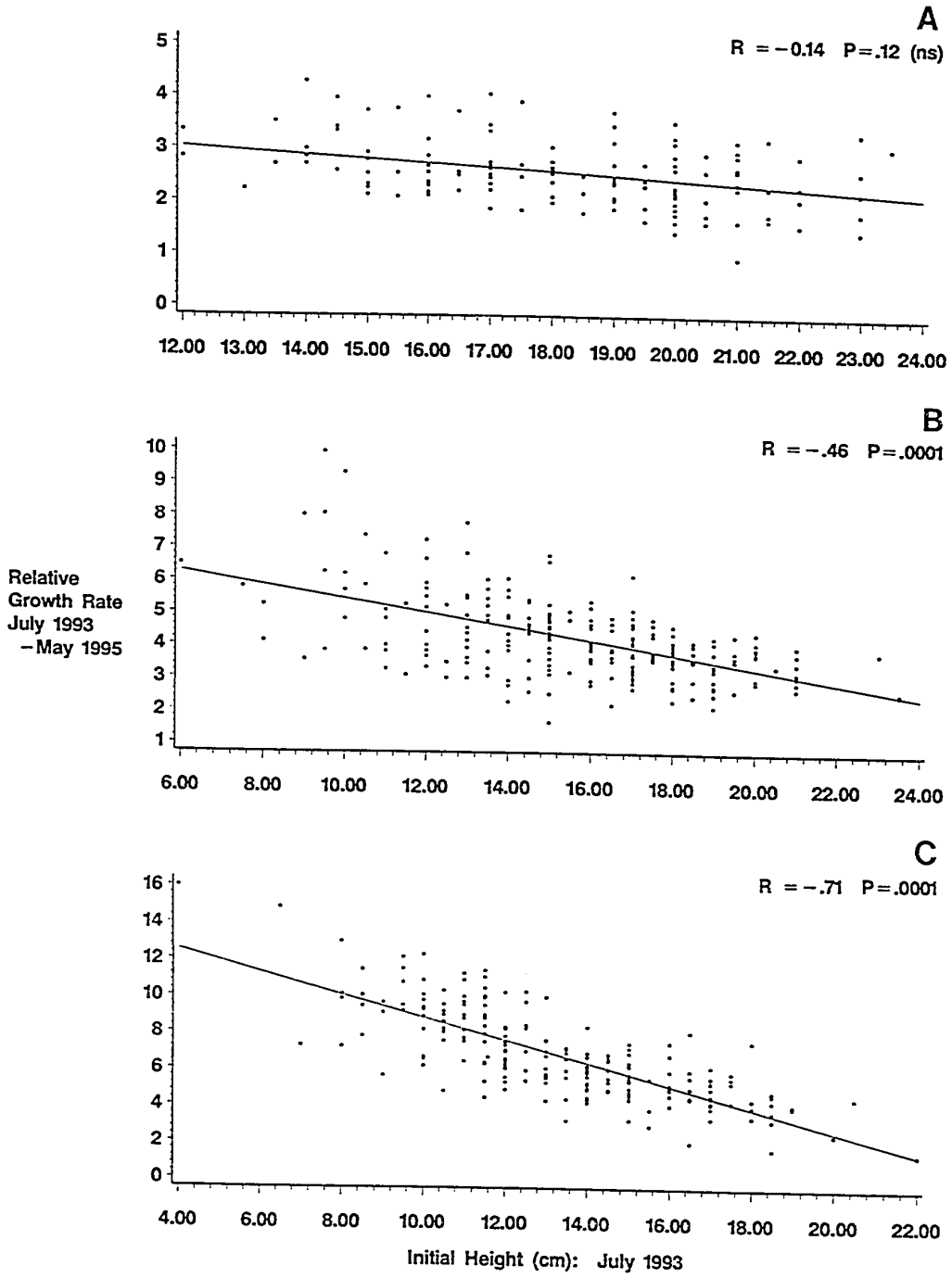

A
R = −0.14 P=.12 (ns)
5
4
3
2
1
0
12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00 22.00 23.00 24.00
B
R = −.46 P=.0001
10
9
8
7
6
5
4
3
2
1
6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00
Relative Growth Rate July 1993 −May 1995
C
R = −.71 P=.0001
16
14
12
10
8
6
4
2
0
4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00
Initial Height (cm): July 1993

Figure 3-6. Correlation between initial height and height in May 1995 for trees surviving to May 1995 in A) high density treatment, B) medium density treatment, and C) low density treatment.

