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EFFECTS OF THINNING REGIMES ON GENETIC VARIATION OF WHITE OAK (*QUERCUS ALBA* L.) IN UPLAND OAK FORESTS OF EASTERN KENTUCKY

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EFFECTS OF THINNING REGIMES ON GENETIC VARIATION AND RELATEDNESS OF WHITE OAK (*QUERCUS ALBA* L.) IN UPLAND OAK FORESTS OF EASTERN KENTUCKY

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in Forest and Natural Resource Science
in the College of Agriculture, Food and Environment
at the University of Kentucky

By

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2020

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ABSTRACT OF THESIS

EFFECTS OF THINNING REGIMES ON GENETIC VARIATION AND RELATEDNESS OF WHITE OAK (*QUERCUS ALBA* L.) IN UPLAND OAK FORESTS OF EASTERN KENTUCKY

This research assesses the effects of silvicultural thinnings, varying in residual basal area and number of thinnings, on genetic variation and relatedness of white oak (*Quercus alba* L.). The objectives were to (1) develop microsatellite DNA markers (simple sequence repeats, SSRs) for assessing the genetic variation of white oak and (2) determine if thinnings significantly change the degree of diversity and relatedness in white oak stands. For objective 1, 84 primer pairs from previously predicted genomic SSRs of white oak were evaluated for consistent PCR amplification and polymorphism. Twenty of the 84 primer pairs amplified consistently and were confirmed to be polymorphic. For objective 2, 16 markers were amplified across 225 white oak trees sampled from seven half-acre plots from a thinning experiment on the Daniel Boone National Forest. Measurements of heterozygosity, total and effective allele numbers, deviation from Hardy-Weinberg equilibrium, and pair-wise genetic relatedness were calculated using GenAlEx 6.5. Analysis suggests that thinning decreases observed heterozygosity relative to expected and decreases relatedness between trees in thinned treatment plots compared to plots that were not thinned. Consideration of genetic effects should be made when thinning white oak stands to maintain genetic diversity while enhancing reproductive potential and future stand establishment.

KEYWORDS: *Quercus alba* L., Silvicultural Thinning, Microsatellite Markers, Genetic Diversity, Forest Management, Artificial Selection

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05/05/2020

Date

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DEDICATION

To my mother, father and most importantly, the Creator. Without whom, none would be possible.

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CHAPTER 1. LITERATURE REVIEW

1.1 Executive Summary

Artificial selection due to management practices and industry demands can produce significant effects on the characteristics and extent of genetic diversity in natural tree populations. White oak (*Quercus alba* L.), a keystone species of economic and ecological importance to eastern hardwood forests, is potentially subject to genetic degradation due to specific selection of timber-type phenotypes by the forest industry. This research project assesses the effects of silvicultural thinnings, varying in intensity and timing, on the genetic diversity and genetic relatedness of white oak (*Quercus alba* L.) utilizing microsatellite DNA analysis, specifically genomic simple sequence repeat (gSSR) markers. Our research objectives are to (1) test and validate microsatellite markers (genomic SSRs) for assessing diversity of natural and managed *Quercus alba* forest plots and to (2) determine if silvicultural thinnings significantly change the degree of heterozygosity and genetic relatedness in white oak stands, leading to a change in population diversity. For objective 1, 84 formerly predicted genomic gSSR (gSSRs) primer pairs of white oak were evaluated for consistent PCR amplification. Twenty of the primer pairs were identified as potentially good markers and 16 of these were utilized in the objective 2 study. For objective 2, existing long-term thinning experiments of a white oak dominated study site established by the USDA Forest Service, in 1961 on the Daniel Boone National Forest (Jackson County, KY, USA), provided seven half-acre plots varying in post thinning basal area and number of subsequent thinnings. Foliage tissue samples were collected for DNA extractions and gSSR marker analysis from all mature white oaks within the study plots for an assessment of genetic variation. Measurements in heterozygosity (observed and expected), total and effective allele numbers, deviation from Hardy-Weinberg Equilibrium and pairwise genetic relatedness, were calculated using GenAlEx 6.5 for 12 SSR markers reliably scored on 225 white oak sample trees. Our analysis suggests that thinning influenced some of the markers, primarily in decreasing observed heterozygosity relative to the expected in the thinned stands. In addition, we found a general reduction in genetic relatedness occurring in thinned plots,

especially for the higher thinning intensity plots. Consideration of genetic effects should be made when thinning white oak stands to maintain genetic diversity while enhancing reproductive success and future stand establishment.

1.2 Introduction

Environmental stressors (biotic or abiotic) impose natural selection pressures that lead to changes in the genetic composition of species, for adaptation to their environment, over time (McKinney *et al.*, 2014). In contrast, artificial selection is the intentional selection by humans of desired traits in a population. Due to its high demand in timber markets, white oak (*Quercus alba* L.) has been subjected to many artificial selection pressures, such as forest stand thinnings and logging (Abrams, 2003). In recent years, a significant drop in white oak regeneration has been noted in the Eastern and Central Hardwood Forest Region, leading to efforts to identify the underlying reasons for this decline and to develop management strategies to reverse this trend and preserve the species diversity (Dey, 2014). As with many species, erosion of genetic diversity over time can lead to population crashes and problems with sustainability (Aldrich *et al.*, 2005). Considering the high value of white oak both economically and as a key forest tree species, we were interested in assessing whether logging and conventional silvicultural practices had impacted the population's genetic diversity. The objectives of our project were to (1) identify a set of microsatellite markers (gSSRs) that exhibit sufficient allelic variation to enable population diversity assessment in white oak stands and 2) employ these markers to determine if silvicultural thinning in a subset of these stands has significantly impacted the degree of heterozygosity and genetic relatedness at these marker loci. To situate our research, we discuss the literature for population genetics and genomics in a forest genetics context, then survey and synthesize the current literature of population genetics and genomics research on *Quercus alba*. Finally, we review and discuss studies looking at the impact of forest management practice on the diversity of forest tree populations and the implications of these findings with respect to our objectives.

1.2.1 The Importance of Genetic Diversity

Genetic diversity, the genetic variation within a species, is the substrate for evolution and adaptations of species driven by environmental forces, which prevents population size declines and local extinctions (Keller and Waller, 2002; Ratnam *et al.*, 2014). Factors such as natural selection, gene flow and genetic drift jointly affect the genetic diversity of populations, while also impacting local and range-wide adaptation. Trees, being some of the most genetically diverse organisms, adapt to their local environments creating a mosaic of intraspecies diversity across their ranges (Ratnam *et al.*, 2014). This difference of local adaptations for survival creates a montage of varying degrees for key fitness-related traits such as resistance to climate change, diseases and pests (Savolainen *et al.*, 2007). Ultimately, this collective genetic resilience becomes critical for the long-term survival of natural tree populations and species. With each forest tree species possessing different forest genetic resources and genetic variation within species ranges, it becomes increasingly important to assess the genetics of ecologically and economically valuable species for long term management. It has been shown that forest trees adapt locally to photoperiod, water availability, pathogens, pests and temperature for optimized survival (Holliday *et al.*, 2017). As an example, recent studies have assessed how trees will be able to respond to an increase in local droughts as global temperatures increase and the distribution of rainfall is altered (Holliday *et al.*, 2017). Specifically, drought resistance and adaptation are characterized by certain phenotypic traits such as leaf thickness, root morphology and leaf shedding (Polle *et al.*, 2019). This means that local adaptation for traits associated with drought resistance adds to the collective diversity and resilience of the species. Other studies have proven that deforestation, forest degradation and forest fragmentation cause a reduction in genetic variation within and among populations of tropical forest trees (Bawa and Dayanandan, 1998). These factors are responsible for causing the extinction of genetically unique populations, increasing inbreeding and inhibiting gene flow of forest trees. Maintaining forest genetic resources has become increasingly important as we combat and mitigate the effects of future threats to our wild tree populations (Schueler *et al.*, 2013). Arguably, assessing local adaptation and genetic variability becomes important for documenting and preserving the collective forest genetic resources of a species across its range.

1.2.2 Natural and Artificial Selection

Natural selection is a process that results in an organism better adapting to its environment through the selection of traits that increase survival and reproductive success, creating changes in its genotype over generations. In contrast, artificial selection is a process by which humans choose to selectively breed or perpetuate only those individuals with desirable inheritable characteristics (Sterrett, 2002). It is a widely held belief that natural selection plays a critical role in how forest trees adapt to their local and range-wide environments (Smouse and Sork, 2004; Sork *et al.*, 2013). For example, a study conducted in Chile on three forest tree species identified a continuum of water use strategies explaining species distribution along a small-scale, 0.1 to 36 ha moisture gradient (Negret *et al.*, 2013). This study suggested that natural selection shaped how tree species adapted to moisture availability, causing intraspecies partitioning matching the moisture gradient on a small scale and species niche specialization. However, it is unclear how artificial selection influences the genetic resources of forest trees. Researchers have been able to assess the effects of natural and artificial selection on the genetic structure of some forest tree species. It has been hypothesized that artificial selection, when mimicking natural processes, can have a positive effect on genetic variation within forest tree populations (Koskela *et al.*, 2012). However, when artificial selection does not mimic natural processes and selects continuously for the most highly economically valued individuals (i.e., high-grading), it may lead to a severe reduction in genetic variation within and among populations (Victoria L Sork and Smouse, 2006; Koskela *et al.*, 2012; Soliani *et al.*, 2016). In a later section, we will examine studies like ours, that test the effects that forest management and silvicultural practices on the genetic diversity of forest trees.

1.2.3 Gene Flow

The movement of genetic material through mechanisms such as pollen flow (i.e. migration) from one population to another is called gene flow. In *Quercus,* gene flow occurs via wind-driven pollen dispersal. Pollen flow, especially in wind-pollinated forest tree species, is often thought to be responsible for homogenization, distribution, and maintenance of genetic variation among populations (Valbuena-Carabaña *et al.*, 2005).

Conventionally, the effective dispersal of pollen via wind was thought to maintain the genetic connectivity between populations of forest trees (Valbuena-Carabaña *et al.*, 2005; Pluess *et al.*, 2009). In specific, white oak traditionally was modeled to have a large pollen dispersal distance (Ducousso *et al.*, 1993) but more recently, white oak's pollen movement was modeled to be more restricted than previously thought (Smouse *et al.*, 2001; Dyer *et al.*, 2004). Based on a study of *Quercus alba* in the Missouri Ozarks, researchers found that the effective pollen dispersal must occur within 16.77 meters (55.02 feet) on average of a mature masting female, with an idealized number of 18-20 pollen donors to maintain genetic diversity in the population (Smouse *et al.*, 2001). It has been hypothesized that gene flow could have a negative homogenizing effect when a low diversity gene pool is substantially spread across multiple populations (Dyer *et al.*, 2004). As a result, forest managers are concerned about the effects of limited gene pools spreading into natural populations of forest trees from plantations originating from a single seed stock or severely isolated wild populations from habitat fragmentation (Larcombe *et al.*, 2013). Studies have shown that pollen flow among populations, specifically long-distance pollen flow, is not enough to mitigate a loss of genetic diversity with added geographic barriers, forest fragmentation and heavy selection management practices.

Studies have shown that the decay rate of gene flow within tree species, or the pollen flow rate, creates isolation of genes and alleles by distance, thus creating genetic structure among and within the species range (Sork, 2016). A study, based on data from white oak populations in the Missouri Ozarks, modeled that white oak demonstrated significant genetic variation in association with the environmental heterogeneity on a smaller scale. The effective pollen dispersal area was found to be 0.0884 ha (0.25 ac) even though some pollen does travel larger distances. This small effective pollen dispersal creates isolation and a heterogeneous landscape of many subpopulations with their own unique genetic structure. It was also suggested that pollen flow among the populations was not enough to homogenize the genetic variation in the face of artificial selection (Smouse and Sork, 2004). Further, a study in northeastern Illinois found that there was a significant difference between white oak allele frequencies in populations, suggesting that spatial barriers do affect this wind pollinated species (Craft and Ashley,

2006). Moreover, it has been shown that long-distance pollen dispersal is not enough to counteract a strong impact of selection pressures (Sork, 2016). This led researchers to hypothesize, that both the strength of the selection pressure and the presence of a fine-scale genetic structure counter the homogenizing effects of gene flow. These studies countered earlier research that wind-pollinated species promote extensive gene flow among and between populations of forest trees.

1.2.4 Genetic Drift

Genetic drift is the chance disappearance of certain genotypes in populations resulting from loss of alleles in individuals due to death or inability to produce offspring for the next generation. Just like natural selection and gene flow, genetic drift is a naturally occurring phenomenon in wild populations. Due to the extensive gene flow of many forest trees, resulting from highly outcrossed populations in the absence of geographic barriers and lack of highly heterogeneous landscapes, genetic drift plays a much smaller role in forest tree population genetics (Sork, 2016). The exception to this generalization only occurs in small or extremely isolated populations of forest trees (Thomas, 1988). However, increasingly high amounts of deforestation and habitat fragmentation are raising the number of small and isolated forest tree populations (Young *et al.*, 1996; Dobeš *et al.*, 2017). Isolation and fragmentation of forest tree populations can make them more vulnerable to the loss of genetic variation through limited pollen flow (Pluess *et al.*, 2009). In addition, limited pollen flow could lead to a loss of fitness through genetic drift and inbreeding in small, isolated populations and possibly local extinctions (Thomas, 1988). As mentioned previously, the white oak pollen movement and dispersal is much less than originally hypothesized by researchers. Thus, the distance restricted gene flow of white oak could lead to more isolated, less genetically variable populations in the face of deforestation, urbanization, and habitat fragmentation. Ultimately, white oak and other forest tree populations could be more sensitive to the effects of genetic drift and inbreeding because of anthropogenic influences.

1.3 Forest Trees as Model Systems

Natural tree populations provide some degree of livelihood for an estimated 1.6 billion people worldwide and constitute 82 percent of the continental biomass (Neale and Kremer, 2011). A combination of population genetics fundamental theories, genomic tools, and landscape ecology is necessary to effectively assess the genetic variability of forest trees across landscapes and species ranges. Genetics and genomics research of forest trees has been hindered due to their long-life cycles, large genomes and limited funding for research (White *et al.*, 2007; Neale and Kremer, 2011). Recently it has been argued that forest trees do serve as a prime candidate for research on the relationship between phenotypic and genotypic diversity due to their large natural populations, outbreeding, domesticated and undomesticated populations, and large infrastructures for phenotyping (Ingvarsson and Street, 2011). Traditionally, natural genetic variation of forest trees is studied using the common garden approach, which estimates genetic variation based on the measurement of phenotypic variation (Kramer *et al.*, 2008). Common gardens are easily replicated within diverse environments and, as a result, population geneticists can estimate genotypes based on environmental and quantitative variables (Kramer *et al.*, 2008). This method of study, however, has limitations as individual genes that underlie complex adaptive traits in most organisms cannot be studied (Cappa *et al.*, 2013). In recent decades, microsatellites or simple sequence repeats (SSRs) have become prevalent molecular markers for describing genetic variation and genetic structure for a wide variety of organisms (Tereba *et al.*, 2017; Tsykun *et al.*, 2017). Microsatellites are repeated nucleotide sequences of DNA, located at a specific locus. Analysis using microsatellites involves polymerase chain reaction (PCR), amplification of the repeating DNA, using fluorescently died labels and primer pairs. SSRs are often used for genotyping plant species because they are multi-allele codominant markers, and they are easily experimentally reproducible (Vieira *et al.*, 2016). It has been noted that microsatellite markers are especially helpful in studying gene flow; recombination rates, evolution, and linking phenotypic with genotypic variation (Agarwal *et al.*, 2008).

The development and use of genetic markers for studying natural genetic variation in forest tree populations allow researchers to characterize genetic drift and

migration. However, depending on the marker type, their use in assessing complex adaptive traits is limited (Ingvarsson and Street, 2011). Complex trait dissection was first tackled utilizing quantitative trait locus (QTL) mapping but due to the low resolution of QTL maps, essential underlying genes for complex traits could not be identified (Sewell *et al.*, 2000; Neale and Ingvarsson, 2008). However, the use of single nucleotide polymorphisms (SNPs) and association genetics provided the greater map resolution and methods necessary to identify genes underlying complex traits (Neale and Ingvarsson, 2008; Grattapaglia and Resende, 2009; Ingvarsson and Street, 2011). The abundant nucleotide diversity in forest tree genomes makes them ideal candidates for association genetics studies because association mapping requires a high density of SNPs in the populations being studied (Ingvarsson and Street, 2011). It is the current hope of researchers that large numbers of SNPs across genomes, paired with new genome sequencing tools will allow them to describe geographic patterns of adaptive genetic variation in natural populations (Sork *et al.*, 2013). Contemporary forest managers and researchers hypothesize that new genomic and genetic discoveries in forest tree research will ultimately lead the way in the global enhancement of forest health (White *et al.*, 2007).

1.4 Impacts of Forest Management Practices on Tree Genetics

Forest management practices are often implemented to optimize sustainable production of timber and less so for non-timber forest resources (Ledig, 1988; Ratnam *et al.*, 2014). Silvicultural management practices (e.g., stand thinning) as well as exploitive, non-silvicultural activities like high grading are regularly the result of societal demands, public input, and fluctuating markets to create the highest output of resources. Although forest management practices have influenced genetic diversity of forest trees throughout the interaction of humans and forests, little is known about the effects of these practices on past or present populations. As in ancient times, the genotypic structure of natural populations of forest trees is often unintentionally altered by forest managers (Savolainen and Kärkkäinen, 1992). However, it is hypothesized that, due to their substantial genetic resources, diversity of many forest species may not be affected significantly by management activities except for in rare, low genetically diverse species (Kramer *et al.*,

2008). In order to address this, we summarize studies focused on assessing the genetic effects of silvicultural thinning and the selective removal of trees on natural populations.