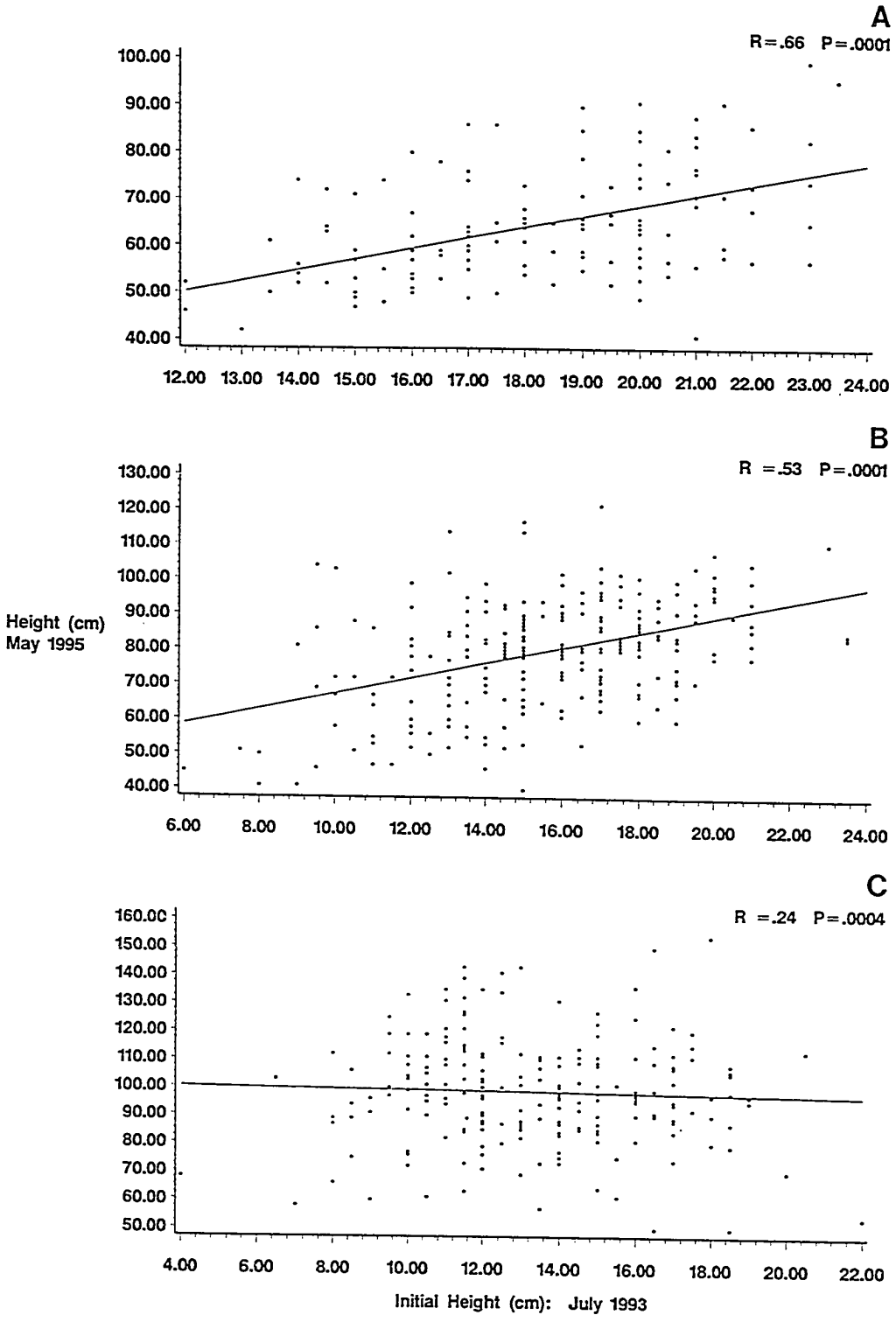

A
R=.66 P=.0001
100.00
90.00
80.00
70.00
60.00
50.00
40.00
12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00 22.00 23.00 24.00
B
R =.53 P=.0001
130.00
120.00
110.00
100.00
90.00
80.00
70.00
60.00
50.00
40.00
Height (cm)
May 1995
6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00
C
R =.24 P=.0004
160.00
150.00
140.00
130.00
120.00
110.00
100.00
90.00
80.00
70.00
60.00
50.00
4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00
Initial Height (cm): July 1993

Figure 3-7. Genotype classes for GOT2. The Y axis is the observed/expected Hardy-Weinberg ratio. A) The total high density population before thinning events took place. After approximately 1/2 o fhte seedlings died, the genotypic ratios of the B) dead population, and C) the surviving population.

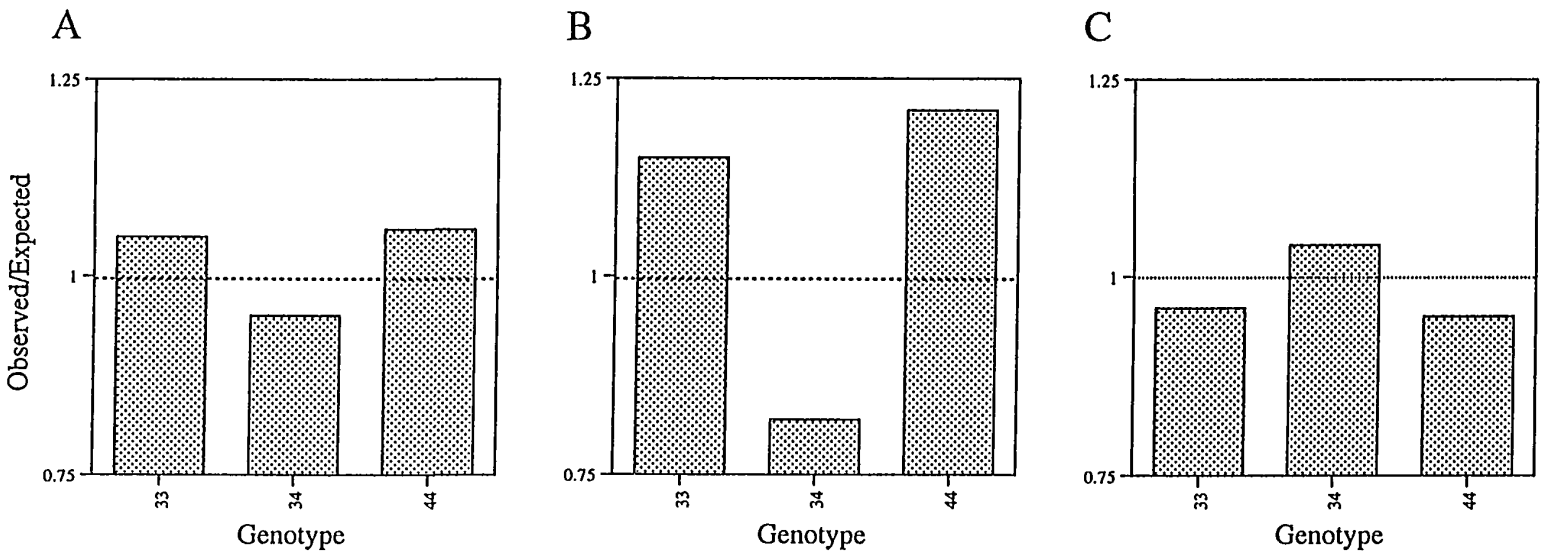
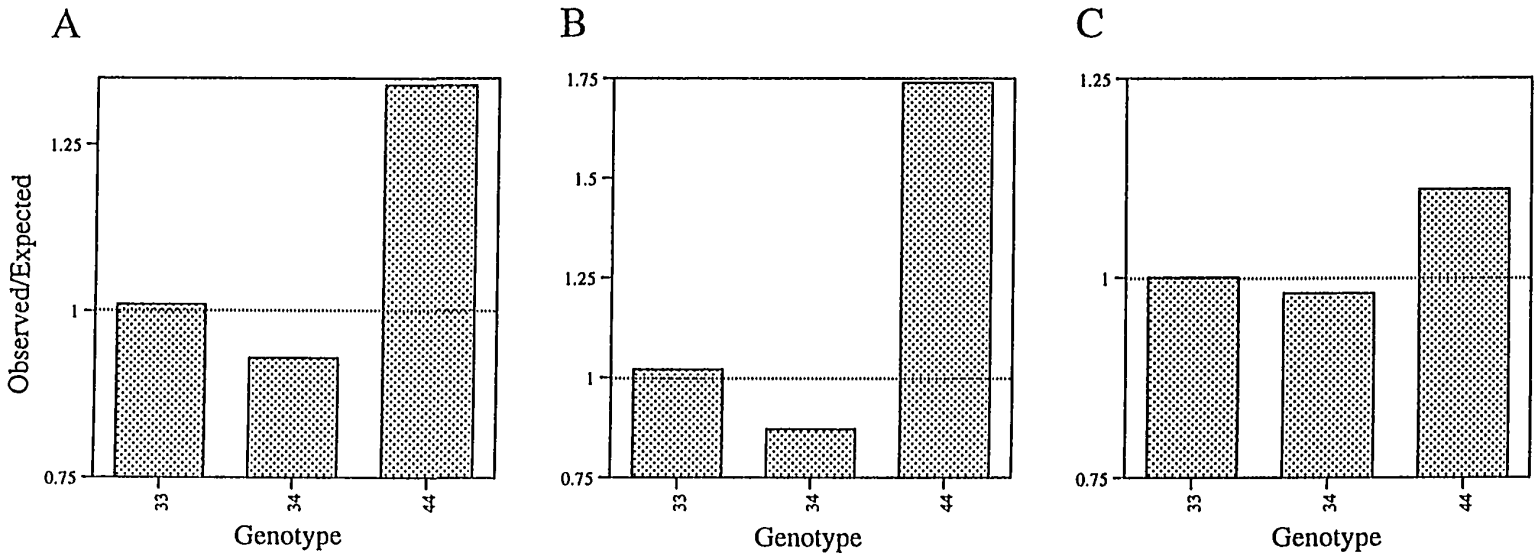

A
B
C
Observed/Expected
1.25
1
0.75
33
34
44
Genotype

Figure 3-8. Genotype classes for GDH. The Y axis is the observed/expected Hardy-Weinberg ratio. A) The total high density population before thinning events took place. After approximately 1/2 of the seedlings died, the genotypic ratios of the B) dead population, and C) the surviving population.


A
B
C
Observed/Expected
1.25
1
0.75
1.75
1.5
1.25
1
0.75
1.25
1
0.75
33
34
44
Genotype

Chapter 4

The Interaction Between Competition and Heterozygosity in Loblolly Pine: The Duke Forest Study

Introduction

Secondary forest succession has been shown to exhibit numerous trends that are consistent across multiple types of ecosystems. It is presumably driven to a large extent by competition (Christensen and Peet 1984) where growth and survival depend on an individual's ability to acquire needed resources. Although many features of forest stand development have been studied extensively, little is known of genetic changes that accompany it. However, a process such as succession, where differential survivorship plays a key role, provides a potential catalyst for genetic changes that occur as a result of natural selection.

It is possible that competition and the resulting mortality during forest development could initiate stand genetic changes. Forest stand development can be divided into four stages (Oliver 1981), each with varying competitive pressures. The first stage is the establishment stage where many species of herbs and trees become established and begin to grow in an abandoned field. Competition for resources during this stage is low because light levels are high, and soil nutrients are readily available. As the establishment stage progresses, woody tree species, often pines, begin dominating the field. The pines grow and begin competing for light, starting the thinning stage. Thinning will continue until the canopy consists of reproductively mature individuals. As the pines senesce, hardwoods take their places in the gaps, heralding the transition stage. Competition during the transition stage has lessened due to the creation of

gaps. As the gaps are filled by hardwood species, the transition stage is completed, and the steady-state stage begins where hardwoods which die are replaced by other hardwoods.

Of the four stages of forest stand development, the stage most amenable to examining genetic changes due to competitive pressures is the thinning stage. One reason is that the thinning stage is characterized by density-dependent mortality and intense asymmetric competition for light, where taller trees get a disproportionate share of available light (Weiner and Thomas 1986). A size hierarchy is created within the population and the shorter, light-suppressed trees will eventually be thinned from the stand (Knox et. al 1989). This highly competitive period sets the stage for natural selection, but to what degree is selection based on the genotypes of the plants involved? Although genetic change during succession in forest trees has not been previously studied, there is evidence that such changes occur. Several investigators found mean genetic heterozygosity to be higher in older age classes of forest trees as compared to seedling and pole-age populations (Hamrick et. al 1993, Brotschol et. al 1986, Farris and Mitton 1984). This suggests a survival advantage for individuals with higher levels of genetic heterozygosity, and that the more homozygous trees are being lost as the stand ages. Such a discrepancy in frequency of heterozygous individuals might be expected to occur when trees are undergoing intense competition and differential mortality during the thinning stage.