The silvicultural practice of thinning has the potential to modify the genetic structure of forest tree populations due to the selective removal of trees with a certain phenotype and an overall reduction in population sizes (Finkeldey and Ziehe, 2004). Specifically, management regimes that modify the tree density, or trees per acre, and the size or age class structure of forest stands, at multiple occurrences during the stands rotation, have the potential impact genetic diversity of natural populations (Ratnam *et al.*, 2014).

Studies on thinning of forest trees, utilizing genetic markers, have been somewhat contradictory. Earlier studies on Norway spruce and European beech (*Fagus sylvatica* L.) found that management practices did not alter the allele and genotype frequencies (Hosius *et al.*, 2006). A more recent study on the effects of low versus high thinning level on beech in Europe also found no effect on the genetic structure (Buiteveld *et al.*, 2007). Similarly, a study on Northern red oak (*Quercus rubra* L.) in eastern deciduous forests of North America found that there was no loss of allelic richness between managed and unmanaged stands, attributing the lack of genetic change to the plasticity of the species (Grant, 2010). Also, a study on the effects of selective logging of best trees on lenga beech (*Nothofagus pumilio* L.) in Argentina found no significant difference in genetic diversity between managed and natural populations (Soliani *et al.*, 2016). Likewise, a research project in Australia found no significant difference in allelic richness, effective number of alleles or expected heterozygosity between management treatments of silvertop ash (*Eucalyptus sieberi* L.) (Glaubitz *et al.*, 2003). On the other hand, a study in Southern China on Diao Luo Shan Qing Gang (*Quercus tiaoloshanica* L.), a *Quercus* species with a relatively narrow range, found that a significant drop in alleles per locus occurred with selective logging (Zheng *et al.*, 2005). In Brazil, a study on the effects post-logging on the genetic diversity of big-leaf mahogany (*Swietenia* macrophylla L.) found a significant reduction in the number of alleles, observed heterozygosity, and distinct multilocus genotype number between post-logged sites and natural populations (André *et al.*, 2008). Another study in Brazil assessing the effects of reduced impact logging on West-Indian locust (*Hymanaea courbaril* L.) reported a reduction in the

distance of the spatial genetic structure in the reproductive population from about 800 to 200 m, reducing the gene pool of the populations (Biscaia De Lacerda *et al.*, 2008). Collectively, all the previous studies stressed the importance of multigenerational studies to fully evaluate the impact of logging and silviculture practices overtime. The long rotation cycle of forest trees makes it difficult to study the long-term effects of silvicultural regimes on the genetic structure of wild tree populations.

The current lack of research on the impacts of harvesting (including both silvicultural treatments and exploitive high-grading activities) on genetic structure is prevalent across global forestry practices, but even more so for populations in eastern deciduous forests of North America. Thus, to evaluate the long-term impact of logging practices on genetic diversity of forest trees, it is critical to utilize areas of high management impact, affected over long periods of time, with comparable unmanaged reference forest tree populations. This becomes especially important for timber species with high demands and artificial selection pressures, such as white oak. To date, there are no studies on the effects that forest management practices have on the genetic diversity and structure of *Quercus alba* populations.

1.5 *Quercus alba* Genome Sequencing

It is the goal of recent projects, such as the *Fagaceae* Genome and the Hardwood Genomics projects, to provide genomic resources for members of the *Fagaceae* family. Currently, the provision of genetic and physical maps, transcriptomic data, functioning analyses, and data mining tools are at the forefront of forest tree genomics research. The Fagaceae Genome project focuses on eight organisms within the *Fagaceae*, white oak included. To date for white oak, 203,206 expressed sequence tags (ESTs), that are short sub-sequences of complementary DNA sequences, are available that comprise 41,453 over lapping sequences that collectively represent continuous regions of DNA (contigs). In addition, a white oak genomic DNA resource is available that contains 4,350 predicted microsatellites with primer pairs designed for 84 of those that are predicted to make informative SSR markers. As stated previously, there is a shortage of genomics-based research for forest trees (Neale and Kremer, 2011), including white oak. More research is needed in the study of association genetics coupled with utilizing new sequencing tools,

to begin to understand the complex adaptive traits of forest trees. Current research on complex adaptive traits of the *Fagaceae*, have barely begun to understand species-specific characteristics (Staton *et al.*, 2015). An example of the application of genomics and association genetics in oaks is a study comparing the quantitative trait loci for adaptive traits between oak and chestnut. Specifically, the researchers found that variation of bud burst times for members of the *Quercus* genera were under high levels of natural selection, while height growth was not under genetic reference (Casasoli *et al.*, 2006). Studies such as these for the *Quercus* genera, among all forest tree genera, are expected to increase in occurrence over the next decade (Neale and Kremer, 2011).

1.6 Summary and Conclusions

Forest trees offer several ecological services and support extensive markets worldwide (Muller *et al.*, 2018). Historically, the long-life spans and large genomes of forest trees made genetics and genomics research difficult (Neale and Kremer, 2011). Now, measuring genetic diversity in tree populations is much easier with genetic markers (e.g., SSR markers) being developed that are highly informative codominant, multi-allele and easily reproducible markers. Genetic diversity in forest tree populations is responsible for adaptation to local climates and microclimates, shifting climates, diseases, pests, pathogens and collectively optimizes survival. Processes such as natural selection, gene flow and genetic drift drive and protect the forest genetic resources of a species. Although, when populations of forest trees become isolated, fragmented and experience high levels of artificial selection, natural processes are not enough to maintain genetic diversity over time.

Studies have reported mixed results on whether forest management practices and silvicultural regimes affect the genetic diversity of forest tree populations. For example, studies on Norway spruce, European beech, northern red oak, lenga beech and silvertop ash reported no significant change to genetic diversity (Glaubitz *et al.*, 2003; Hosius *et al.*, 2006; Buiteveld *et al.*, 2007; Grant, 2010; Soliani *et al.*, 2016). While studies on other species such as Diao Luo Shan Qing Gang, bi-leaf mahogany and West-Indian locust, species that are subject to heavy management practices, forest fragmentation and

isolation, did report losses in genetic diversity of these forest tree species (Zheng *et al.*, 2005; André *et al.*, 2008; Biscaia De Lacerda *et al.*, 2008). It is the consensus of forest researchers and managers, that understanding the long-term genetic effects of management practices and the compounding effects of a changing climate, should get full consideration when developing strategies for the conservation and preservation of our natural forest tree populations (Savolainen and Kärkkäinen, 1992). Forest genetic resources need to be conserved for the long-term management and successful recruitment of future forest tree populations. With genomic resources making their way to the forefront of genetic diversity studies, it is now considerably easier to investigate the mosaic of genetic variation, coupled with complex adaptive traits, across landscapes and species ranges (Savolainen *et al.*, 2007; Sork *et al.*, 2013). Particularly, it is expected that the application of population genetics with landscape genomics will be used extensively to evaluate the effects of forest management practices, habitat fragmentation, deforestation, and climate change on forest tree populations.

The newly emerging field of landscape genomics, or the spatially explicit study of the geographic pattern of genome-wide genetic variation, yields promising research opportunities (Sork, 2016). Potentially, the integration of traditional population genetics theory, bioinformatics tools, and geospatial genetic analysis would grant researchers the opportunity to assess complex adaptive traits of forest tree species across large scales (Sork *et al.*, 2013). The primary application of landscape genomics could be the study of the effects that anthropogenic influences have on the genetic variation and complex adaptive traits of forest tree species within and among populations and across entire species ranges. Another potential application of landscape genomics is modeling geospatial genetics analysis to predict the adaptive responses of trees that are pertinent to forest genetic resource management and conservation for future climates (Sork *et al.*, 2013), human population growth, and pathogens. In considering the advancement in technology and research methods, scientists and conservationists hope that the application of the ideas and methods discussed can be combined to better assess and understand the interactions influencing genetic variation of our world's forest tree populations.

Despite white oak's ecological and economic importance, there is a current lack of literature focusing specifically on white oak genomics. However, some studies have emphasized the *Fagaceae* genera due to the importance of American chestnut conservation and preservation, in the face of the detrimental chestnut blight. This movement has allowed researchers to successfully cross reference genomes of members of the *Fagaceae* (Casasoli *et al.*, 2006; Vieitez *et al.*, 2012). It is our hope that this study will help by identifying genetics tools for assessing and describing the genetic diversity of extant forest tree populations and the effects of silvicultural practice on the genetic structure of forest tree species, specifically white oak. Due to heavy artificial selection pressures via harvesting activities, it is important that we have the tools necessary to assess the effects of these practices on genetic variation for the long-term conservation and best management practices of white oak. This study aims to (1) amplify and evaluate predicted microsatellite markers (gSSRs) for white oak and then to (2) use a subset of the validated markers to assess the impact of silvicultural thinning treatments on the genetic variation and relatedness in white oak dominated stands. To address the first objective, we amplified 84 predicted gSSR primer pairs designed for white oak and then evaluated these SSR primer pairs for consistent PCR amplification and polymorphism. Twenty of the 84 primer pairs consistently amplified products that were polymorphic and could potentially be used as markers for the second objective of this study. In the second objective, we utilized the most reliable 12 SSR markers for an assessment of genetic variation and pair-wise relatedness of white oak trees growing on seven half-acre plots from an existing long-term thinning experiment of a white oak dominated site. Estimates of heterozygosity (observed and expected), total and effective allele numbers, deviations from Hardy-Weinberg equilibrium and pair-wise genetic relatedness were calculated with GenAlEx 6.5 using the marker data collected on genomic DNA samples of 225 white oak tress. The statistical analyses used in this study shows a reduction of observed heterozygosity relative to expected and a reduction of genetic relatedness in thinned plots compared to unthinned plots.

CHAPTER 2. MICROSATELLITE MARKER DEVELOPMENT

2.1 Introduction

Oaks (*Quercus* spp.) are a keystone genus in eastern deciduous forests of North America (Brooke *et al.*, 2019), and white oak (*Quercus alba* L.) specifically is a keystone species due to its disproportionate effect on other species, community structure, and key ecosystem processes (Fralish, 2004). The white oak species range extends from southern Quebec to northern portions of Florida and westward to eastern Kansas (Buchanan and Hart, 2012). White oak is extensively sourced as lumber for furniture, veneer, paneling, and flooring, but most notably harvested for staves to make bourbon and wine barrels (Tirmenstain, 1991). Silvicultural thinnings are a tool used by forest managers to meet the demand for forest resources from the timber industry and individual landowner objectives. Silvicultural thinnings commonly aim to reduce the long rotation age, promote positive effects on mean annual growth and/or increase recruitment into overstory canopy classes of forest trees (Dale, 1984). It is important to have the genomic tools necessary to assess the effects of artificial selection such as thinning and other silvicultural activities on the genetic diversity of managed forest tree species. The attributes of microsatellites or simple sequence repeats (SSRs) make them especially useful for measuring genetic diversity in populations of forest trees (Echt and Josserand, 2018). Microsatellite DNA markers are tandemly repeating nucleotides consisting of 1-6 base pairs. Individual microsatellite containing loci are easily amplified using polymerase chain reaction (PCR), while being abundant, highly polymorphic, and easy to reproduce (Grover and Sharma, 2016). To assess the genetic diversity of white oak populations under artificial selection pressures (silvicultural thinnings), we needed to develop polymorphic microsatellite markers for the white oak species due to a lack of validated markers. For white oak, researchers had previously used low coverage genomic sequencing to develop sequences for SSR discovery. From these sequences they designed primer pairs for 84 gSSR sequences that were predicted to make high quality genetic markers (Staton *et al.*, 2015). Their gSSR discovery pipeline, the SSR sequences and primer pairs are available at the Hardwood Genomics Project. After evaluating the 84 predicted gSSR primer pairs for polymorphism with PCR and electrophoresis, we found

20 primer pairs that consistently amplified interpretable alleles and thus appeared suitable as markers for general use in conservation genetic analyses. In this chapter we report the results of our screening experiments including the evaluation of 16 of those most reliable primer pairs over a large population sample of white oak. Further population genetic analyses are presented in Chapter 3, where we studied the genetic consequences of thinning treatments in a white oak population using 12 of these SSR markers.

2.2 Methods and Materials

Leaf tissue samples were collected from white oak sample trees using either a hand pruner, pole pruner or firearm in a preexisting study site on the Daniel Boone National Forest near McKee, Kentucky. The samples were stored and transported on dry ice, in coolers, to an ultra-cold (-70 C) freezer at the University of Kentucky Forest Health Center Lab in Lexington. The study site is the location of an ongoing thinning study of white oak implemented by the United States Forest Service (USFS) in 1961 (Dale, 1973; Lhotka, 2017). Located within the Cumberland Plateau region, the stands were thinned in 1961, varying residual basal area and number of subsequent thinnings. For our SSR marker testing and validation objective, we selected and analyzed three white oak genomic DNA samples, referred to as reference trees or samples, as a subset from the total 225 samples collected from the McKee study site. During preliminary primer pair testing PCRs and gel electrophoresis we used two of the three reference samples but used all three reference samples during the final PCRs and capillary electrophoresis runs (see below). White oak genomic DNA was extracted from foliage tissue samples of the reference trees at the University of Kentucky Forest Health Center Lab using the DNeasy Plant Mini Kit following the standard protocol from Qiagen. The reference sample white oak genomic DNA quality and concentration was visualized using a NanoDrop™ spectrophotometer (Thermo Scientific™) for each of the three reference samples following factory guidelines.

From the results of a low coverage genome sequencing of ten hardwood species by the Hardwood Genomics Project, researchers ended up with 84 predicted gSSRs (45 di, 38 tri and 1 tetra motif lengths) for which unique, high-quality PCR primer pairs were

designed using Primer 3.0 (Table 2.1) (Staton *et al.*, 2015). The predicted 84 gSSRs and associated primer pairs from Staton *et al*. were utilized as our set of SSRs to evaluate for consistent amplification and polymorphism. We began by preparing all of the primer pairs with tails on the 5' ends of the forward and reverse primers to facilitate fidelity of amplification and fluorescent labeling for analysis (Echt *et al.*, 2011). Specifically, forward primers were 5' tailed with the M13 forward (-29) sequence CACGACGTTGTAAAACGAC to facilitate labeling and the reverse primers were 5' tailed with the sequence GTTTCTT to improve amplification fidelity. To evaluate the predicted white oak gSSR markers, we first performed polymerase chain reactions (PCR) on the white oak genomic DNA extracts of the two reference trees (M11-46 and M4-142) using 84 putative SSR primer pairs. For the PCRs, we used the following PCR reagent protocol and corresponding concentrations: in a 25 µl reaction volume: 4-6 ng of white oak genomic DNA, 0.6 of µl forward primer (0.04 uM), 0.6 µl of reverse primer (0.16 uM), 2.5 µl of TAQ buffer 10x (1.8 mM), 0.2 µl of dNTP mixture (1 mM each; 4 mM total all 4 bases), 0.5 µl of TAQ DNA polymerase 500U (5U/ul) and sterile nuclease-free water as needed to bring the total volume to 25 µl. These PCRs were amplified using the following thermocycling protocol (John Carlson's lab, Penn State University, as published on http://www.hardwoodgenomics.org): 5 min at 95ºC; followed by 35 cycles of 30 s at 95ºC, 45 s at 60ºC, and 1 min at 72ºC; and finally, 10 min at 72ºC. Only simplex PCRs were used, such that each PCR had only one primer pair and one reference DNA sample. After PCR amplification, we evaluated the amplification quality using 2% agarose gel electrophoresis.

After our preliminary PCR amplification and gel electrophoresis, we identified 28 SSR markers that exhibited signs of consistent amplification and potential polymorphism. From the 28 SSR markers, 20 of the most consistently amplifiable markers from the gel electrophoresis screening were amplified one marker at a time for three reference tree (M11-46, M4-142, and C2-1) DNA samples and then separated by capillary gel electrophoresis on an ABI PRISM 3130xl Genetic Analyzer (Life Technologies Corporation) as per manufacturer guidelines. ABI PRISM LIZ600 (Life Technologies Corporation) was used to create an internal size standard via loading within the same cell or lane. Allele sizes, in base pairs, were called using GeneMapper® software version 5.0

from the exported capillary data files. An allele scoring and naming protocol was used to call and maintain allele names over capillaries and runs within this study (Deemer and Nelson, 2010). We found that 20 primer pairs yielded distinguishable alleles/locus and used 16 of these to generate population-level polymorphism data to complete and utilize the microsatellite marker set. Subsequently, the 16 microsatellite primers pairs were analyzed on the complete set of 225 white oak foliage samples at the McKee site using PCR and capillary electrophoresis conditions utilizing four pooled SSR markers per capillary channel on an ABI PRISM 3630xl Genetic Analyzer. For capillary electrophoresis multiplexing, sets of four markers were organized into pools so that fragments would not overlap and then loaded into a capillary channel. Within a pool, only one marker per color (fluorescent dye FAM, VIC, NED, or PET) was included so they could be individually scored. In addition to only one color per primer pair per pool, these pools were composed so their expected allele sizes, based on bioinformatic and agarose gel information across a larger population, would not likely overlap. Four of the 5 pools (16 markers) were run against the larger sample of 225 trees and these results are reported in this chapter (summary statistics for genetic diversity of the markers) and in Chapter 3 (genetic analyses of the thinning treatments).The allelic data were analyzed using the add-in GenAlEx 6.5 in Microsoft Excel software following the same protocol previously mentioned and utilized SAS procs to autoscore alleles based on the reference sample calls (Deemer and Nelson, 2010). Specifically, script 1 and script 2 used in our analysis were designed by Deemer and Nelson and can be found in their supplementary materials. Allele and genotype frequency metrics were used to assess genetic diversity in the white oak population and sub-populations (thinning treatments). For each marker and sub-population, we calculated number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He) and Hardy-Weinberg Equilibrium (HWE) p values.