Selection for heterozygous individuals could be based on the amount of competition a stand experiences. Competition depends on the density of trees, with low density stands experiencing less pressure and thereby less mortality than dense stands. If individuals with higher levels of heterozygosity are being selected during the thinning stage, lower mortality, as would be present in an initially low density stand, should result in a mature stand that is more genetically like a young stand. Such a stand should have lower levels of heterozygosity because less mortality of homo-

zygous individuals has occurred.

In this study, I explore the relationship between heterozygosity and the intense competition trees experience during the thinning stage of forest stand development. Loblolly pine was chosen as the study species for several reasons. First, in the Piedmont region of North Carolina, the thinning stage is often dominated by loblolly pine, making genetic analysis simpler than if more than one species was involved. Second, I had available a mature stand of loblolly pine with permanent sample plots representing a range of initial densities with detailed records of growth dating back to 1933. The different densities of the stand represent varying levels of competition experienced by the trees during the thinning stage. This allowed me to test whether intensity of competition, as assessed by initial density and density-dependent mortality, affects growth and mortality among individuals with different levels of heterozygosity. Allozyme analysis on surviving trees was used to relate heterozygosity to growth and mortality of these trees before and during the competitive thinning stage. Three questions were tested: 1) is heterozygosity positively correlated with growth rate, 2) can mortality due to intra-specific competition create changes in population genotype frequencies, and 3) does intense competition and high mortality select for individuals with higher levels of heterozygosity?

Methods

Study Site

The study site is located in the Durham Division of Duke Forest, an area of low, rolling hills in the eastern Piedmont of North Carolina. Permanent plots were established in 1933 to study the effects of different densities of loblolly pine stocking on harvestable timber. The twelve 0.1 acre subplots were established in a heterogeneous stand of eight-year-old pines (Figure 4-1) by Clarence Korstian as Duke Forest Permanent Sample Plots 12-23. Plots were remeasured by personnel for the Duke

School of Forestry in the employ of R.K. Peet and N.L. Christensen. The entire area encompasses approximately 4 acres. The site is on a south-facing slope, and soil conditions and moisture levels vary slightly across the site. Prevailing wind direction and location of the presumed parent stand (subsequently harvested) is noted on the figure. The subplots had a broad range of naturally seeded initial densities, but some were artificially thinned to further increase the range of densities. Height and diameter were measured for each tree in each subplot approximately every five years starting in 1933. Summary statistics, including initial density, ending density, number of trees sampled for allozyme analysis, and mean heights and diameters are shown in Table 4-1.

Originally, identification numbers were painted on trees with blaze paint, but in the early 1980's, field crews placed metal tags with ID numbers on each tree. Trees surviving in 1993 were located for sampling for allozyme analysis. Table 4-1 shows summary statistics for the population, including initial density, ending density (1993), number of trees sampled, and mean heights and diameters for 1933 and 1992. Survivorship ranged from 26 trees in plot 21 to 10 trees in plot 23.

The trees in the study plots were on average 30 meters tall, and lowest branches with needle tissue averaged 15 meters above the ground. Needle tissue samples were collected using a 20 gauge shot gun with #4 shot. The gun was pointed into the canopy of each tree from directly under the tree and fired. Small branchlets were collected as they dropped from the canopy. These branchlets were placed into ziploc bags marked with the tree and plot identification numbers. The bags were kept in the shade until all samples were collected in a given morning, and then were taken back to the lab where they were stored in a cold room at 4°C until they were prepared for electrophoresis. Samples were stored up to two weeks before processing.

Allozyme Analysis

Allelic and genotypic frequencies of surviving trees in each subplot were investigated. Twelve loci polymorphic in loblolly pine were resolved, although two of these were monomorphic in this population. Polymorphic loci used in the analysis were PGD, PGI1, PGI2, GDH, IDH, DIA1, FE, MDH2, GOT2, and SAD. Studies to establish that loci were not linked were not possible in this study, due to the absence of controlled crosses. Previous work suggests the loci are not linked (Bush and Smouse 1991, Roberds and Conkle 1984, Conkle 1981, and Adams and Joly 1980).

Statistical Analysis

To examine the relationship between heterozygosity and growth, number of heterozygous loci in each individual within each subplot was regressed against diameter at breast height (DBH) for each of the years in which data were collected for the stand. Due to the small sample sizes within each subplot, separate regressions yielded nonsignificant results. The model was analyzed as a split plot design with plot and number of heterozygous loci as class variables (SAS proc GLM):

$$D = S\ h\ S*h$$

where D is diameter at breast height, S is subplot, and h is the number of heterozygous loci. Type three sums of squares were used due to the unbalanced sample number in each plot and heterozygosity class. All analyses of variance were carried out using SAS 6.08 (SAS Institute Inc. 1990).

Initially I hoped to compare population level heterozygosity (H) between subplots, to determine whether initial density affected the degree of heterozygosity in the resulting stand. Although the study plots were started at different initial densities which might allow me to compare the effect of the level of competition on genetic

responses, the plots were less than optimal for several reasons. 1) The sample size in each subplot is too small. Survivorship varies from 10 to 26 individuals in each of the seven subplots. This number is insufficient to overcome the random artifacts associated with gene frequencies which would result in highly biased H statistics. 2) Parentage is unknown. This is a naturally-seeded stand resulting from open pollination in a mixed mating system. Differences in gene frequencies among subplots may stem from different maternal trees due to spatial heterogeneity, and whether the seed resulted from a selfed or outcrossed pollination. These problems are especially challenging in comparing small subsample sizes where results may be skewed if one subplot has an excess of inbred or outcrossed individuals, or one maternal tree had a rare genotype compared to the other subplots. 3) Initial density was patchy. Competitive responses of plants change according to proximity and number of neighbors, and although average stand density is given, the type of interactions each plant experienced, such as whether each had several years of no competition or grew in a competitive patch, are unknown. To try to get around these problems, I calculated H statistics (Zaykin and Pudovkin 1993) by pooling subplots into four overlapping categories: low density (subplots 12, 14, 15, 17), medium low density (14, 15, 17, 19), medium high density (15, 17, 19, 21), and high density (17, 19, 21, 23). Each pooled category encompassed an average of 70 individuals. Although it would be desireable to not have plot 17 shared by all categories, this was necessary to provide sufficient sample size. Mean H for a category was calculated by averaging the H statistics of the four subplots. This method increased sample size to a more appropriate level, decreased the spatial heterogeneity factor because plots were spread over a larger area, reduced the possible effect of a maternal tree with a rare genotype, and helped to smooth noise associated with mating system and presence or absence of competitive patches.