2.3 Results and Discussion

In order to develop reliable SSR markers for white oak, we evaluated primer pairs designed to amplify 84 predicted gSSR markers for consistent amplification and polymorphism in a subset of three trees (referred to as reference trees) from a population

sample of 225 white oak trees. The reference samples are important in that they are used to calibrate all other allele calls in later Genemapper sessions (Deemer and Nelson 2010). In addition, if the tree and its DNA are maintained, they can be used as references for future studies. Of the 84 gSSR primer pairs tested, 28 (~33.3%) were deemed as exhibiting signs of consistent amplification and potential polymorphism from 2% agarose gels (Figure 2.1 and Figure 2.2). Of these 28, 20 were found to be polymorphic among the three reference samples that were genotyped using capillary electrophoresis. The allele calls (length of the amplified fragment in base pairs) for the three reference samples with the 20 polymorphic microsatellite markers are shown in Table 2.2. Genetic diversity metrics for each of the 16 markers that were tested over the entire 225 samples from a population of white oak are presented in Table 2.3. For the 16 markers run across the 225 samples, the number of alleles ranged from five to 19 (mean 11.17), and the number of effective alleles ranged from 1.955 to 10.045 (mean 5.349). Observed and expected proportion of heterozygotes varied from 0.51 and 0.488 to 0.82 and 0.9, respectively, across the whole sample of 225 trees. The range in missing data across the 16 markers was from 0% to 32% (mean 9%). The four markers with the most missing data (from 18% to 32%) may need additional modifications in PCR conditions to improve their performance in population studies. In addition, the four markers of the 20 that were not run across the whole population sample should be further tested for their usefulness in population studies of white oak. Across the whole population the observed and expected number of heterozygotes differed significantly ($p<.05$) in eight of the 16 loci. The reduction in the number of heterozygotes relative to expected was also apparent in the Hardy-Weinberg values, with the same eight loci being out of equilibrium.

The predicted white oak gSSR primer sets tested, proved to be a reliable source of genetic markers for white oak. The markers tested and filtered in this study should prove to be quite useful for examining the population genetic structure of white oak, specifically in the Cumberland Plateau region and likely throughout the range of white oak. Studies examining SSR markers for white oak are not common and usually have only a few primer pairs tested. A study on population differentiation among three species of white oak in northeastern Illinois (Craft and Ashley, 2006) used 5 SSR markers developed for *Quercus bicolor* and *Quercus macrocarpa* but none for white oak *Quercus*

alba. This is due to a lack of SSR marker development and research for white oak specifically. The number of SSR markers that are now available for white oak has been expanded with the edition of these 16 markers. Additionally, the primer sets that did not pass the screening rounds, could perhaps yield additional polymorphic markers with further optimization of primer/PCR conditions. In conclusion, with the 16 highly polymorphic microsatellite loci (gSSRs) developed in this study, our data suggest that these markers will be extremely useful for white oak conservation programs that require population genetic data and analysis. In the next chapter, we use the most reliable and consistent 12 microsatellite marker primer pairs to (1) assess the genetic structure and diversity of the white oak population with varying thinning intensity levels and timing and (2) assess if silvicultural thinnings cause a degree of change in the genetic relatedness of white oak. The results will be useful for management, conservation, and breeding plans for the longevity of genetic resources of white oak and the sustainability of an economically important forest tree species.

Table 2.1 Predicted Microsatellite Marker Primer Pairs

The 84 predicted microsatellite (gSSR) marker primer pairs for white oak with respective motif pattern, forward and reverse primer sequences, and fragment size in base pairs. Locus names with an associated * indicates that the marker was in the top group of 20 polymorphic markers, ** indicates within the top group of 16 markers, and *** indicates the 12 markers used in the final analysis of chapter 3.

Locus	Motif	# Repeats	Forward Primer	Reverse Primer	Fragment Size
1T	gaa	8	CCGGTCTTCTCAACCACTACC	GGGTAGTACGTGCTATAAGGG	272
2T	tct	7	GCCGTTGATTTCACCGATCG	GGATTGAGGTTGAAGATGTTAGACG	118
3T	acc	9	TGAACTTGGCTATTGTGCTGC	ATGGCCAGGAGCTTAATGGG	334
4T	tct	8	CGAAACGCTACGACATACGC	AAATCTCATCCGCTTCAACC	225
5T	gaa	7	TGCTTTACATCAATTCTGAGAAGGG	CAGCCTCCAAGACTTTCAAGG	150
6T	ctc	8	ACTCAGGCCAAATCTCCACC	TGGATGCTTTGGGAGTTACG	258
7T	tgg	7	CTCAGAGCGAGTACCATCGC	GGTCCGCTCTTCGACTTAGG	246
8T	cac	10	TGGGTTGTGTCTCTCAATGG	GACAGGATTTCTGACCCGGG	213
9T	tac	8	GTAACTGTGGCCTAGACCGG	CCCACCAGTTTCCTGAGTCG	326
10T	tat	9	GATCATCCAACAACGACGCC	GTAAACCCAAACCCAGCAGC	169
11T	ttg	7	CAACTTCACTAGAATTCTCATTCCC	GCTTCACCAGTTCTTGCAGC	277
12T	aga	7	TTGGACGGAGGAAGAACAGC	CTCATTCCTCTCCTCTGCCC	203
13T	tct	7	GTTACGTGCCTCTCGAAAGC	GCCCTTCTTTGAATTCTCCAAGC	174
14T	gaa	9	TCGGTGTGCCATAGATTCCG	CAGAGTTGGAGAGTACAAGC	196
15T	act	7	TTTCCTCCTCTGCTTGGTCC	AACCACTGTGACCAGATCCG	259

Table 2.1 (continued)

16T* **	gaa	10	CACCAGCACCTCACTTCTCC	GGATTTGGGTTTGGGTTGGG	231
17T	aga	8	TGGTAATTTCCTAATTTCCCGG	GATCTGGGTACGGGTATGCC	350
18T	cac	8	CCCTCCAGGTCCCATTATAAGC	TCTAAGGTTCTGAGAGTGCACC	107
19T* **	tta	10	GGGCTTGCTTGCATTACTCC	AGATTTGTTCTCTCAATCTCGCC	136
20T	ttc	7	GGACGTGTAGATGAGCCAGC	ACCCACATCTGTACCAAGCG	105
21T	tat	11	GCTACCACTGCCACTATCGG	TTCTTCACCTCACCGCTTCC	151
22T* **	aag	16	CGCACTCTCTCTCAAGGACC	GCTTTCCTTCATGAGCCG	237
23T	aac	8	CTCTACACCCACCAACCACC	ACCCAATATCCATGGCCTTAGC	313
24T	cca	8	ACAGTCTCTCTCACCAAACCC	TGGCTGATTTGTGGGTAGCC	212
25T	ctt	7	TTCTGACCATTGCCTCCACC	GGTACCTTTGTTTAGCTTCTGC	102
26T	cac	8	TCCTGTGACCATTCCTTCAGC	AGTGCAGAACAAGCCTTTGG	191
27T	caa	7	CTCTGATGTCAAAGCCAGCC	TGAAGGCTTGGAGAAAGATGGG	130
28T	ggt	8	GTACTAGTCCCTGGCAGACC	CGCGGAAGAAATCGAATCCG	274
29T	gaa	9	CTTCTCCCACCAATCCACCC	ATGCGTCCACCAACACAACC	175
30T* **	gtt	10	TTATCCTGTGGTGCCGTAGC	GCGTTGTCGAACTAGAACCG	116
31T* **	gaa	8	CTTGCATTGGAAGCAGTCGG	TCATGAAGAACACACACGATGC	195
32T* **	gtg	7	GATTCGATTTCTTCCGCGGC	CTGGAACTCCACGCTAAAGC	116
33T*	agt	9	AGTACTCGAAGCTGGTTGGC	CGAATCCGACTCCGATTTCC	179
34T	ttc	7	ACCACCACCTCATCGATTCG	TGGCAACTCTCACGATAGCG	231

Table 2.1 (continued)

35T	tgg	7	CCTTTGCCACGCTTGTAACC	CCAGAAGATAAAGTAGAAACACACGC	154
36T*	agg	8	CCACCATCACTCACTCCACC	CTTCCAGCCTCTATAGCCGC	235
37T	tta	9	TCTTTATGGGTTTGAGCAGTAGG	TGTACATGCCCATACTAACAGC	202
38T	agc	7	GGTCAGAGGAGGAGAACACC	TTCGAATTCGGCACTGCACC	127
39T	ct	9	ATGTGGCTTGTTTAATCGCC	GGAAATTAACTAGTTTGGCCATCCC	101
40T	ag	10	AAACCCAAACACAGAGCACC	ACGAACACAACTCCGAGACC	212
41T	ct	8	GCTGCATTTGTAATTTGAGTGACC	GATGTCCCGTCACTGGTAGG	294
42T	ct	10	GCTGAAGAGGTCAACAGTGC	TGAGACTTCTGGTTGTATCAAGC	177
43T***	ct	8	TGGAGAACGCGAAGATGAGG	CACCGACACTGCTACATTTGC	272
44T**	ta	10	TCTGAGAGACGAGGCTGAGC	ACATTACAGCCATTCCCTTTGC	167
45T***	ag	10	CAGAATCTCCTTCTCCGCC	ACGTAAGGAGAACCGTAATCAGG	236
46T	ag	9	AAAGAGAAACACCGCTGTGC	CCAAGTGGTGTTGGTGTTGG	209
47T	ag	13	AGTCCTAGTACCTAATTTACCCAGC	GCGTTGTTTGGTACCTCTGC	125
48T	at	13	ACTCACCAAGTCATCATGGC	ACATCCAATCACCATGAATCTTGC	137
49T	ag	8	CACTACTACAATTGAAATAGACCAGGG	ATTGAGGGAGCGATCGATGG	200
50T	ac	8	ATTGAAGGAGGCCAAGGTCG	TTCTCTCGTGACGGAGATCG	270
51T	tc	13	TCCTTCTGTGAAACTTTCATGCC	AGTAAGTACGAACCGAAACACC	157
52T	ct	9	TGCAAACCCATCAAAGCACG	CAACCCGATAGATCCTCGCC	280
53T	ga	13	ATAGGCTTGTCAGGTCGAGC	TACGGATTCTCCAAAGCCCG	315

Table 2.1 (continued)

54T	ag	10	TTATCTAGGAGACGAGGTAGC	CCTTCTTGTCCCTCCGTTCC	215
55T	ag	8	AGGGACTATGGACTATATAGGTGG	TGTCAATTCCTGTTTCTGGG	205
56T	ag	9	GAAGCTGAAAGTGTAGTGGGC	AGGCCCGCATAGATTTCTCC	119
57T	ag	10	CCGTAAGAGCAAGGCTTTGG	ACCCTGACGTCAGTTGTTCC	230
58T	tg	14	TGTCAACTAGTCAGTTCCTTGTAGG	CTACCCATTAGGCTGGTGGC	318
59T	at	13	TTTGCTGGCCTGGAGAGTGG	ATGTGAGGCAAGTCGTGAGG	313
60T	tc	14	AGTCGTTTCCTCTGGCATCG	ACTCTTCTCATTTGAATTCCGC	119
61T**	ga	11	GGAGGGTGGTAGTACATGCG	ACAGCTACACACACAAACCC	226
62T**	ct	8	GGAGGCCCTGGTAGAAATGC	AGGTTAACTGGTTATAGCCCACC	158
63T	ta	11	ACTCAGGCCAAATCTCCACC	TGGATGCTTTGGGAGTTACG	258
64T	tg	13	TCTTTAACAAGATCCACGCAACC	AGTGTGATTGCTCTAACCCG	235
65T	ct	9	CCAGTCATTCCCACCACTGG	TGTCCCTTGCCAGTAGAACG	252
66T**	tc	9	TAGGAACTTCAACGCCACGG	CAAACAGGCCAACTCTAACCC	107
67T	ga	9	CCTTGTGCTGCTGTGAATCC	ACACTCACACAAACTCAAGG	216
68T*	ga	8	AAGTGCAAGAGTCCACAGGC	ACTCAGGCATGGGCTACAGC	294
69T	ag	11	TGGTGGATCTAAGGTGAATGG	GGATGAGCTTGCTGGAGAGG	218
70T	tc	9	TGCCTTCAATTCGGATAAGG	CTGGGTTGGTTCTGGAGAGG	281
71T	ct	9	TCTTCATCCACCTTCACCACC	GGAAATGGTGGGCATTGTGC	227
72T	ga	11	AGTTGTGTTGGGAAGTTAACC	ACATCCTCAAGCTGTCACGG	171

Table 2.1 (continued)

73T**	ag	11	TCCTTCACTTGCTGCTGTGG	GTGTGTGTGAGTCTTCGTCG	137
74T	ag	10	CAAAGCCTCCCTCTCTAATCCC	GCCCTGTTAACAGTCCTCCG	308
75T***	tc	16	TGGAGAACGCGAAGATGAGG	CACCGACACTGCTACATTTGC	272
76T***	ag	8	ACTAAGAGGAGCACCAACGC	TGGCTGTAGAAAGATGGTGTGG	148
77T	ag	11	TGGTGATGAGCTAGCTAGTGG	ACAACTGCAACACCAACACC	134
78T	ag	14	TGAGGGATTGTGAGTTGTGGG	TGCCATTAGAGACCCATATCCC	107
79T	tc	9	TCATCCTTTGGTGGTGAGCC	ACAGCAAAGAAGAGGAGGCG	239
80T	at	13	CACCCATGCCAATTTCCACC	CGCCATGGTCAATACATGCC	114
81T	at	10	TTCGTGGAGAGAAGCAAAGG	TGTTGTGTCTCTGTCTCTCACC	294
82T***	ag	10	TTCAACACCTCAACTTCACG	TCAAACCCGTGACATGACCC	264
83T	ga	9	TTGCAAGGCAAGTTTGGTCG	AAATGATCCCACCCTGCTCG	330
84T	tcca	6	TGGTTCATCCATCCATTCATCC	ACTAAAGGAAGCCATGATGC	232

Table 2.2 Allele Call by Locus

Allele calls (estimated fragment length in bp) by marker across three reference samples (C2-1, M11-46, M4-142) in screening for consistent amplification and polymorphism. Each pool is a grouping for electrophoresis in the ABI PRISM 3730xl and panels are the marker names.

Pool1	**C2_1**	**M11_46**	**M4_142**	**C2_1**	**M11_46**	**M4_142**	**C2_1**	**M11_46**	**M4_142**	**C2_1**	**M11_46**	**M4_142**
Panel	19T	19T	19T	31T	31T	31T	45T	45T	45T	66T	66T	66T
Allele 1		159	168	207	210	210	268	264	268	130	130	130
Allele 2		162		210			272				132	134
Pool2	C2_1	M11_46	M4_142	C2_1	M11_46	M4_142	C2_1	M11_46	M4_142	C2_1	M11_46	M4_142
Panel	22T	22T	22T	32T	32T	32T	76T	76T	76T	82T	82T	82T
Allele 1	249	246	237	140	133	133	170	178	174	284	281	283
Allele 2		255	249	143	140		176	200	182	286	283	286
Pool3	C2_1	M11_46	M4_142	C2_1	M11_46	M4_142	C2_1	M11_46	M4_142	C2_1	M11_46	M4_142
Panel	30T	30T	30T	43T	43T	43T	61T	61T	61T	62T	62T	62T
Allele 1	139	139	143	290	284	292	247	252	257	183	181	181
Allele 2		219		296	296	298	252	259	261			183
Pool4	C2_1	M11_46	M4_142	C2_1	M11_46	M4_142	C2_1	M11_46	M4_142	C2_1	M11_46	M4_142
Panel	16T	16T	16T	44T	44T	44T	73T	73T	73T	75T	75T	75T
Allele 1	252	252	249	193	193		166	148	152	290	284	292
Allele 2			255	200	195		168	166	158	296	296	298
Pool5	C2_1	M11_46	M4_142	C2_1	M11_46	M4_142	C2_1	M11_46	M4_142	C2_1	M11_46	M4_142
Panel	33T	33T	33T	36T	36T	36T	68T	68T	68T	77T	77T	77T
Allele 1	198	198	198	249	249	249	315	315	323	157	149	157
Allele 2	201				252		325	328		165	157	163

Table 2.3 Results by Locus

Results by marker/locus. N (number of trees, sample size), Na (number of alleles), Ne (number of effective alleles), Ho (observed heterozygosity), He (expected heterozygosity), HW P-value (Hardy-Weinberg Equilibrium p-value). Numbers in bold mean that the p-value was statistically significant.

Locus	N	Na	Ne	Ho	He	HW P-value
T16	217	6	1.955	0.51	0.488	0.654
T19	221	11	4.696	0.52	0.787	**0.000**
T22	217	11	4.285	0.82	0.767	0.279
T30	219	8	2.567	0.59	0.61	**0.000**
T31	219	5	3.009	0.65	0.668	0.981
T32	221	5	2.163	0.46	0.538	**0.000**
T43	201	15	9.18	0.73	0.891	**0.000**
T44	179	10	5.842	0.6	0.829	**0.000**
T45	196	14	7.399	0.58	0.865	**0.000**
T61	176	14	8.017	0.7	0.875	**0.000**
T62	184	9	3.191	0.64	0.687	**0.000**
T66	222	11	3.597	0.74	0.722	0.881
T73	153	13	5.97	0.79	0.833	**0.000**
T75	208	18	9.899	0.77	0.899	**0.001**
T76	222	19	10.05	0.78	0.9	**0.000**
T82	225	11	5.404	0.75	0.815	**0.002**

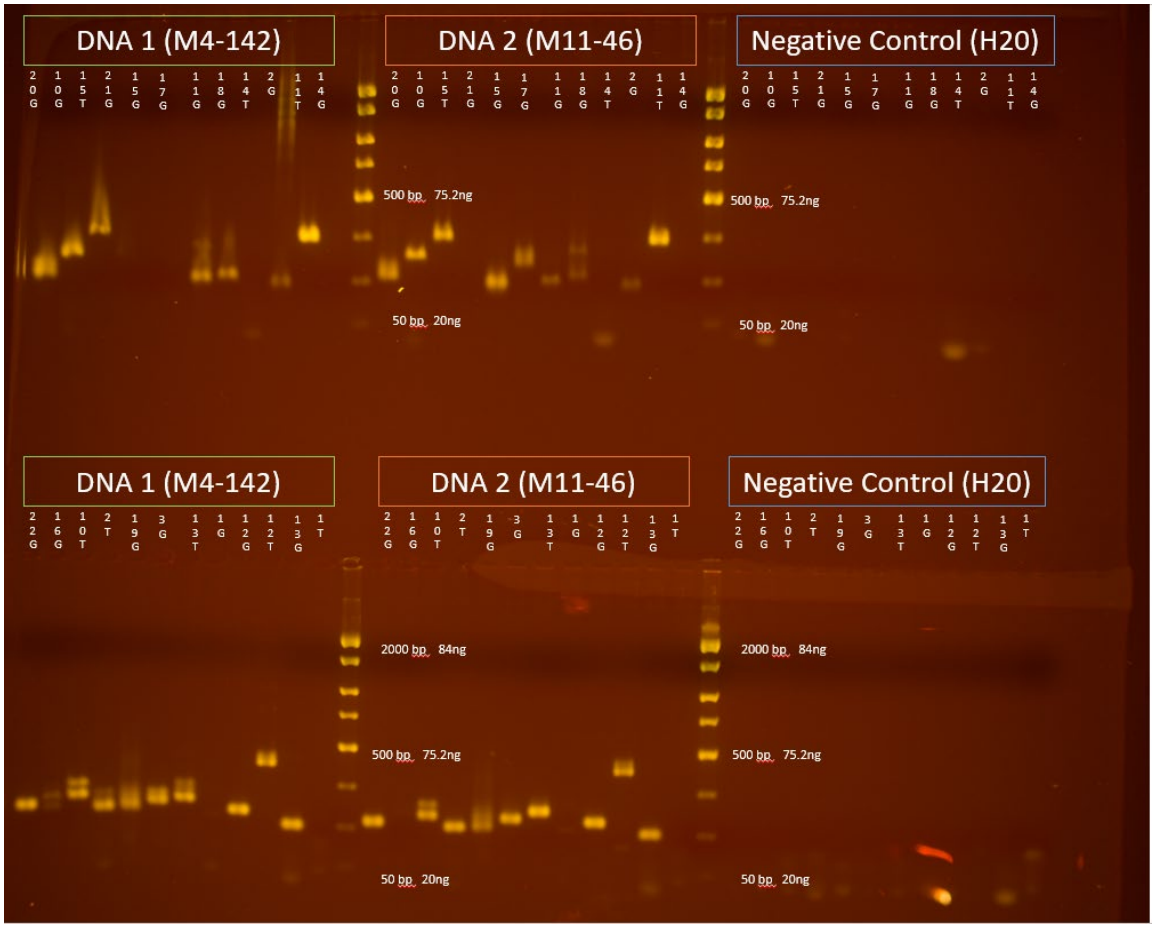


Figure 2.1 Preliminary Agarose Gel

This figure shows amplification results for 24 of the 84 predicted microsatellite (gSSR) primer pairs on 2% agarose gels at 100 volts for 3 hours. Each primer pair label is at the top of the lanes. Each primer pair is tested against two reference samples and a negative control, shown here labeled at the top of the primer pair labeling. In addition, the size standard in the last lane of each sample is labeled in base pairs for easy interpretation.

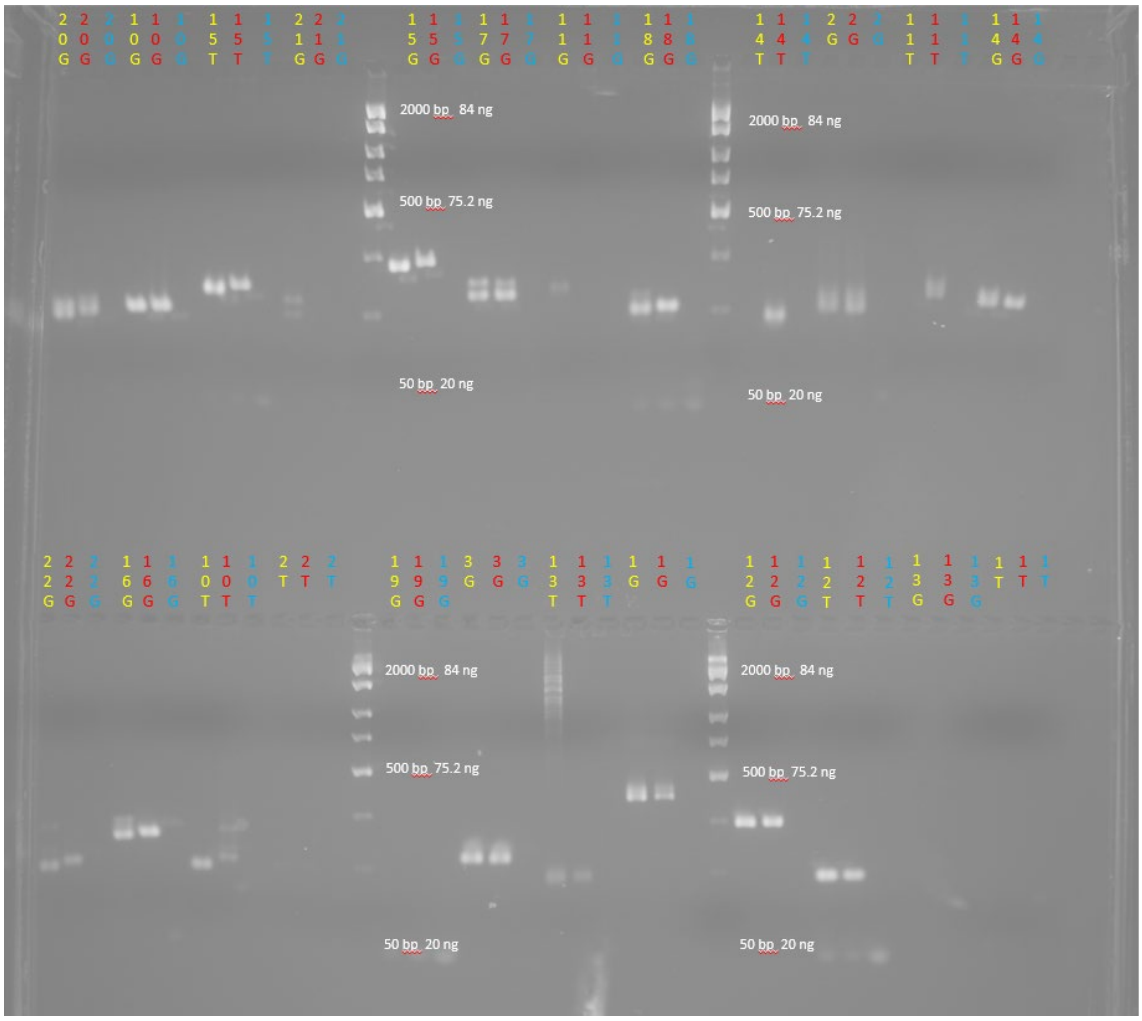


Figure 2.2 Secondary Agarose Gel

This figure shows amplification results for 24 of the 84 predicted microsatellite (gSSR) primer pairs on 2% agarose gels at 100 volts for 3 hours. Instead of the same layout used in Figure 2.1, this figure was designed for easy comparison of the same locus across each DNA and negative reference sample. Each primer pair is tested against two reference samples and a negative control. Reference sample M4-142 is shown in yellow, reference sample M11-46 is shown in red and the negative water reference is shown in blue. The size standard is labeled in base pairs for easy interpretation.

CHAPTER 3. GENETIC DIVERSITY ANALYSIS OF THINNING TREATMENTS

3.1 Introduction

Genetic diversity, in forest tree populations, is responsible for maintaining the short-term viability and the long-term evolutionary potential of forest tree species (Buiteveld *et al.*). Natural selection, genetic drift and geneflow (i.e. migration) jointly affect the genetic diversity of forest tree populations. Natural selection is responsible for fostering rapid local adaption and is attributed to explaining the vast genetic diversity in forest tree species along with the immense distribution of key fitness-related traits such as survival, resistance to drought and pests, resistance to pathogens and growth (Ratnam *et al.*, 2014). Genetic drift can lead to local and range-wide extinctions due to inbreeding depression. While gene flow can be responsible for restoring genetic diversity via pollen flow from a genetically diverse population or can genetically degrade populations if gene flow spreads from a low genetically diverse population (Ratnam *et al.*, 2014). Although forest trees are some of the most genetically diverse organisms on the planet, they are still potentially subject to genetic diversity losses. As societal demands increase, it becomes increasingly important to preserve forest genetic resources through forest management practices for the longevity of forest tree species. The diverse public pressures and market demands on forest tree populations create a variety of silvicultural management practices (Kimmons, 2008). Silvicultural thinnings are the most frequently used technique to increase the health and commercial value of forest tree stands (Zeide, 2001). During silvicultural thinnings, commonly the most phenotypically valuable trees are selected to (i.e., to not be cut) optimize stand growth and commercial value by the removal of trees with inferior phenotypes. It has been hypothesized that thinning stands alters forest tree genetic resources, especially when the traits selected for or against are at least under some form of genetic control or do not mimic natural selection (Finkeldey and Ziehe, 2004).

In view of recent studies, it is becoming more apparent that the effects of forest management practices on the genetic resources of forest trees are dependent on species, climate, local adaption, and anthropogenic influences. Genetic studies on the effects of

management practices cannot be applied broadly across species or sometimes even within species ranges. For example, a study in Southern China on Diao Luo Shan Qing Gang (*Quercus tiaoloshanica* L.), a narrow range *Quercus* species with genetic isolation due to habitat fragmentation and urban sprawl, found that a significant drop in number of alleles per locus occurred with selective logging (Zheng *et al.*, 2005). In comparison, a study on Northern red oak (*Quercus rubra* L.), a species with a large range, reported no loss of allelic richness between managed and unmanaged stands, accrediting the lack of genetic change to the plasticity and genetic diversity within the species (Grant, 2010). Traditionally, studies on the effects of forest management practices on the genetic resources of forest trees have focused on economically important trees such as poplar, mahogany, conifers and the *Eucalyptus* (Buiteveld *et al.*, 2007). More recently, studies have expanded to more forest tree species not only of economic importance. A study in Brazil found a significant reduction in the number of alleles, observed heterozygosity, and distinct multilocus genotype numbers between post-logged sites and natural populations of big-leaf mahogany (*Swietenia macrophylla* L.) (André *et al.*, 2008). While a similar study in Brazil on the effects of reduced-impact logging of West-Indian locust (*Hymanaea courbaril* L.) stated that a reduction of about 800 to 200 m in the distance of the spatial genetic structure in the reproductive population reduced the effective population size, but did not result in a reduction of genetic diversity (Biscaia De Lacerda *et al.*, 2008). With studies on the impacts of timber management practices for forest trees having been somewhat contradictory, it becomes increasingly important to study the effects across species ranges regardless of their economic importance. Studies assessing the effects of forest management practices, such as the ones previously mentioned, become important across species ranges and for each type of silvicultural practice. Extrapolating data on the effects of change on genetic diversity of one species to another may be difficult. Currently, there are no studies to date on the effects of thinning regimes on the genetic diversity of white oak (*Quercus alba* L.).

Historically, oaks were the dominant forest tree genus in the eastern United States, with white oak being one of the most common dominant species (Abrams, 2003). A member of the Fagaceae family, white oak ranges from southern Quebec to northern portions of Florida and westward to eastern Kansas (Buchanan and Hart, 2012).

Generally growing on a wide range of soil types, the greatest limiting factors of growth for white oaks are latitude and topography (Honeycutt *et al.*, 1982). Regardless of its habitat generalization, white oak does not grow well on extremely dry, shallow-soil ridges, poorly drained flats, or wet bottom lands (Rogers 2010, Silvics of North America: Volume 2. Hardwoods, white oak chapter pp. 601). White oak is a monoecious species, meaning that the flowers of both sexes are present on a single individual. The flowers of white oak are in the form of catkins, produced during the late spring (Rogers, 2010). Pollination typically occurs via wind within a short, three-day period given that the appropriate environmental conditions of relative humidity and wind strength are met (Williamson, 1966). The acorns reach maturity approximately 120 days post pollination, during September and October, and begin germinating as soon as they fall (Brooke *et al.*, 2019). During masting years, which occur only every four 4 to 10 years, up to 10,000 acorns can be produced by a dominant, forest-grown tree (Rogers, 2010). White oaks reach maturity between 50 and 200 years, and studies have shown that an exceedingly small proportion of the acorn crop completes germination (Sork and Bramble, 1993). The seedlings require adequate sunlight, soil moisture, and moderate litter cover for successful germination and recruitment into higher canopy classes (Rogers, 2010). Research on successful recruitment of white oak seedlings has shown that the number of new seedlings produced each year is lower than most of the other oak species (Abrams, 2003). With a reputation for slow growth and hindered recruitment from seedling to higher canopy classes, silvicultural rotation ages are typically long and sometimes can reach more than a hundred years (Rogers, 2010). Thinning is implemented in white oak stands to improve and promote oak regeneration (Aldrich *et al.*, 2005) because of their positive effects on the mean annual growth of mature trees and overstory tree recruitment (Dale, 1984). White oak's ecological significance has been attributed to its large masting characteristics. Its acorn crop serves as food for wildlife, while its large branching patterns and flaking bark provide habitat for other wildlife species (McShea and Schwede, 1993; Hutchinson and Lacki, 2000). With the many ecological services that white oak provides, it has been considered a keystone species due to its strong contribution to community structure; maintenance of critical or key ecosystem processes; and disproportionately large effect on the existence of other species (Fralish, 2004).

Economically speaking, white oak is the most valuable North American oak. Notably, white oak is used for staves in bourbon and wine barrels, wood for furniture, veneer, paneling, and flooring (Tirmenstain, 1991). Studies on the forest genetic resources of white oak are rare, with studies usually explicitly focusing on geneflow. There is currently no research on the difference of genetics and genomics data in managed versus undisturbed stands of white oak.

In the previous chapter (chapter 2), we applied a series of tests to validate 84 predicted microsatellite markers (gSSRs) and confirmed 16 to be reliable and highly polymorphic in a population of white oak. In this chapter (chapter 3), we will examine the impact of free thinning forest management practices on the genetic diversity of a white oak population. Free thinning is the removal of trees to control the stand density and favor desired phenotypes (i.e. crop trees) using a combination of thinning criteria, typically disregarding crown position of individual trees (Nyland, 2016). A long-term thinning study utilizing free thinning treatments at the McKee site on the Daniel Boone National Forest, served as the sampling site for our study (Dale, 1973). The half-acre plots at the McKee study site varied in residual basal area post thinning and the number of subsequent thinnings after the initial (completed in 1961), creating two experimental treatment factors for our assessment. In a broad sense, we wanted to examine if artificial selection (i.e. free thinnings) produce significant effects on the characteristics and extent of genetic diversity in the white oak population of residual (non-cut) trees. We examined the different treatment effects on the degree of heterozygosity (observed versus expected) at the 16 polymorphic microsatellite loci confirmed in chapter 2 and the genetic relatedness (estimated with these loci) between tree pairs in the sampled population. We focused our analysis on comparisons among loci at plots differing in thinning treatments as categorized by their residual basal area and number and timing of removals. In addition, we examined the pairwise genetic relatedness between thinned and unthinned (i.e., reference or natural selection stands) treatments. Through our analysis, we show that there is a general trend for a reduction in observed heterozygosity relative to expected and genetic relatedness in the thinned stands of white oak. Ultimately, additional analyses to refine significance testing and treatment delineation is needed for a complete interpretation of the thinning effects.

3.2 Methods and Materials

3.2.1 Study Site and Sampling

Our study took place on the Daniel Boone National Forest near McKee, Kentucky (see Figure 3.1). Thus, the study's population is designated the McKee Site throughout the research project and written thesis. The area is an upland oak forest type, part of the Northern Cumberland Plateau ecological section (Cleland *et al.*, 2007) and dominated by white oak (Lhotka, 2017). Plots from a 1961 thinning research study by the USDA Forest Service on the effects of stand density on wood production, growth and quality of residual white oak trees served as the foundation for this project (Dale, 1973). Sample trees were all white oak trees that were residual from the free thinning treatments in 1961 and were identified by painted tree ID numbers in the field. The original thinning study by the Forest Service did not include reference plots (or control plots) that did not receive a thinning treatment. We followed the original plot layout methods from Dale 1973 and added two reference plots to the study to compare thinned versus non thinned plots. Both reference plots are located within the same watershed and were a part of the same stand or population of trees (see Figure 3.1). Areas selected for reference plots were based on management history and were known not to have been thinned, or logged, in a considerable amount of time. Thus, the reference plots represent a sub-population of trees that have not been subjected to artificial selection (i.e., thinning) during the experiment or for a considerable amount of time prior to the experiment. In total, 6 thinned plots (M3, M4, M10, M11, M17 and M18) and 2 unthinned (reference) plots (C1 and C2) were sampled for our analysis.

White oak leaf tissue samples were collected from each previously marked white oak in all eight plots between July 2016 and August 2016 in thinned plots. Although only seven plots are included in our analysis, due to insufficient extracted genomic DNA from plot 17, we did sample all eight The reference plot trees were first tagged with flagging tape delineating them as a sample tree because they were not painted with ID numbers as they were not part of the original study. White oak sample trees in the reference plots included any tree that was at or above pole timber size (i.e. above 6 inches diameter breast height). A total of 275 leaf tissue samples were collected by hand, pole pruner, or firearm from the thinned plots and reference plots combined. The foliage samples were

kept cold on dry ice coolers and brought to the University of Kentucky for storage at -70 degrees Celsius for later lab analysis. Tree locations were taken for all 275 white oak trees from a given plot's corner using a Laser Technology Mapstar System Electronic Compass and Laser Technology Impulse laser hypsometer. This digital compass and laser range array mapped the azimuth and distance from the corner to the first tree, and from each successive tree to the next tree. A GPS coordinate from the starting corner (lower right corner oriented with North as the top boundary of the plot) was taken using a Garmin handheld GPS and this spatial location was used to georeference each tree from the collected azimuth and distance data. Individual phenotypic tree data has been collected every 5 years post the original thinnings in 1961 by the Forest Service in plots that received a thinning treatment. The data collected and available includes individual tree data such as species code and diameter breast height DBH and plot level data such as basal area BA and trees per acre. Phenotypic data does not exist in perpetuity for the trees in the reference plots as they were not delineated during the thinning experiment implementation. During sampling, reference plot tree DBH and GPS location were recorded but later the data were lost and not able to be included in the final analysis.