Hardy-Weinberg proportions were calculated (Zaykin and Pudovkin 1993) for

each enzyme to determine whether observed ratios fit the expectations of the model. Deviations from expectations in the direction of heterozygote excess could indicate preferential survival of heterozygotes. These calculations were only carried out for the population as a whole, because of small sample size of the individual subplots.

Results

The regression model produced significant results for heterozygosity during the early years of the study. From 1933 to 1938, number of heterozygous loci was significantly correlated with diameter (Table 4-2). Figure 4-2 shows a graph of the correlation for 1938, the strongest correlation, with each subplot shown individually. Although each subplot shows at least a slightly positive trend (Table 4-3), variations in slope between subplots are probably due to small sample sizes and are not indicative of competitive response. During the years of World War II, no data were collected. When measurements were resumed in 1946, the relationship was borderline significant ($p < .08$). After this time, heterozygosity was no longer correlated with diameter.

The fact that the correlation between heterozygosity and diameter is significant through the first part of the study stresses the robustness of the correlation. However, it is important to note that these separate analyses are not independent of one another. If the correlation which existed between DBH and heterozygosity for 1933 happened to be merely spurious, the correlation for 1935 would tend to be similar due to the fact that the same trees were measured. However, the combination of the consistent correlation through time and the obviously positive slopes of each of the subplot lines in Figure 4-2 suggest the results are real.

The model also shows the strong influence of plot density on diameter (Table 4-2). Figure 4-3 shows that as density increases, mean diameter decreases. The influence of density on diameter is obvious through time, as even at the end of the

study when each subplot reached a similar ending density, mean diameter was generally less in plots with high initial densities. This agrees with Lanner's (1985) findings of decreased diameter at high densities, although another possible explanation for this trend is that diameters are lower for the higher number subplots at the top of the slope where soil depth is lower.

Hardy-Weinberg analysis yielded significant deviations from the expected in four of the ten cases. Two of these cases, DIA and PGI1, appear to be due to the small sample size, as the significant deviations for these two loci depend on the presence or absence of just a few individuals. However, PGD and SAD show deviations in high frequency genotypes (Table 4-4). PGD shows an excess of heterozygotes and a corresponding paucity of homozygotes, while SAD is significant due to a paucity of heterozygotes. These deviations may still be due to the small sample size or they may be present due to preferential selection of genotypes. Since Hardy-Weinberg analysis did not yield overwhelming results in a consistent direction (homozygote or heterozygote excess) for both enzymes involved, it is difficult to interpret these results as significant.

Raw data for H statistics in each plot for each enzyme, as well as enzyme and plot means are given in Table 4-5. H statistics analyzed according to density category yielded interesting results. Figure 4-4 shows that at both the low and high density ends, heterozygosity (H) was lower than in the middle categories. The highest level of ending heterozygosity appears to be in the medium-low density category. The analysis of variance test, however, shows that the means are not significantly different from each other.

Discussion

As competition for a resource increases, a lower proportion of individuals will be successful at obtaining adequate amounts of it. Ledig et. al (1983), after observing

age-dependent correlations between heterozygosity and growth rate, suggested that the differential abilities of plants may be evident only under competitive conditions. However, Thomas and Bazzaz (1993) found that genotype explained much more variance in size when clonal individuals of the herb *Polygonum pensylvanicum* were not competing than when they were grown at high densities. Plants grown individually in like conditions were able to express genetically determined growth rates. When plants were grown at high densities, initial differences in germination time, suitability of microsite, and stochastic processes had a greater effect on plant performance than genetic make-up. This pattern may apply also to forest trees, and may account for whether or not genetic heterozygosity is correlated with fitness traits.

In loblolly pine stands, competition intensity increases as a cohort of seedlings grows, leading to the thinning stage where mortality of suppressed individuals takes place (Knox et. al 1989). Early in stand development, when trees are small, asymmetric competition for light is not intense. In patches where several trees are growing, competition may be quite intense, but because conditions are patchy with some trees growing alone, the mean level of competition is lower than later on when trees become larger and crowns overlap. As the crowns enlarge, the trees require more resources and capture increasing light, causing competitive interactions to increase. The results indicate that early on in stand development, diameter was correlated with heterozygosity. During the early stages of growth, when competition for light was less intense, the trees were able to grow at their own inherent growth rates, which are prompted by the level of genetic heterozygosity. Lanner (1985) found that tree diameter growth was severely reduced by intense competition. The implication here is that after the onset of intense asymmetric competition, the effect of heterozygosity was no longer significant to a tree's diameter growth. Once competitive interactions began, the influence of genotype on diameter growth ceased to have an effect. This is evidence that competition can alter the effect of genotype on individual growth.

It could be predicted that the longer a tree goes without competitive influences, the more its genetic make-up will influence its growth and morphology. As interactions with other plants begin, the competitive conditions alter genetic performance. Hamrick, Platt, and Hessing (1992), found that heterozygosity was higher in mature stands of longleaf pine than in juvenile stands. Longleaf pine grows in especially patchy conditions and during the "tussock grass" stage does not compete with neighbors for light. Growth without competition during the early years could predispose individuals in a cohort with higher genetic growth rates to an advantage from avoiding competition-induced alterations in morphology and growth.

For loblolly pine, growth without competition, as may be found in an initially low-density stand, would allow genetic differences between individuals to influence the outcome of the self-thinning stage. This early respite from competition could result in higher success of more heterozygous individuals later on as competitive processes commence during the self-thinning stage. Weiner and Fishman (1994) found that height was most important in determining competitive success, but it should not be interpreted as contradictory that height is not correlated with heterozygosity throughout the years in this study, as the dead trees are missing from the allozyme analysis. It is, therefore, impossible to determine whether heterozygosity among those trees was lower. An early advantage could predispose heterozygous individuals to higher growth rates later on as competition for light ensues, if they have a height advantage when crowns begin to overlap. As self-thinning begins, if the more heterozygous individuals have a height advantage, they will have a higher probability of survival, because due to asymmetric competition for light, taller individuals suffer less mortality. This could account for the higher levels of heterozygosity in mature vs. juvenile natural stands reported by other investigators (Hamrick, Platt, and Hessing 1992; Brotschol et al. 1986; Farris and Mitton 1984).

Density analysis by category showed the nonsignificant trend that heterozygosi-

ty is lower at both the low and the high end of the density gradient. This could indicate that the level of stand heterozygosity can be altered by differences in initial density and therefore the amount of competition a stand undergoes during development. At the low end of the density gradient, lower mortality results in a higher proportion of the original members dominating the stand. Inbred individuals, which are often less vigorous than outcrossed individuals, have a higher probability of survival under the reduced competition, yielding a lower heterozygosity (H) value. At the high end of the density gradient, severe competition results in high mortality. Stochastic influences early on, such as microsite quality and germination time have a greater effect on growth, so that height at an early age is random with respect to genotype. If the plants begin to compete early, genotype plays a small role. In the medium range, especially at the medium-low end, the lack of competitive interactions during early growth allowed trees to overcome stochastic effects such as germination time and resource patchiness, and grow at inherently-determined growth rates. Once competition began at the medium low range, heterozygous individuals already gained a foothold and were able to better outcompete the more homozygous individuals.

Ledig et al. (1983), in presenting age-dependent correlations in *Pinus rigida*, predicted that different genetic abilities of trees would only be expressed under highly competitive conditions. I argue that the absence of competition allows genetic differences in growth to be expressed and measurable. This study suggests that a period of low competition is needed to establish genetic differences between individuals. Asymmetric competition for light occurring after this initial phase may magnify genetically-determined morphological differences leading to higher levels of heterozygosity once the stand matures.

Table 4-1. Duke Forest Plot Statistics.
Mean heights and Diameters for both 1992 and 1933 are based on individuals surviving to ending density, 1993.

	Plot 12	Plot 14	Plot 15	Plot 17	Plot 19	Plot 21	Plot 23
Initial Density (#/0.1 acre)	25	51	79	149	236	431	1173
Ending Density (#/0.1 acre)	16	17	19	19	15	26	10
# Trees Sampled Allozymes	16	17	19	19	15	26	10
Mean Ht & SD 1933 (Ft.)	4.1 0.5	4.5 0.9	5.0 0.5	4.6 0.3	4.8 0.4	4.6 0.4	4.1 0.4
Mean Ht & SD 1992 (Ft.)	33.4 2.4	29.4 2.1	32.9 2.8	29.4 3.0	29.0 5.3	27.6 4.5	27.5 2.5
Mean DBH & SD 1933 (Inches)	7.2 1.0	7.9 2.2	7.6 1.2	6.5 0.8	6.6 1.3	5.9 1.0	4.0 0.8
Mean DBH & SD 1992 (Inches)	38.1 7.4	30.8 6.0	33.1 6.3	28.7 7.0	29.6 8.3	24.7 7.8	29.9 5.8

Table 4-2. Analysis of Variance: DBH = Plot Heterozygosity

Type III Sums of Squares
Dependent Variable = Diameter at Breast Height

Year	p (PLOT)	p (Heterozy.)	slope	R^2	p
1933	0.0001	0.04	0.18	0.48	0.0001
1935	0.0001	0.02	0.26	0.68	0.0001
1938	0.0001	0.02	0.36	0.75	0.0001
1946	0.0001	0.07	0.37	0.75	0.0001
1950	0.0001	0.26	0.27	0.70	0.0001
1955	0.0001	0.45	0.20	0.68	0.0001
1959	0.0001	0.70	0.11	0.65	0.0001
1966	0.0004	0.97	-0.01	0.58	0.0001
1977	0.02	0.68	-0.18	0.43	0.0001
1984	0.04	0.72	-0.18	0.35	0.0001
1988	0.08	0.70	-0.20	0.32	0.0001
1992	0.18	0.47	-0.40	0.28	0.0005

Table 4-3. Intercepts and Slopes for Heterozygosity vs. Diameter in 1938

Plot #	Intercept	Slope
12	17.70	0.49
14	16.15	0.16
15	13.51	0.63
17	12.20	0.18
19	10.49	0.59
21	9.62	0.32
23	7.50	0.15

Table 4-4. Hardy-Weinberg Analysis for PGD and SAD

PGD Genotype	obs	exp	X^2
33	49	55.8	0.8
34	58	46.0	3.1
35	9	7.4	0.3
44	4	9.5	3.1
45	2	3.1	0.4
55	0	0.2	0.2
			8.1 > 7.81

SAD Genotype	obs	exp	X^2
22	14	7.6	5.3
24	33	45.7	3.5
44	75	68.6	0.6
			9.47 > 3.84

Table 4-5. Population-level Heterozygosity (H) Statistics for Plots and Enzymes

Enzyme	Plot 12	Plot 14	Plot 15	Plot 17	Plot 19	Plot 21	Plot 23	Mean
DIA	0	.06	.05	.05	0	.04	0	.03
FE	.37	.17	.41	.16	.53	.38	.20	.32
GDH	.31	.47	0	.26	.47	.42	0	.32
GOT2	.31	.53	.74	.21	.40	.46	.30	.43
IDH	.06	.06	.06	.05	0	.11	0	.06
MDH2	0	.12	.05	.05	.07	0	.10	.05
PGD	.56	.65	.58	.63	.60	.46	.50	.57
PGI1	.12	0	.06	.05	.07	.08	0	.06
PGI2	.50	.65	.53	.53	.67	.65	.60	.59
SAD	.31	.41	.16	.26	.27	.23	.30	.27
Mean	.209	.313	.264	.225	.308	.283	.200	.257

Figure 4-1. Loblolly pine subplots in Duke Forest. The plot slopes from north, northwest to south, southeast. Subplots measure 20.1 m on a side. Scale is approximate.