3.2.2 Treatments

The original Forest Service thinning study included 15 thinned plots varying in residual basal area, periodic increase in stocking percent to be cut in subsequent thinnings (i.e. degree of thinning) and number of subsequent thinnings. For our study, we selected 6 of the original 15 plots for sampling and data analysis. The 6 thinned plots selected vary in thinning intensities with differences in residual basal area, number of subsequent thinnings and the degree of thinnings. The variation in residual basal area and number of total thinnings produced two treatment factors that were used for our analysis. Residual basal area in 1961 for the plots selected varied from least thinning (plots 3, 10 and 17) that were thinned to ~75 ft2/acre residual BA and thinning to below full stocking (plots 4, 11, 18) that were thinned to ~ 45 ft2/acre residual BA (see Table 3.1). Thinning timing was categorized with two plots (plot numbers 3 and 18) thinned once in 1961 but received no subsequent thinnings. Two plots (plot 10 and plot 17) were thinned twice, once in 1961 and again in 1987. Two plots (plots 4 and 11) were thinned three times, once in 1961, 1971, and 1987. The degree of thinning varied in subsequent thinnings by

the periodic increase in stocking percent removed from none (plot 3 and plot 18), half (plot 4 and plot 17) and all (plot 10 and plot 11). As a part of our treatment delineation we calculated the number of individual trees removed during each thinning to get a better understanding of how many trees were removed from each plot (see Table 3.2). The number of individual white oaks removed is valuable because it tells us how many trees were actually selected against (i.e., cut) during the thinning implementation. However, the exact number of white oaks removed in the initial thinning implemented in 1961 to all plots (except our reference plots) cannot be determined. We have data for the number of white oak post thinning in 1961 but not prior to thinning. We calculated the total basal area per acre and number of white oaks for each plot in 1961 (following initial treatment implementation) and 2016 as a percent of white oak basal area (see Table 3.2) to further contribute to descriptive statistical evaluation of artificial selection level and delineation of treatments. The combination of residual basal area and number of subsequent thinnings were delineated as medium thinning intensity and high thinning intensity. The medium thinning intensity plots vary in residual basal area but do not have more than 1 subsequent thinning. The high thinning intensity plots also vary in residual basal area and are generally thinned more than once. It should be noted that although we analyzed our data based on the thinning intensity treatment delineation in Table 3.1, we also provide an alternate thinning intensity treatment delineation based on the proportion of white oak trees removed from the plots and balancing sample size groups (see Table 3.10). We feel that this alternative treatment delineation would be valuable in further analysis because it is based on the proportion of white oak trees removed (directly related to selection intensity) and not thinning treatments (intended level of selection). Although the original thinning study delineation is useful, the number of trees removed between treatments is not consistent because plots had variation in site quality, initial tree number and size distributions, thus had denser stands with heavier artificial selection (higher thinning intensity) to reach the target BA.

The original study's thinning prescription by Dale 1973, serves as the artificial selection in our analysis. The free thinning specific silvicultural prescription used gives us an idea of which traits were selected for or against during the thinnings process. During thinning implementation marking rules were to (1) cut older "wolf" trees (or

those generally over 9 inches diameter breast height DBH,), (2) cut cull and defective trees, (3) space trees uniformly over the plot, (4) cut undesirable species, and (5) cut trees of poor form or quality. Traditionally in forestry, a wolf tree is generally considered to be an individual tree that takes up a disproportionate amount of space in a stand, shading out or towering over the other trees (Elliott, 1945). Similarly, current literature refers to wolf trees as those that grow faster than surrounding trees, utilizing a larger growing space than their economic value justifies, may be older and have larger crowns than other trees in the stand (Elliot, 1945; Makrickiene *et al.*, 2016). Ultimately, wolf trees are considered undesirable because they cause a reduction in stand timber quality and potential for volume growth. Cull and defective trees are trees that show undesirable timber characteristics and display a significant amount of timber volume lost. Undesirable timber characteristics commonly found in cull trees include rotten or missing material, sound dead wood and/or a form defect (i.e. lightening scar). The spacing is implemented to not only give each residual tree ample room, but also efficiently space trees as to maximize space utilization. Undesirable tree species were black gum (*Nyssa sylvatica* L.), red maple (*Acer rubrum* L.), dogwood (*Cornus florida* L.), sourwood (*Oxydendrum arboretum* L.), serviceberry (*Amelanchier arborea* L.) and scarlet oak (*Quercus coccinea* L.). Trees of poor form or quality are generally considered to be trees with poor branch and stem structure (i.e. multiple codominant stems or epicormic branching).

3.2.3 DNA Extractions and Marker Data Evaluation

Genomic DNA was extracted from leaf tissue of each sample tree with the DNeasy Plant Mini Kit following the standard protocol from Qiagen with modification to the tissue disruption step with the use of a FastPrep machine to homogenize tissue for half of the samples. The other half of the samples were homogenized by hand using liquid nitrogen, mortar, and pestle. Estimation of DNA quality (O.D. 260/280, and 260/230) and concentration (O.D. 260) was done using a spectrophotometric measurement of UV absorption at wavelengths of 230, 260, and 280 nm. Plot 17 samples were dropped from further analysis due to degraded quality and concentration of genomic DNA, reducing total sample count from 275 to 225.

The 16 polymorphic SSR marker primer pairs were amplified using PCR across the 225 samples. Please see chapter 2 for details concerning polymorphic microsatellite testing and amplification. The resulting PCR products were then separated by electrophoresis on an ABI PRISM 3730xl Genetic Analyzer (Life Technologies Corporation) as per manufacturer guidelines. ABI PRISM LIZ600 was used as an internal size standard. During electrophoresis, the markers were pooled four to a capillary channel, each marker with a different fluorescent dye (FAM, VIC, NED or PET), and grouped into sets so that expected allele size ranges in the population would not overlap. Allele sizes, in base pairs (bp), were generated following a protocol from the Southern Research Station in Saucier, MS using a SAS proc to build a cumulative binset from reference samples in Genemapper® software version 5.0 (Deemer and Nelson, 2010).

Allelic data was analyzed using the add-in GenAlEx 6.5 in Microsoft Excel software. Due to substantial missing data (>15%) 4 microsatellite markers were dropped, leaving 12 loci (see Table 3.3) for analysis. Population allele frequency metrics were used to assess genetic diversity in the white oak populations. For each marker we calculated number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He) and Hardy-Weinberg Equilibrium HWE associated p-values (Peakall and Smouse, 2012). The number of alleles is the number of observed alleles that amplified at each microsatellite marker locus. The effective number of alleles is the number of alleles that you would need to observe of equal allele frequencies to see the same level of observed heterozygosity. Heterozygosity can be defined as an individual having two different alleles for a gene. In our analysis, the expected number of heterozygotes was determined based on the allelic frequencies at each locus using the Hardy Weinberg Equilibrium Theorem (HWE). The HWE states that given a set of assumptions the allele frequencies will not change from one generation to the next (Andrews, 2010) and the genotypic frequencies can be determined from them. HWE assumes that there is no selection is acting on a locus, there is no mutation or migration of new alleles, the population size is infinite, and that mating is occurring randomly. If the observed genotype numbers of a population deviate (i.e., p-value < .05) from the calculated HWE expected frequencies at given locus, we say that the locus is out of HWE. We used a chi-square goodness-of-fit test to determine the significance of the

deviation from HWE (Andrews, 2010). In addition to evaluating heterozygosity with tests for HWE, we used the genetic relatedness analysis function in GenAlEx6.5 to calculate the Lynch and Ritland Mean (i.e., LRM) estimator of pairwise genetic relatedness for all pairs of trees in all sample plots (Lynch and Ritland, 1999). The pairwise genetic relatedness analysis compares the genotype of each individual tree to all the other trees in the population individually. The genetic relatedness coefficient (i.e., LRM) ranges from a -1 to 1 with 0 not being related at all other than being the same species, to 1 full-siblings or parent-offspring. Note that LRM1 and LRM2 values calculated in GenAlEx 6.5 are used to calculate the mean relatedness estimator value (i.e., LRM) (Lynch and Ritland, 1999). Histogram and fitted probability density function of LRM pairs were generated for each plot, across plots within the reference treatment or natural selection plots, and across plots within the managed or artificial selection treatment. Additional statistical analysis and modeling were conducted to examine how pairwise genetic relatedness could be affected by free thinning. What follows is a detailed description of the statistical analysis used do assess our research questions.

3.2.4 Statistical Analysis

The objectives of our microsatellite marker analysis of white oak (*Quercus alba* L.) were to address several questions pertaining to the effects of forest management practices, specifically free thinnings, on genetic diversity. This section presents the null and research hypotheses for the six research questions to assess the effects of the specific treatments in our study on the white oak population. Our analysis addressed (1) whether heterozygosity of loci among thinned plots differ from that among reference plots, (2) how heterozygosity of loci differ among plots varying in number of subsequent thinnings, (3) how heterozygosity of loci differ among plots varying in residual basal area, (4) how heterozygosity of loci differ among plots delineated selection intensities determined from a combination of number of thinnings and residual basal area, (5) whether pairwise genetic relatedness means differed between thinned plots and reference (non-thinned) plots, and (6) how the response of pairwise genetic relatedness to geographic distance may be affected by various free thinning regimes.

To assess the effects of thinning regimes on the SSR allelic variation among our treatments, we used a chi-square goodness of fit tests as implemented in GenAlEx 6.5 software. Our first four research questions were addressed by comparing the expected and observed number of heterozygotes, with Hardy-Weinberg Equilibrium (HWE) test statistics (chi-squared values) and corresponding p-value being calculated for each locus among the treatments. Using a .05 significance level, we determined if there is a significant difference between the expected and observed number of heterozygotes (i.e., we can reject HWE) at each locus within each treatment type. For our null hypothesis, we stated the observed and expected number of heterozygotes will not differ significantly at loci between the specified treatments in white oak stands (i.e., HWE cannot be rejected). Thus, there will be no difference in the number of loci out of HWE with corresponding p-values above .05. As an alternative hypothesis, we stated that the observed and expected number of heterozygotes will differ significantly (i.e., HWE can be rejected). at loci between the specified treatment in white oak stands. Thus, there will be a difference in the number of loci out of HWE with corresponding p-values less than .05.

For our fifth research question examining genetic relatedness, we asked if the LRM (i.e., pairwise genetic relatedness) differs significantly between the pairs from reference plots experiencing natural selection (only) and the pairs from managed plots experiencing artificial selection (thinning). Our null hypothesis stated that the degree of genetic relatedness (LRM) will not differ significantly between the pairs from reference plots and pairs from managed plots. Alternatively, our research hypothesis stated the mean of genetic relatedness differs significantly between the mean LRM of pairs from reference plots and pairs from managed plots. We conducted one-way ANOVA in R using the *aov* function of the *stats* package to examine how the mean LRM of pairs in which both trees are from reference plots, pairs in which one tree is from a reference plot and the other tree is from a managed plot, and pairs in which both trees are from managed plots might differ. Pairwise differences in mean LRM between any two levels of the pairs type were assessed with the Turkey HSD method using the *TurkeyHSD* function of the *stats* package with a 95% confidence interval. Because there are multiple plots within the reference and managed treatment, pairs within the same treatment type may be further differentiated by whether the two trees are from the same plot or not. Therefore, we

further conducted a two-way ANOVA analysis to examine how both the treatment and plot affect the mean LRM for the subset of all pairs in which the two trees are either both from reference plots or both from managed plots. We used the *Anova* function of the *car* package to compute type-II and type-III analysis of variance for linear models in which LRM is a linear function of treatment type (reference vs. managed) and plot origin (same plot vs. different plot). To assess if there is a significant interaction between the treatment and plot effects, we used *Anova* function of the *stats* package to compare a linear model in which the interaction term is included and another linear model in which the interaction term is not included. We further used the *interaction2wt* function of the *HH* package to plot all main effects and two-way interactions in a four-panel graph.

To examine how spatial structure of LRM within a plot might be affected by thinning regimes, scatter plots of Euclidean (geographic) distance and LRM (genetic relatedness) of all possible pairs within each managed plot were generated. Ordinary least squares (OLS) regressions and quantile regressions were performed to examine how mean, 90%, 95% and 97% quantile of LRM may change as Euclidian distance of the two trees in a pair increases using the *lm* function of the *stats* package and the *rq* function of the *quantreg* package respectively. The fitted slope coefficients of various regressions were compared among various thinned plots to assess how the response of pairwise genetic relatedness to geographic distance may be affected by various thinning regimes.

3.3 Results

The 12 microsatellite (gSSR) markers were amplified across 225 samples (see Table 3.3) with the numbers of samples that each marker amplified ranging from 196 samples for marker T45 (13% missing data), to all 225 samples for marker T82. The number of alleles (observed) across all samples ranged from 5 alleles at markers T30 and T31, to 19 alleles at marker T76. The number of effective alleles, or the number of equally frequent alleles that it would take to achieve the same expected heterozygosity as in the study population, ranged from 1.955 alleles at marker T16 to 10.045 alleles at marker T76. Significantly, there were fewer heterozygotes observed for 8 of the 12 markers when compared to the expected number of heterozygotes. This is supported by

the significant p-values ($p<.05$) for deviation from Hardy-Weinberg Equilibrium. We then examined the 12 microsatellite markers delineated per plot instead of across the entire population. In general, more markers were out of HWE with a reduction of heterozygosity for plots with higher amounts of thinning (see Table 3.2 and Table 3.4). Some managed plots (i.e., plots that received a thinning), for example plot 10 that is delineated as a high thinning intensity plot, had substantially more markers out of HWE when compared to other plots and the reference plots. On the other hand, plot 18 which was delineated as a medium thinning intensity plot had more loci significantly out of HWE than any other plot in the study. Appendix A and Appendix B present the allele frequencies by loci and molecular analysis of variance by loci between reference and managed plots, respectively. With respect to allele frequency changes the following alleles generally decreased in frequency between reference and managed plots; Marker T19, alleles 159 and 165; Marker T30, allele 139; Marker T43, alleles 297, 301 and 303; Marker T45, alleles 247 and 257; Marker T75, alleles 291, 301 and 303; Marker T76, alleles 172, 181, 184 and 188; Marker T82, allele 285. It should be noted that the 7 previously listed markers that experienced a reduction in allele frequency were also 7 of the 8 markers that experienced a deficiency in numbers of heterozygotes and were not in HWE. With respect to the analysis of molecular variance, most of the genetic (allelic) variation exists within individual organisms and among individuals within populations (i.e., treatments in our study). Variation was low (1-3%) for all loci among treatments, meaning that little to no impact due to the reference -vs- managed (i.e., thinned) on the genetic structure of the delineated populations was observed. Overall, only 1 percent of variance was attributed to treatment among all loci (mean~1%) (see Figure 3.2).

Between reference and managed plots, the number of loci experiencing a reduction in heterozygosity relative to expected generally increased in the managed (i.e., thinned) plots compared to the reference (i.e., unthinned) plots (see Table 3.5). The reference plots had 3 loci not in Hardy-Weinberg Equilibrium and the managed plots had 8 loci (significant p-value $<.05$) not in HWE. This difference in the number of loci not in HWE (3 vs. 8) provides evidence for rejecting the null hypothesis. Our conclusion based on this evidence suggests that thinning vs. no thinning causes a reduction in heterozygosity as indicated by the larger number of loci with fewer heterozygotes relative

to the expected in the managed plots vs. the number of loci with fewer heterozygotes relative to expected in the reference plots. A statistical test of the difference in the number of loci out of HWE is needed to verify the observed difference's significance.

The effect that the number of thinnings had on heterozygosity was assessed based on our previously stated research question. We found that there is a general difference in the number of loci out of HWE between thinned once and thinned twice plots with a residual basal area post thinning of 75ft²/acre (see Table 3.9). Thinned once plots had 5 loci experience a significant reduction of heterozygosity relative to expected and thinned twice plots had only 1 locus experience a significant reduction in heterozygosity relative to expected. This suggested that the number of thinnings per se (1 vs. 2 to get to ~75 ft²/acre) was not likely causing the apparent effect (thinning vs. no thinning) noted above. That is to say the thinned vs. non-thinned effect, if determined significant, is not due to the number of thinnings. We then compared plots thinned once versus plots thinned three times, both with a residual basal area of 45ft²/acre (see Table 3.8). Thinned once plots had 3 loci experience a significant reduction of heterozygosity relative to expected and plots thinned three times had 5 loci experience a significant reduction in heterozygosity relative to expected. These results suggest that we would reject our null hypothesis for question 2, concluding that observed and expected number of heterozygotes relative to expected does differ significantly at loci between plots of white oak with different numbers of subsequent thinnings (i.e., 1 vs 3 to get to 45ft²/acre). Overall the number of thinnings effect is difficult to assess as we could compare 1 vs. 2 thinnings to get to a relatively higher residual basal area (75 fts/acre) and 1 vs. 3 thinnings to get to a lower basal area (45 ft2/acre). In one case, fewer thinning resulted in more loci to be out of HWE and in the other case more thinnings resulted in more loci to be out of HWE. This provides further evidence that the number of thinning per se are not impacting the degree of heterozygosity at least in any consistent manner. Next, we analyzed the effect of target residual basal area on heterozygosity.