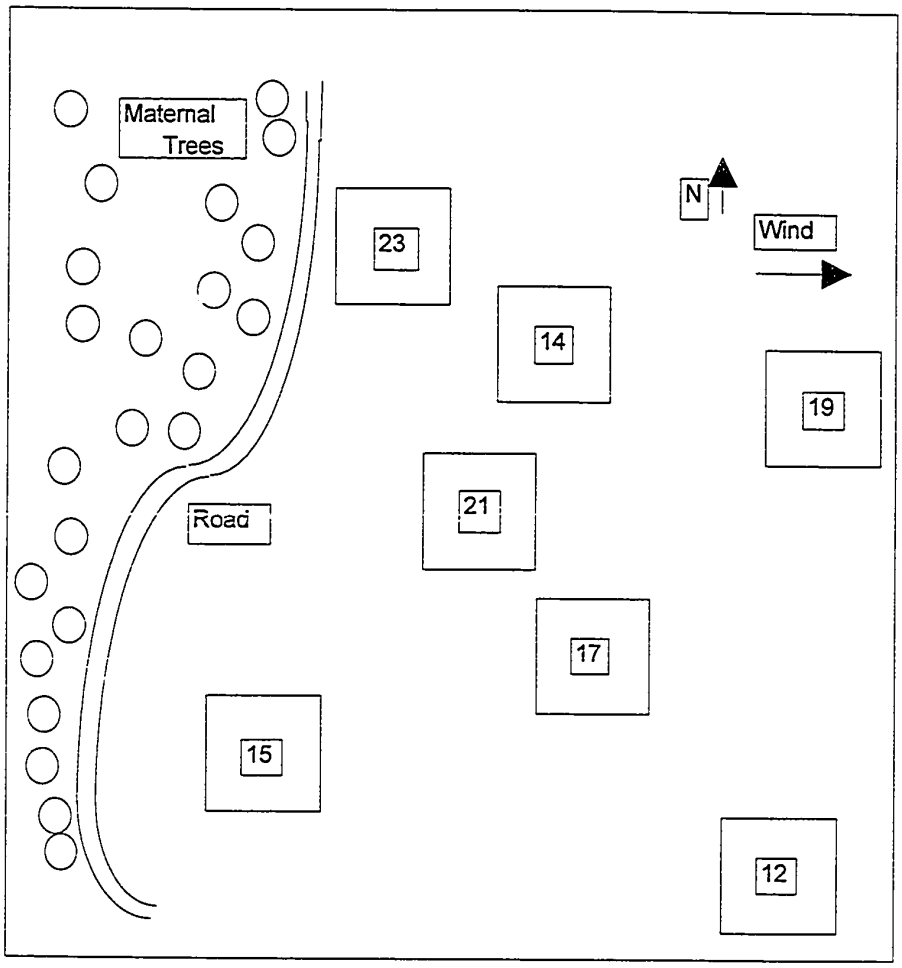

Maternal Trees
N
Wind
23
14
19
21
Road
17
15
12

Figure 4-2. Relationship between the number of heterozygous loci and diameter at breast height (DBH) in 1938.