We compared expected and observed heterozygosity at loci between the 45ft²/acre and 75ft²/acre residual basal area plots (see Table 3.7). There were 5 loci on plots with a residual basal area of 45ft²/acre and 5 loci on plots with a residual basal area of 75ft²/acre

experiencing a significant reduction in heterozygosity relative to expected. Meaning, both treatments had the same number of loci out of HWE. This provided evidence that thinning to different levels of residual basal area (i.e., 75 ft²/acre vs. 45 ft²/acre) does not impact the degree of heterozygosity with respect to number of loci having fewer heterozygotes than expected under HWE assumptions. For our final evaluation of the effects of artificial selection on heterozygosity, we combined treatments for number of thinnings with the residual basal area treatments to create thinning intensity categories, medium thinning intensity and high thinning intensity (see Table 3.2 and Table 3.6). The number of loci experiencing a reduction in heterozygosity relative to expected increased slightly as the thinning intensity increases. The reference plots had 3 loci not in Hardy-Weinberg Equilibrium, the medium thinning intensity had 6 loci not in HWE and the high thinning intensity had 5 loci not in HWE (significant p-value <.05 in bold). The overall reduction in heterozygosity relative to expected as measured by number of loci being out of HWE between reference (i.e., no thinning) and thinned (i.e., medium and high intensity levels) led us to conclude that the thinnings were reducing the heterozygosity. The reduction of heterozygosity at the loci does not appear to differ significantly between selection levels of medium and high thinning intensities. Meaning that the level of the thinning does not seem to matter (at the levels we tested), only if it was thinned to these levels or not. This result is consistent to our result above when comparing managed (i.e., thinned) plots to reference (i.e., unthinned) plots.

To address question 5 (in white oak stands, does the degree of genetic relatedness, as measured by LRM, differs significantly between pairs of trees from reference (not thinned) plots and managed (thinned) plots?), we found the following results. Appendix C shows a series of histograms and fitted probability density functions of LRM within each plot's pairs of trees and within each treatment's pairs of trees. While some plots do show general trends, there is no significance differences in these distributions. Across all plots of reference vs. managed treatments, LRM had the greatest density around 0, which is expected due to all sampled individuals being of the same species and on average unrelated. Negative values show pairs of trees that are less related than average. An LRM above 0.1 between a pair of trees can be considered a substantial level of relatedness in an outcrossing species such as white oak. Specifically, an LRM near 0.5 suggests a level

of relatedness expected for half-sibling relationship and near 1 for either full-siblings or parent and offspring. There does seem to be a general trend for the reference plots to have higher amounts of related pairs at the level expected for closer relatives as shown by a minor bump greater than 0.3 LRM (see Figure 3.3). Full siblings and parent offspring relationships are not distinguishable in this analysis, which is not an issue since these trees are an even-aged stand, so the parent-offspring relationship are unlikely. It should be mentioned that the reference plot C2 was the only plot between managed or reference plots that had an LRM value at the 0.5 level (see Figure 2 of Appendix C). One-way ANOVA shows that mean LRM differs among the pairs from reference plots (CC), managed plots (MM), and from mixed types (CM) with p-value of the corresponding *aov* function is less than 0.001. The 95% confidence interval Tukey HSD shows that the pairs from the reference plots (CC) had a significantly higher mean LRM than the pairs from managed plots (MM) as MM-CC is less than 0 (see Figure 3.4). The two-way ANOVA shows that both the treatment type (reference versus managed) and plot origin (trees from the same plot versus different plots) have a significant effect on the LRM. However, the interaction between the treatment and plot is not significant (see Figure 3.6). It also shows that whether pairs from the same plot or not explains a greater amount of variance than whether the pairs are from reference or managed treatment groups.

The LRM scatter plots over Euclidean distance shows that the LRMs decrease as the geographic distance increases for each managed plot (see Appendix D). All three quantile regressions of 97%, 95% and 90% showed a general decreasing trend in LRM as the distance between the pair increased within each managed plot and between all managed plots (see Figure 3.4) with most crossing the 0.1 level of relatedness at around 40 meters. This result indicates that related tress are not typically more than 40 meters from one another, forming related family groups across the landscape. It should also be noted that plot 10 had an abnormally steep slope compared to the other thinned plots (see Figure 3 of Appendix D). Plot 10 was considered to be a high thinning intensity plot but the other high thinning intensity plots did not have as steep of slope (see Table 3.2). However, a closer examination of number of white oak trees removed shows that plot 10 had the highest percentage (90%) of white oak trees removed among all high thinning intensity plots (Table 3.10). When plotting the 97% quantile regression slope coefficients

against the percentage of white oak trees removed across the five managed plots, it shows a general trend of decreasing the slope (i.e., steeper negative slope) as the percentage of white oak trees removed increases (see Figure 3.7). A simple univariate linear regression shows that this trend is marginally significant with p-value 0.058 and adjusted R-squared 0.67. The results show that genetic relatedness would be more quickly decrease as the pair's distance increases in the plots with higher artificial selection pressure. The results from both the one-way and two-way ANOVA analysis of LRM led us to reject our null hypothesis in question 5, the degree of genetic relatedness does differ significantly between the mean LRM of pairs from thinned plots and from unthinned plots.

3.4 Discussion

Selection, genetic drift, gene flow (migration) and mutations are the mechanisms that cause changes in allele frequencies and genetic structures of populations over time. When one or more of these forces are acting in a population, the population may violate the Hardy-Weinberg Equilibrium assumptions. The Hardy-Weinberg Equilibrium Theorem provides a null model for the study of evolution and the focus of population genetics is to understand the consequences of violating these assumptions (Hansson and Westerberg, 2002). Natural selection occurs when individuals with certain genotypes are more likely than individuals with other genotypes to survive and reproduce, and thus to pass on their alleles to the next generation. On the other hand, artificial selection (i.e., stand thinnings or harvests) may select for good or bad timber quality phenotypes [i.e., stand improvement or regeneration selects for good, while high grading selects for bad (Finkeldey and Ziehe, 2004)]. In this regard, fitness (i.e., the reproductive success of a genotype) is not considered in artificial selection but instead potentially, unintentionally reduced in the population with the selection against heterozygote individuals. Artificial selection due to management practices and industry demands can produce significant effects on the characteristics and extent of genetic diversity in natural tree populations. White oak (*Quercus alba* L.), a species of economic and ecological importance to eastern hardwood forests, is potentially subject to genetic degradation, via a reduction in heterozygosity and change in relatedness patterns in stands, due to preferential harvesting of timber-type. Low heterozygosity typically, and generally, means little genetic

variability and reduced fitness (Hansson and Westerberg, 2002). In this research project, we assessed the effects of silvicultural free thinnings, varying in intensity and timing, on the genetic diversity and relatedness patterns of white oak (*Quercus alba* L.) utilizing microsatellite marker analysis. Our research objective was to determine if free thinnings significantly change heterozygosity and genetic relatedness in white oak stands, leading to potential negative consequences for stand regeneration and white oak recruitment.

Between managed and reference plots, the managed plots had a total of 8 markers showing a significant deficiency in heterozygosity relative to expected and were out of HWE, while the reference plots had 3 markers that showed a deficiency in heterozygosity and were out of HWE. This broad look at the entire population supports our hypothesis that white oak thinnings do influence the number of heterozygous individuals relative to expected in thinned plots versus non-thinned plots. We examined thee breakdowns in thinning intensity and timing prescription, specifically how these different regimes affect heterozygosity and HWE in our white oak study population. When broken down between thinning intensities, the medium thinning intensity plots had 6 markers and high thinning intensity plots had 5 markers that showed a reduction in heterozygosity relative to expected compared to the reference plots that had 3 markers. Overall delineation of combined thinning intensities and timing into two categories of high thinning intensity and medium thinning intensity showed that they are relatively equal in that the medium selection intensity only had one more locus that showed a reduction in heterozygosity relative to expected. When residual basal area grouping is compared, thinnings with a residual basal area of 45ft²/acre (thinned more heavily) and 75ft²/acre (thinned less heavily) both had 5 marker loci showing a reduction in heterozygosity relative to expected and were out of HWE, suggesting no effect of 45ft²/acre vs. 75ft²/acre of residual basal area on number of loci not in HWE. When the number of thinnings is compared, 3 repeated thinnings resulted in 5 loci with a reduction of heterozygosity relative to expected and were out of HWE. While 1 thinning, initial thinning only, resulted in 3 loci showing a loss of heterozygosity relative to expected and being out of HWE. All thinnings in this comparison were thinned to a residual 45ft²/acre. In the analysis of 2 repeated thinning versus 1 thinning both to 75ft²/acre, twice thinned plots show 1 locus with a reduction of heterozygosity relative to expected and plots thinned

once had 5 loci showing a reduction in heterozygosity relative to expected. This result should be taken with caution, considering that data for thinned twice only exists in one plot with only 17 trees. We suggest that this result should be reanalyzed with additional sampled trees as we only had one plot data for 2 thinnings because plot 17 DNA samples were not available for analysis. We also suggest preforming Exact tests of HWE (Raymond and François, 1995) instead of the chi-square tests implemented in GenAlEx 6.5 to better evaluate the significance of the departures from HWE. In addition, the statistical significance in the numbers of loci showing significant HWE test between treatments should be determined, potentially with resampling based tests.

In summary, our heterozygosity results show that free thinning in white oak dominated stands within the Cumberland Plateau Region has the potential to decrease the genetic diversity of natural populations via the preferential removal of heterozygous individuals. Although we did not find a significant change in allele frequencies attributed to thinning treatments, we did see a significant reduction in heterozygosity relative to that expected under HWE at the loci level when comparing reference and thinned plots. In addition, the techniques and methods applied in this study could inform similar studies in other economically and ecologically important species assisting in conservation and preservation of our forest resources. Future studies should aim to build upon this study to look at multiple age classes of white oak and at multiple populations of white oak.

When observed heterozygosity is lower than expected, we seek to attribute the discrepancy to forces such as reduced population sizes increasing inbreeding and/or selection against heterozygous individuals from artificial selection (Bosse *et al.*, 2019). Inbreeding increases the frequency of homozygotes at the expense of heterozygotes, causing an overall reduction of genetic diversity in the population (Duminil, Hardy and Petit, 2009). Inbreeding is also usually associated with a reduction in growth and survival performance known as inbreeding depression (Savolainen and Kärkkäinen, 1992). However, we do not believe that our results of reduced heterozygosity are caused by inbreeding because the trees sampled are from one age class post thinning. In order to fully evaluate the effects that free thinnings have on inbreeding, the new age class of seedlings should be sampled and compared. A deficiency of heterozygotes could also be

caused by what is termed the Wahlund effect, due to a mix of two sub-populations that mate mostly among themselves but overlap. By selecting for or against certain traits, effectively creating two subpopulations that are interbreeding could occur resulting in a Wahlund effect within the population (Grant, 2010). Also, a multiple age class system is associated with creating a Wahlund effect (i.e., different age classes flowering at the same time) could be caused by free thinnings. However, to attribute our results to a Wahlund effect, like the inbreeding effects, we would need to sample and compare the multiple age classes existing within the population. Another explanation finds its roots in the original thinning prescription implemented in 1961. The first criteria for artificial selection was to remove wolf trees from the plots. As previously mentioned, wolf trees are often faster growing and take up a disproportionate amount of canopy space (Makrickiene, Drossler and Brazaitis, 2016). By targeting the removal of wolf trees, managers could be removing the most fit individuals (from a genetic selection perspective) from the population to reduce competition with higher valued trees. In addition, forest managers may assume that the removal of wolf trees promotes the release of seedlings from the seed bank and the competition of these seedlings results in only the fastest growing and most competitive individuals (i.e., highest associated fitness) eventually making it into the canopy classes. However, we suggest that by removing the wolf trees from stands, forest managers are removing the highest fitness associated genes from the population. Even though the associated fitness of wolf trees does not match the desired characteristics of timber phenotypes, wolf trees still provide valuable genetic attributes to future generations. When writing silvicultural prescriptions, we propose that the long-term maintenance of a proportion of wolf trees might be added to management plans. We suggest that artificial selection processes in thinnings should aim to match natural selection to reduce the overall negative effect on heterozygosity and key related fitness traits such as canopy cover, growth rate and masting, while achieving timber quality and tree spacing criteria.

It was originally hypothesized that gene flow (i.e., wind pollination and acorn movement by wildlife) would be enough to counter any reduction in genetic diversity due to management practices. To assess the geneflow, we would recommend sampling the seedling age class and comparing multi age classes (i.e. multiple generations). A study

assessing the genetic diversity of pedunculate oak (*Quercus robur* L.) found that the naturally generating seedlings did not experience a significant loss of genetic diversity compared to adult cohorts (Vranckx *et al.*, 2013). The researchers did find that the acorn dispersal was restricted and created a fine scale genetic structure across the landscape. We found that the effective pollen dispersal and pair-wise relatedness between trees drop significantly at around 40 meters in our sample plots. To better assess the pollen flow, effective pollen donors and effect pollen movement distance we suggest using the method implemented in TWOGENER (Smouse and Sork, 2004). As habitat fragmentation and isolated populations become more common with increased anthropogenic influences even in the most remote forested regions, it still becomes critical to combat a reduction in genetic diversity. Similar to our results, Vranckx *et al*. (2013) reported an increase in correlated paternity with lower effective population sizes and lower tree densities. Our results showed a general trend for our reference plot pairs to have higher levels of genetic relatedness, meaning more related individuals existed collectively on the reference plots compared to the thinned plots. The reference plots had the lowest tree densities compared to the thinned plots (see Table 3.2). The reference plots had apparent half-sibling (LRM≈.5) and these family relationships were much more apparent in the reference plots than the managed plots. The results from our LRM analysis also indicate that as distance increases the level of genetic relatedness decreases, with related groups (LRM>.15) residing within a short distance (generally <40m). Even though distance has the highest effect on the genetic relatedness of white oak, there is still a general reduction of relatedness in thinned stands compared to non-thinned stands. Meaning, due to the removal of crowded trees, related trees (i.e. half siblings, full-siblings and parent-offspring) are preferentially thinned, leading to the reduction of plot-level relatedness in thinned stands. Another explanation would be that natural selection selects for members from the same heritage as families with favorable adaptations survive and reproduce more. This possibility would also explain part of the reason why we saw a reduction in heterozygosity relative to expected in the thinned stands where artificial selection occurred. If related groups are thinned out and selected against in thinned stands, then we would also see a reduction in the genetic relatedness as in our results.

3.5 Conclusion

Overtime repeated thinning regimes, coupled with fragmentation, could prove to be detrimental to the long-term fitness and survivability of our natural forest resources. Our results presented here show a general trend for a reduction in heterozygosity relative to expected and lower genetic relatedness of thinned stands. We suggest that the removal of high fitness related individuals during the thinnings caused an overall reduction in observed heterozygosity relative to expected. To determine if heterosis or relative fitness is being lost from the population, it is important to sample and compare the next generation of seedlings. To provide a well-balanced experimental design, sample size and accurate depiction of actual selection level treatments (i.e., based on proportion of white oaks selected) we recommend using the alternative treatment delineation (see Table 3.10) for additional analyses. Initially, we delineated treatment based on the thinning prescription per plot, but the alternative delineation provides a more accurate analysis of the selection that took place. We recommend re-evaluating the allelic data based on the new delineation and adding Exact tests for HWE and resampling methods to compare the number of loci experiencing a loss of heterozygosity between treatments.

Table 3.1 Plot Design and Layout

Treatment delineation for the McKee study site sample plots. Thinning intensity grouping by plot ID, including the target basal area (BA) post thinning, the amount thinned in subsequent thinnings as the degree of thinning (0 meaning none removed, 1 meaning half of the increase in percent stocking removed and 2 meaning all of the increase in percent stocking removed), the number of times the plot was thinned, the years the thinnings occurred and the type of selection per plot. An * indicates that the plot was sampled but not used in subsequent analysis due to low amount of high enough concentration and quality genomic DNA.

Thinning Intensity	Plot	Target BA	Degree of Thinning	Number of Thinnings	Thinning Years	Selection Type
No Thinning	Reference1	n/a	0	0	n/a	Natural
	Reference2	n/a	0	0	n/a	Natural
Medium Thinning	Plot 3	75	0	1	1961	Artificial
	Plot 17*	75	1	2	1961, 1987	Artificial
	Plot 18	45	0	1	1961	Artificial
High Thinning	Plot 4	45	1	3	1961, 1971, 1987	Artificial
	Plot 10	75	2	2	1961, 1987	Artificial
	Plot 11	45	2	3	1961, 1971, 1987	Artificial

Table 3.2 Plot Level Basal Area Statistics

Plots and their associated total basal areas post thinning in 1961 and in 2016, the percent of basal area attributed to white oak post thinning in 1961 and in 2016, the number of white oak removed in subsequent thinnings in 1971 and in 1987, the number of white oak present on the plot post initial thinning, the number of white oak present on the plot in 2016, the artificial selection level delineated for our analysis, the number of foliage samples successfully collected from each plot and the number of loci significantly out of Hardy Weinberg Equilibrium (HWE). It is of value to note that the # of white oak in 2016 does not match the number of DNA samples because some individual tree genomic DNA was not of high enough quality and/or concentration.

Plot	Total BA per acre after Initial Thinning 1961	Total BA per acre 2016	% WO BA 1961	% WO BA 2016	WO # 1961	# of WO Removed 1971	# of WO Removed 1987	WO # 2016	Selection Level	# DNA Samples	# of Loci ou of HWE
Reference1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	19	Reference	19	1
Reference2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	18	Reference	18	3
Plot 3	66.17	119.56	76.60%	70.92%	634	0	0	65	Medium	57	1
Plot 18	44.86	119.45	83.90%	73.65%	394	0	0	60	Medium	43	5
Plot 4	43.07	123.48	93.45%	81.73%	400	42	21	56	High	52	3
Plot 10	72.19	146.24	45.30%	19.35%	394	0	2	20	High	17	4
Plot 11	48.51	116.54	53.76%	41.15%	200	46	25	20	High	19	4

Table 3.3 Twelve Microsatellite Loci Over Whole Population

Twelve microsatellite markers (gSSRs) across 225 samples with locus microsatellite marker name; FWD, forward primer sequence; REV, reverse primer sequence; motif with number of repeats; N, number of samples that the primer pair amplified on; Na, number of alleles; Ne, number of effective alleles; Ho, observed heterozygosity; He, expected heterozygosity; HW P-value, p-value significance for Hardy-Weinberg Equilibrium.

Locus	FWD and REV	Motif	# Repeats	N	Na	Ne	Ho	He	HW P-value
T16	FWD-CACGACGTTGTAAAACGACCACCAGCACCTCACTTCTCC REV- GTTTCTTGGATTTGGGTTTGGGTTGGG	gaa	10	217	6	1.955	0.512	0.488	0.654
T19	FWD-CACGACGTTGTAAAACGACGGGCTTGCTTGCATTACTCC REV- GTTTCTTAGATTTGTTCTCTCAATCTCGCC	tta	10	221	11	4.696	0.52	0.787	**0.000**
T22	FWD-CACGACGTTGTAAAACGACCGCACTCTCTCTCAAGGACC REV- GTTTCTTGCTTTCCTTCATGAGCCG	aag	16	217	11	4.285	0.82	0.767	0.279
T30	FWD-CACGACGTTGTAAAACGACTTATCCTGTGGTGCCGTAGC REV- GTTTCTTGCGTTGTCGAACTAGAACCG	gtt	10	219	8	2.567	0.589	0.61	**0.000**
T31	FWD-CACGACGTTGTAAAACGACCTTGCATTGGAAGCAGTCGG REV- GTTTCTTTCATGAAGAACACACACGATGC	gaa	8	219	5	3.009	0.648	0.668	0.981
T32	FWD-CACGACGTTGTAAAACGACGATTCGATTTCTTCCGCGGC REV- GTTTCTTCTGGAACTCCACGCTAAAGC	gtg	7	221	5	2.163	0.462	0.538	**0.000**
T43	FWD-CACGACGTTGTAAAACGACTGGAGAACGCGAAGATGAGG REV- GTTTCTTCACCGACACTGCTACATTTGC	ct	8	201	15	9.18	0.731	0.891	**0.000**
T45	FWD- CACGACGTTGTAAAACGACCAGAATCTCCTTCTCCGCC REV- GTTTCTTACGTAAGGAGAACCGTAATCAGG	ag	10	196	14	7.399	0.577	0.865	**0.000**

Table 3.3 (continued)

T66	FWD-CACGACGTTGTAAAACGACTAGGAACTTCAACGCCACGG REV- GTTTCTTCAAACAGGCCAACTCTAACCC	tc	9	222	11	3.597	0.743	0.722	0.881
T75	FWD-CACGACGTTGTAAAACGACTGGAGAACGCGAAGATGAGG REV- GTTTCTTCACCGACACTGCTACATTTGC	tc	16	208	18	9.899	0.774	0.899	**0.001**
T76	FWD-CACGACGTTGTAAAACGACACTAAGAGGAGCACCAACGC REV- GTTTCTTTGGCTGTAGAAAGATGGTGTGG	ag	8	222	19	10.045	0.779	0.9	**0.000**
T82	FWD-CACGACGTTGTAAAACGACTTCAACACCTCAACTTCACG REV- GTTTCTTTCAAACCCGTGACATGACCC	ag	10	225	11	5.404	0.751	0.815	**0.002**

Table 3.4 Twelve Loci by Each Plot

Twelve markers across 7 plots with: locus, marker name; N, number of samples that the primer pair amplified on; Na, number of alleles; Ne, number of effective alleles; Ho, observed heterozygosity; He, expected heterozygosity; HW P-value, p-value significance for Hardy-Weinberg Equilibrium. Values in bold indicate significant deviations from H-W Equilibrium.

Plot	Locus	N	Na	Ne	Ho	He	HW P-Value
Plot 10	**T16**	49	5.000	2.253	0.571	0.556	0.733
	T19	50	8.000	4.583	0.540	0.782	**0.000**
	T22	51	9.000	3.245	0.765	0.692	0.999
	T30	51	7.000	3.748	0.784	0.733	0.892
	T31	49	4.000	2.902	0.694	0.655	0.872
	T32	52	4.000	2.118	0.365	0.528	**0.000**
	T43	49	13.000	9.509	0.776	0.895	0.056
	T45	38	14.000	6.926	0.579	0.856	**0.000**
	T66	52	8.000	2.424	0.577	0.587	0.972
	T75	50	14.000	10.893	0.820	0.908	0.298
	T76	52	12.000	7.097	0.750	0.859	**0.022**
	T82	52	10.000	4.499	0.808	0.778	0.327
Plot 11	**T16**	16	3.000	2.024	0.438	0.506	0.772
	T19	16	5.000	4.267	0.438	0.766	**0.024**
	T22	16	5.000	3.459	0.813	0.711	0.768
	T30	16	3.000	1.210	0.188	0.174	0.982
	T31	17	3.000	2.995	0.647	0.666	0.984
	T32	17	3.000	2.359	0.588	0.576	0.887
	T43	16	11.000	9.481	0.938	0.895	0.769
	T45	14	8.000	5.521	0.571	0.819	0.636
	T66	17	7.000	4.158	0.941	0.760	0.536
	T75	16	11.000	9.309	0.938	0.893	0.407
	T76	17	11.000	5.558	0.765	0.820	0.910
	T82	17	7.000	4.940	0.706	0.798	0.286
Reference1	**T16**	19	4.000	1.462	0.368	0.316	0.987
	T19	19	6.000	5.049	0.579	0.802	0.143
	T22	19	6.000	3.422	0.789	0.708	0.658
	T30	19	5.000	1.989	0.421	0.497	0.566
	T31	19	3.000	2.168	0.368	0.539	0.111
	T32	19	4.000	2.292	0.684	0.564	0.903
	T43	17	10.000	4.857	0.765	0.794	0.678
	T45	18	11.000	5.635	0.667	0.823	0.329

Table 3.4 (continued)

	T66	18	9.000	4.629	0.889	0.784	0.938
	T75	19	10.000	5.348	0.474	0.813	**0.011**
	T76	19	11.000	6.505	0.737	0.846	0.813
	T82	19	9.000	6.389	0.842	0.843	0.312
Reference2	**T16**	19	4.000	1.778	0.421	0.438	0.728
	T19	19	8.000	4.628	0.579	0.784	**0.000**
	T22	17	7.000	3.461	0.941	0.711	0.953
	T30	19	4.000	1.470	0.211	0.320	**0.036**
	T31	19	4.000	3.297	0.895	0.697	0.150
	T32	18	3.000	2.182	0.611	0.542	0.500
	T43	16	9.000	6.481	0.750	0.846	0.703
	T45	19	11.000	6.333	0.684	0.842	0.060
	T66	18	6.000	3.146	0.667	0.682	0.870
	T75	14	10.000	6.759	0.786	0.852	0.806
	T76	18	10.000	6.056	0.722	0.835	**0.027**
	T82	19	8.000	3.741	0.579	0.733	0.516
Plot 3	**T16**	17	4.000	2.000	0.471	0.500	0.094
	T19	18	6.000	4.101	0.556	0.756	0.238
	T22	17	8.000	4.817	0.765	0.792	0.375
	T30	16	3.000	1.759	0.438	0.432	0.849
	T31	18	3.000	2.571	0.556	0.611	**0.042**
	T32	17	3.000	2.072	0.529	0.517	0.849
	T43	12	9.000	6.400	0.667	0.844	0.205
	T45	16	10.000	5.020	0.625	0.801	0.096
	T66	18	6.000	3.176	0.611	0.685	0.389
	T75	14	9.000	6.877	0.857	0.855	0.565
	T76	18	11.000	8.757	0.944	0.886	0.313
	T82	18	5.000	3.071	0.667	0.674	0.343
Plot 18	**T16**	56	5.000	1.740	0.500	0.425	0.563
	T19	57	8.000	3.692	0.404	0.729	**0.000**
	T22	57	8.000	4.675	0.895	0.786	0.215
	T30	55	7.000	3.141	0.727	0.682	**0.011**
	T31	56	4.000	3.017	0.625	0.669	0.853
	T32	56	4.000	2.089	0.429	0.521	0.758
	T43	51	13.000	7.347	0.725	0.864	0.121
	T45	53	11.000	7.120	0.604	0.860	**0.000**
	T66	56	9.000	3.874	0.875	0.742	0.704
	T75	54	13.000	7.496	0.796	0.867	**0.001**
	T76	57	13.000	8.428	0.772	0.881	**0.000**
	T82	57	8.000	4.695	0.737	0.787	0.558
Plot 4	**T16**	41	4.000	2.077	0.610	0.518	0.786
	T19	42	8.000	4.292	0.619	0.767	**0.000**

Table 3.4 (continued)

T22	40	10.000	5.161	0.775	0.806	0.935
T30	43	6.000	2.341	0.628	0.573	0.997
T31	41	4.000	2.996	0.683	0.666	0.877
T32	42	3.000	1.708	0.381	0.415	0.858
T43	40	11.000	7.080	0.600	0.859	**0.000**
T45	38	10.000	5.543	0.421	0.820	**0.000**
T66	43	7.000	4.037	0.721	0.752	0.424
T75	41	15.000	8.342	0.732	0.880	0.242
T76	41	15.000	10.639	0.805	0.906	0.294
T82	43	9.000	5.238	0.791	0.809	0.321

Table 3.5 Twelve Loci Between Reference and Managed Plots
Twelve markers across reference (control) or natural selection plots compared to managed or artificial selection plots. Each row shows a microsatellite marker with N, number of samples that the primer pair amplified on; Na, number of alleles; Ne, number of effective alleles; Ho, observed heterozygosity; He, expected heterozygosity; HW P-value, p-value significance for Hardy-Weinberg Equilibrium. Values in bold indicate significant deviations from H-W Equilibrium.

Treatment	Locus	N	Na	Ne	Ho	He	HW P-Value
Control	**T16**	36	5.000	1.888	0.444	0.470	0.282
	T19	37	8.000	4.548	0.568	0.780	**0.000**
	T22	34	9.000	4.250	0.853	0.765	0.584
	T30	35	4.000	1.615	0.314	0.381	**0.024**
	T31	37	4.000	3.012	0.730	0.668	0.161
	T32	35	3.000	2.138	0.571	0.532	0.368
	T43	28	11.000	7.919	0.714	0.874	0.566
	T45	35	12.000	6.049	0.657	0.835	**0.000**
	T66	36	7.000	3.256	0.639	0.693	0.894
	T75	28	12.000	8.711	0.821	0.885	0.666
	T76	36	12.000	8.498	0.833	0.882	0.434
	T82	37	8.000	4.199	0.622	0.762	0.504
Managed	**T16**	181	5.000	1.967	0.525	0.492	0.918
	T19	184	11.000	4.587	0.511	0.782	**0.000**
	T22	183	11.000	4.275	0.814	0.766	0.354
	T30	184	8.000	2.809	0.641	0.644	**0.000**
	T31	182	5.000	3.002	0.632	0.667	0.904
	T32	186	5.000	2.155	0.441	0.536	**0.000**
	T43	173	15.000	9.071	0.734	0.890	**0.000**
	T45	161	14.000	7.423	0.559	0.865	**0.000**
	T66	186	11.000	3.656	0.763	0.726	0.504
	T75	180	18.000	9.866	0.767	0.899	**0.000**
	T76	186	19.000	9.615	0.769	0.896	**0.000**
	T82	188	11.000	5.529	0.777	0.819	**0.015**

Table 3.6 Twelve Loci by Selection Level

High selection level versus medium selection level thinning treatment delineation. Each treatment was analyzed across 12 loci. Each row has the associated treatment; medium selection level or high selection level; Locus, loci microsatellite marker name; N, number of samples that the primer pair amplified on; Na, number of alleles; Ne, number of effective alleles; Ho, observed heterozygosity; He, expected heterozygosity; HW P-value, p-value significance for Hardy-Weinberg Equilibrium. Values in bold signify a significant variation from H-W Equilibrium. The relevant reference (control) treatment results are located in Table 3.5.

Treatment	Locus	N	Na	Ne	Ho	He	HW p-value
High Selection	**T16**	84	5.000	2.033	0.500	0.508	0.971
	T19	85	9.000	5.023	0.529	0.801	**0.000**
	T22	86	9.000	3.587	0.779	0.721	0.987
	T30	86	7.000	2.794	0.593	0.642	0.979
	T31	85	4.000	2.926	0.612	0.658	0.791
	T32	88	5.000	2.214	0.477	0.548	**0.000**
	T43	82	14.000	9.830	0.805	0.898	**0.026**
	T45	70	14.000	7.796	0.600	0.872	**0.000**
	T66	87	9.000	3.121	0.713	0.680	0.512
	T75	85	15.000	10.344	0.765	0.903	0.144
	T76	88	17.000	8.422	0.750	0.881	0.758
	T82	88	11.000	5.867	0.795	0.830	**0.036**
Medium Selection	**T16**	97	5.000	1.899	0.546	0.473	0.353
	T19	99	10.000	3.991	0.495	0.749	**0.000**
	T22	97	11.000	4.929	0.845	0.797	0.459
	T30	98	7.000	2.785	0.684	0.641	**0.009**
	T31	97	4.000	3.016	0.649	0.668	0.947
	T32	98	4.000	2.046	0.408	0.511	0.472
	T43	91	13.000	7.618	0.670	0.869	**0.000**
	T45	91	12.000	6.694	0.527	0.851	**0.000**
	T66	99	10.000	4.128	0.808	0.758	0.647
	T75	95	16.000	8.391	0.768	0.881	**0.002**
	T76	98	17.000	10.316	0.786	0.903	**0.000**
	T82	100	10.000	5.159	0.760	0.806	0.462

Table 3.7 Twelve Loci by Residual Basal Area

Residual basal area treatment. Each row shows treatment; 45ft2/acre basal area or 75ft2/acre basal area; Locus, loci microsatellite marker name; N, number of samples that the primer pair amplified on; Na, number of alleles; Ne, number of effective alleles; Ho, observed heterozygosity; He, expected heterozygosity; HW P-value, p-value significance for Hardy-Weinberg Equilibrium. Values in bold signify a significant variation from H-W Equilibrium. The relevant reference (control) treatment results are located in Table 3.5.

Pop	Locus	N	Na	Ne	Ho	He	HW p-value
45ft2/acre	**T16**	109	5.000	2.039	0.550	0.510	0.961
	T19	111	10.000	4.922	0.577	0.797	**0.000**
	T22	110	10.000	4.025	0.773	0.752	0.760
	T30	113	8.000	2.959	0.664	0.662	0.991
	T31	109	5.000	2.950	0.633	0.661	0.919
	T32	113	5.000	2.132	0.425	0.531	**0.000**
	T43	106	13.000	8.896	0.708	0.888	**0.000**
	T45	94	14.000	6.847	0.532	0.854	**0.000**
	T66	113	11.000	3.399	0.681	0.706	0.988
	T75	110	16.000	9.821	0.727	0.898	**0.002**
	T76	112	17.000	9.442	0.768	0.894	0.089
	T82	114	11.000	5.675	0.807	0.824	0.075
75ft2/acre	**T16**	72	5.000	1.850	0.486	0.460	0.510
	T19	73	8.000	4.010	0.411	0.751	**0.000**
	T22	73	8.000	4.518	0.877	0.779	0.278
	T30	71	7.000	2.533	0.606	0.605	**0.001**
	T31	73	4.000	3.015	0.630	0.668	0.853
	T32	73	4.000	2.158	0.466	0.537	0.793
	T43	67	14.000	8.313	0.776	0.880	0.241
	T45	67	12.000	7.713	0.597	0.870	**0.000**
	T66	73	9.000	3.981	0.890	0.749	0.300
	T75	70	15.000	8.589	0.829	0.884	**0.022**
	T76	74	14.000	8.868	0.770	0.887	0.000
	T82	74	8.000	4.938	0.730	0.797	0.078

Table 3.8 Twelve Loci Between One and Three Number of Thinnings
Plots thinned once versus thinned three times to a total residual basal area of 45ft²/acre. Each row shows the treatment; 3 thinnings or 1 thinning; Locus, loci microsatellite marker name; N, number of samples that the primer pair amplified on; Na, number of alleles; Ne, number of effective alleles; Ho, observed heterozygosity; He, expected heterozygosity; HW P-value, p-value significance for Hardy-Weinberg Equilibrium. Values in bold signify a significant variation from H-W Equilibrium. The relevant reference (control) treatment results are located in Table 3.5.

Pop	Locus	N	Na	Ne	Ho	He	HW p-value
3 Thins	**T16**	68	5.000	2.013	0.515	0.503	0.839
	T19	69	9.000	4.993	0.551	0.800	**0.000**
	T22	70	9.000	3.418	0.771	0.707	0.995
	T30	70	7.000	3.336	0.686	0.700	0.994
	T31	68	4.000	2.882	0.603	0.653	0.793
	T32	71	5.000	2.165	0.451	0.538	**0.000**
	T43	66	13.000	9.066	0.773	0.890	0.009
	T45	56	14.000	7.111	0.607	0.859	**0.000**
	T66	70	9.000	2.862	0.657	0.651	0.986
	T75	69	14.000	9.837	0.725	0.898	**0.025**
	T76	71	15.000	7.743	0.746	0.871	0.153
	T82	71	11.000	5.595	0.817	0.821	0.062
1 Thin	**T16**	41	4.000	2.077	0.610	0.518	0.786
	T19	42	8.000	4.292	0.619	0.767	**0.000**
	T22	40	10.000	5.161	0.775	0.806	0.935
	T30	43	6.000	2.341	0.628	0.573	0.997
	T31	41	4.000	2.996	0.683	0.666	0.877
	T32	42	3.000	1.708	0.381	0.415	0.858
	T43	40	11.000	7.080	0.600	0.859	**0.000**
	T45	38	10.000	5.543	0.421	0.820	**0.000**
	T66	43	7.000	4.037	0.721	0.752	0.424
	T75	41	15.000	8.342	0.732	0.880	0.242
	T76	41	15.000	10.639	0.805	0.906	0.294
	T82	43	9.000	5.238	0.791	0.809	0.321

Table 3.9 Twelve Loci Between One and Two Number of Thinnings
Plots thinned once versus thinned twice times to a total residual basal area of 75ft²/acre. Each row shows the treatment; 2 thinnings or 1 thinning; Locus, loci microsatellite marker name; N, number of samples that the primer pair amplified on; Na, number of alleles; Ne, number of effective alleles; Ho, observed heterozygosity; He, expected heterozygosity; HW P-value, p-value significance for Hardy-Weinberg Equilibrium. Values in bold signify a significant variation from H-W Equilibrium. The relevant reference (control) treatment results are located in Table 3.5.