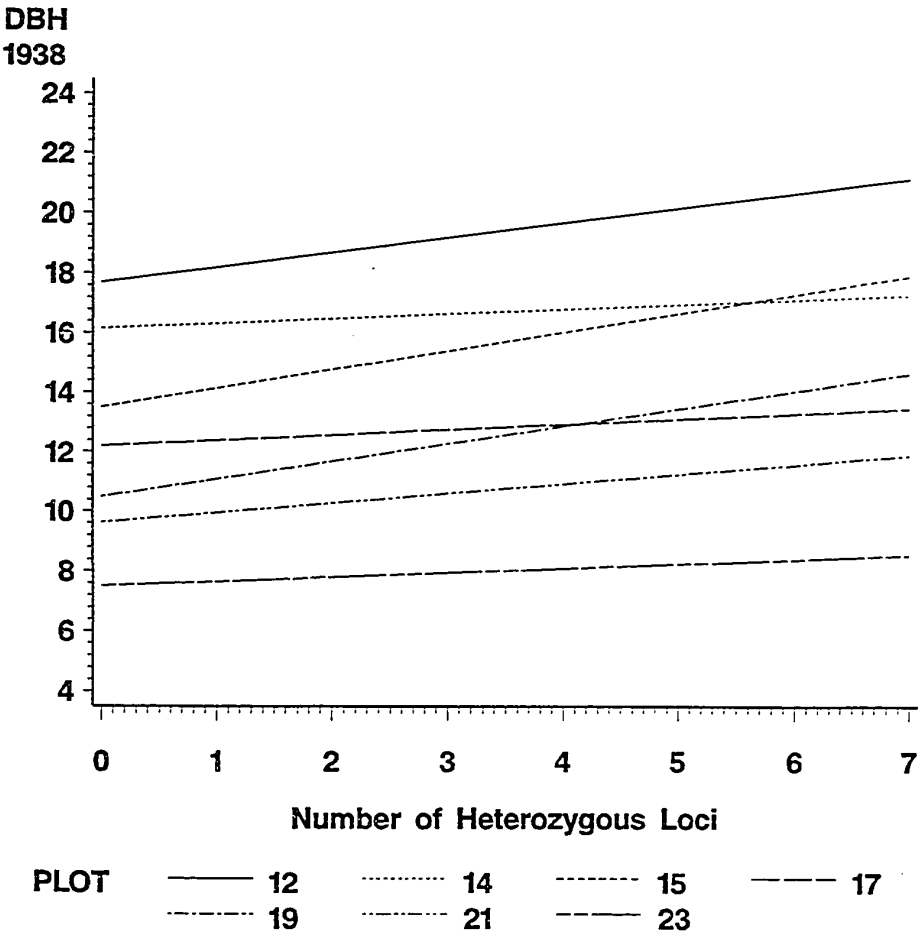

DBH
1938
24
22
20
18
16
14
12
10
8
6
4
0
1
2
3
4
5
6
7
Number of Heterozygous Loci
PLOT
12
14
15
17
19
21
23

Figure 4-3. Relationship between initial density and diameter at breast height (DBH) over time. Subplot numbers and initial densities are as follows: plot 12 (25 trees in 1933), plot 14 (51), plot 15 (79), plot 17 (146), plot 19 (222), plot 21 (427), and plot 23 (1168).

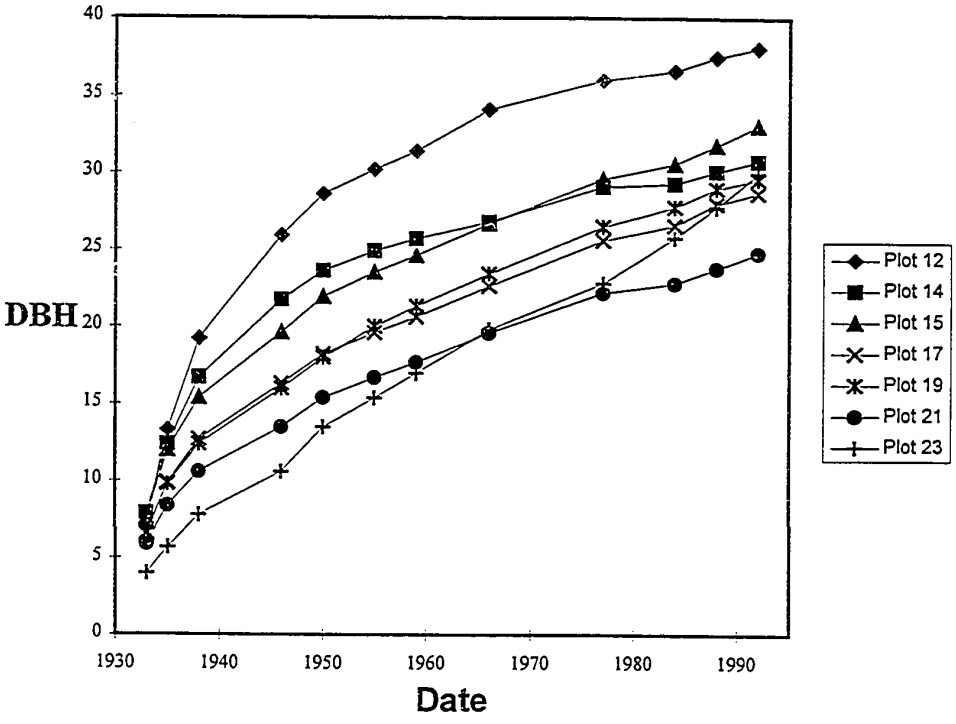
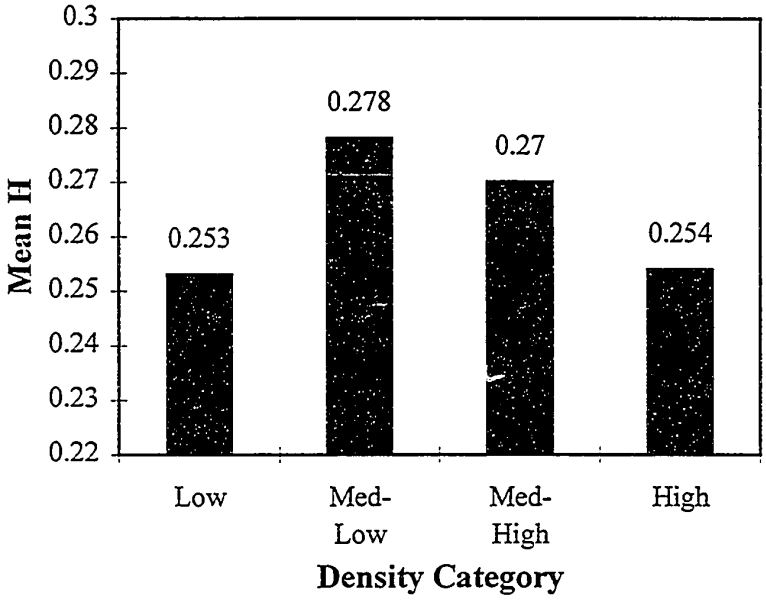

40
35
30
25
20
15
10
5
0
DBH
1930
1940
1950
1960
1970
1980
1990
Date
Plot 12
Plot 14
Plot 15
Plot 17
Plot 19
Plot 21
Plot 23

Figure 4-4. Ending stand H (population-level heterozygosity) statistics for each of the density classes. Classes are as follows: Low (plots 12, 14, 15, 17), Medium-Low (plots 14, 15, 17, 19), Medium-High (plots 15, 17, 19, 21), and High (plots 17, 19, 21, 23).

p=ns


0.3
0.29
0.28
0.27
0.26
0.25
0.24
0.23
0.22
Mean H
0.253
0.278
0.27
0.254
Low
Med-Low
Med-High
High
Density Category

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