Pop	Locus	N	Na	Ne	Ho	He	HW p-value
Thinned 1 Time	**T16**	56	5.000	1.740	0.500	0.425	0.563
	T19	57	8.000	3.692	0.404	0.729	**0.000**
	T22	57	8.000	4.675	0.895	0.786	0.215
	T30	55	7.000	3.141	0.727	0.682	**0.011**
	T31	56	4.000	3.017	0.625	0.669	0.853
	T32	56	4.000	2.089	0.429	0.521	0.758
	T43	51	13.000	7.347	0.725	0.864	0.121
	T45	53	11.000	7.120	0.604	0.860	**0.000**
	T66	56	9.000	3.874	0.875	0.742	0.704
	T75	54	13.000	7.496	0.796	0.867	**0.001**
	T76	57	13.000	8.428	0.772	0.881	**0.000**
	T82	57	8.000	4.695	0.737	0.787	0.558
Thinned 2 Times	**T16**	16	3.000	2.024	0.438	0.506	0.772
	T19	16	5.000	4.267	0.438	0.766	**0.024**
	T22	16	5.000	3.459	0.813	0.711	0.768
	T30	16	3.000	1.210	0.188	0.174	0.982
	T31	17	3.000	2.995	0.647	0.666	0.984
	T32	17	3.000	2.359	0.588	0.576	0.887
	T43	16	11.000	9.481	0.938	0.895	0.769
	T45	14	8.000	5.521	0.571	0.819	0.636
	T66	17	7.000	4.158	0.941	0.760	0.536
	T75	16	11.000	9.309	0.938	0.893	0.407
	T76	17	11.000	5.558	0.765	0.820	0.910
	T82	17	7.000	4.940	0.706	0.798	0.286

Table 3.10 Alternative Thinning Intensity Treatment Delineation

Alternative treatment delineation of the McKee study site sample plots. Thinning intensity grouping by plot ID, including the year it was thinned or year 1961, the number of white oak WO present post thinning, the year data was last collected in 2016 or 55 years later, the number of white oak present in 2016, 55 years passed since the initial thinning, the change in number of white oak, the percentage of white oak removed (cut during thinning) or absent (natural mortality) since 1961, the initial treatment delineation per plot, the sample size of each plot, the new sample size for treatment analysis, the new plot ID for associated treatments and the selection level for the new plot ID. The new sample size is different from the old sample size in that it split the original plots into two plots (i.e., plot M3 became M3a and M3b). The * at plot 17 indicates that the data for this plot is missing due to insufficient quality and quantity of extracted genomic DNA. ** indicates unknown but would only include natural mortality. This would likely be on the order of 10 to 20 %.

Plot ID	WO # Year 0 (1961)	WO # Year 55 (2016)	WO # Change	% WO Removed	Old Selection Level (Table 3.2)	Sample Size	New Sample Size	New Plot ID	New Selection Level
3	634	130	-504	79%	Med	57	29	M3a	Med
							28	M3b	Med
4	400	112	-288	72%	Med	52	26	M4a	Med
							26	M4b	Med
10	394	40	-354	90%	High	17	17	H10	High
11	200	40	-160	80%	High	19	19	H11	High
17*	470	94	-376	80%	High	50	25	H17a	High
							25	H17b	High
18	394	120	-274	70%	Med	43	22	M18a	Med
							21	M18b	Med
Reference1				**	Reference	19	19	C1	None
Reference2				**	Reference	18	18	C2	None

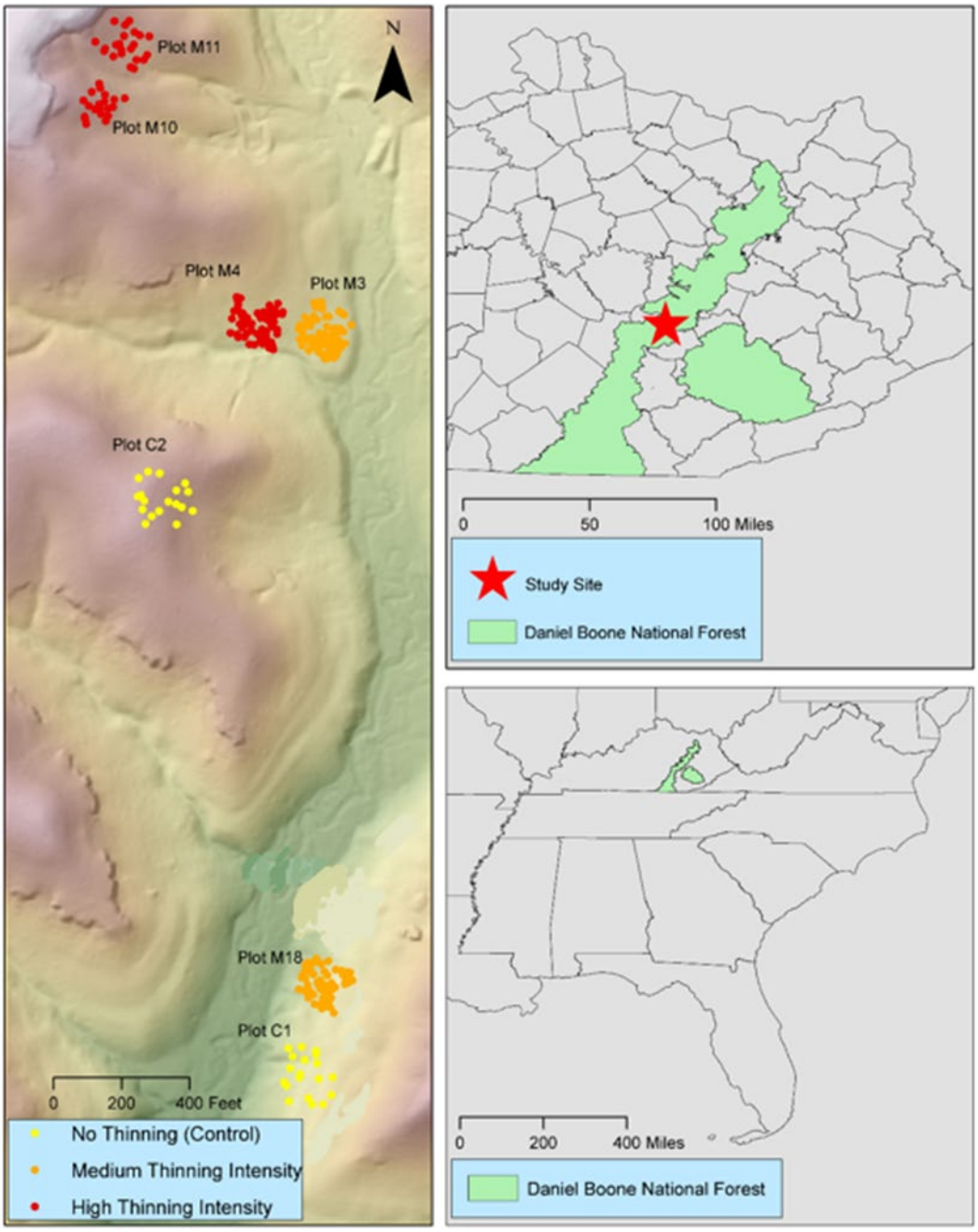


Figure 3.1 Study Location and Plot Treatment
Study location and plot treatments. Study location map; Daniel Boone National Forest in relation to the south east united states, Daniel Boone National Forest in relation to Kentucky counties and specific study location within Daniel Boone National Forest, then the specific plot and tree locations at the study site with corresponding treatments.

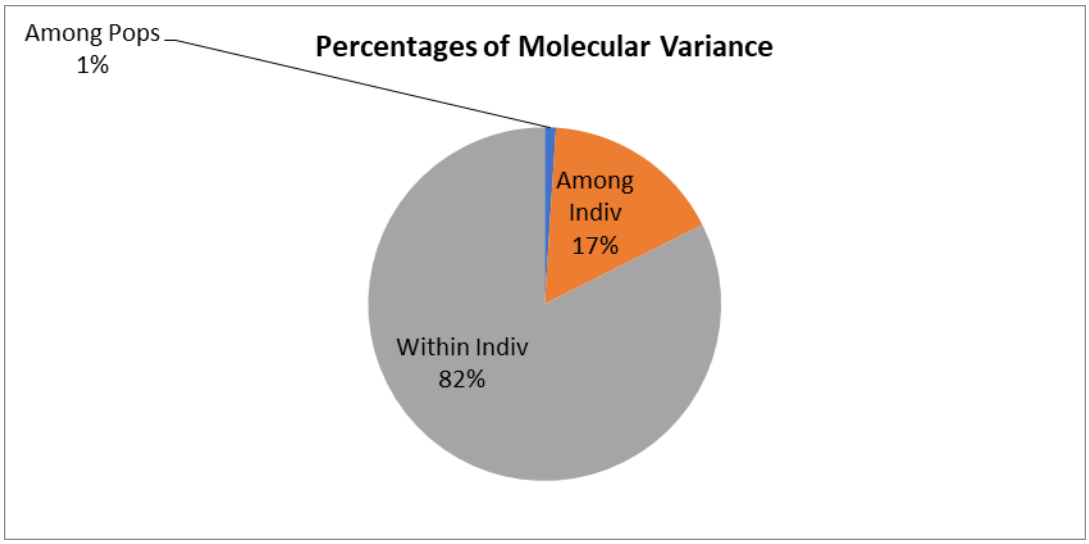


Figure 3.2 Molecular Variance Between Reference and Managed Plots
Molecular variance over all plots and loci between managed or artificial selection plots and reference plots. Specifically, this pie chart shows the percentage of molecular variance over all loci for reference versus managed plots for within individuals (grey), among individuals (orange) and among populations (blue).

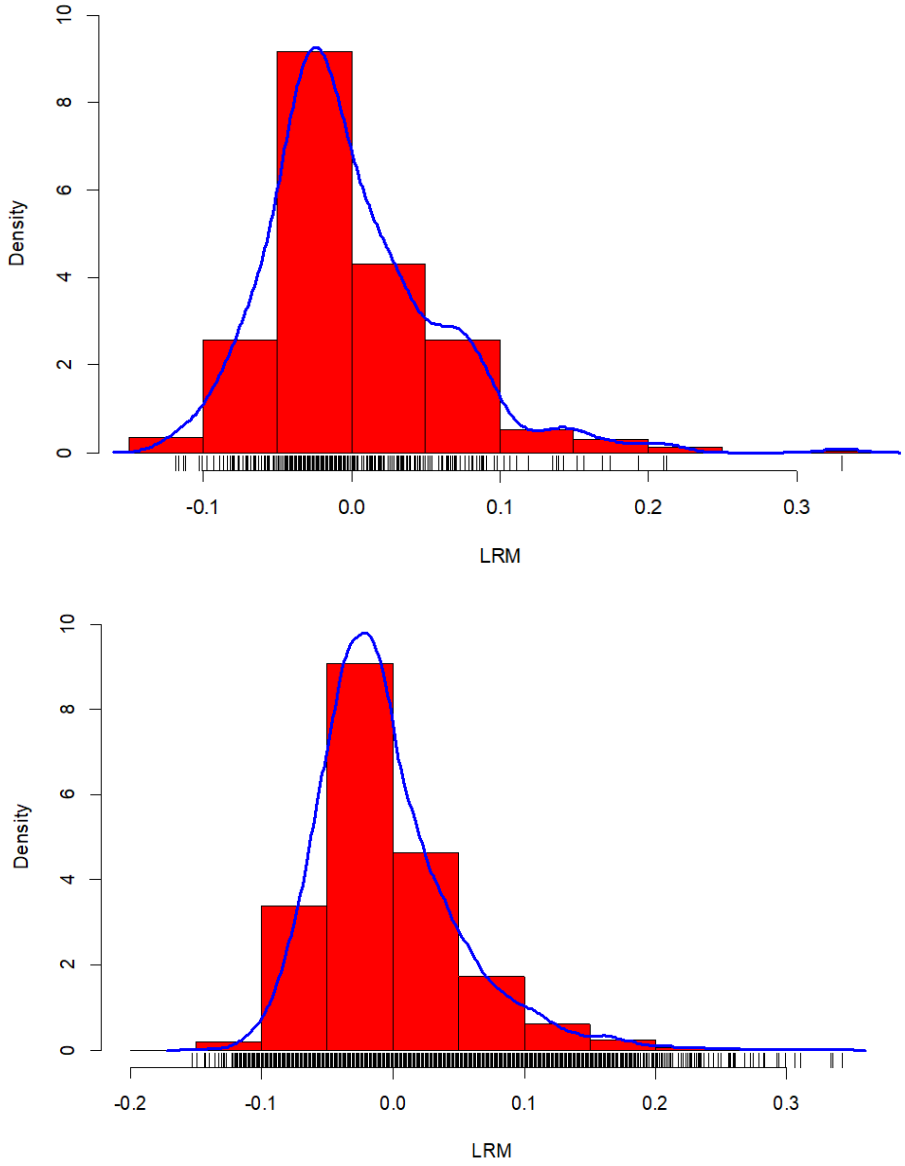


Figure 3.3 LRM Distribution Between Reference and Managed Plots
The LRM histogram and fitted probability density function for each pairwise genetic relatedness between white oak trees across all reference plots (top) and all managed plots (bottom). Each pairs measure of genetic relatedness is shown on the x axis and the density at which that measure of genetic relatedness within the plot occurs is shown on the y axis. An LRM value of above 0.1 is considered significant for relatedness between two trees. An increase in the density of LRM is expected around 0 on the x axis for comparisons of individuals from the same species, as they all share a baseline of relatedness.

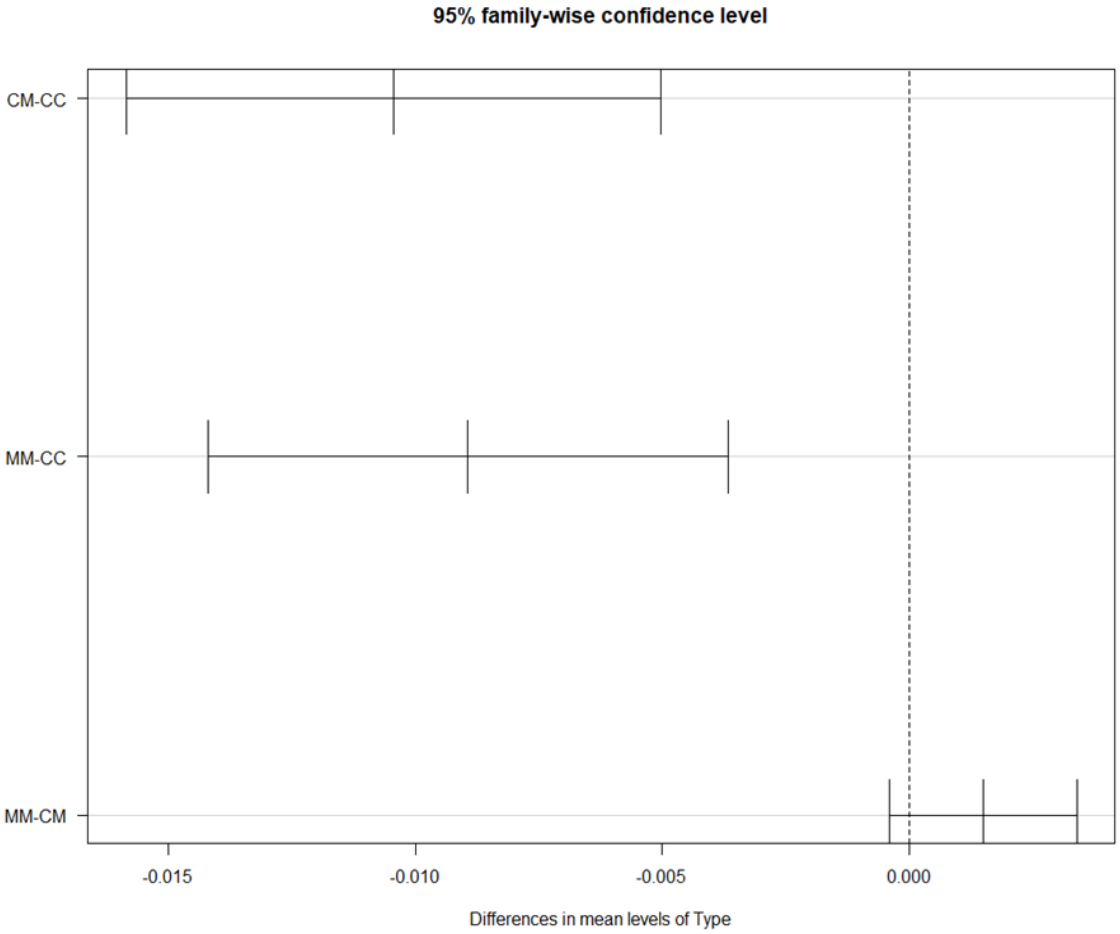


Figure 3.4 Tukey HSD Test of Pair-wise Mean LRM Differences Among 3 Groups
The 95% pairwise differences in mean LRM for each pairwise genetic relatedness between any two levels of the treatment type; Reference vs Reference (CC), Reference vs Managed (CM), and Managed vs Managed (MM). Each pair mean measure of genetic relatedness is shown on the x axis and the treatment type is shown on the y axis. This figure shows that pairs are most related when both individuals come from the reference plots, followed by pairs between the reference and managed plots and pairs from managed plots last.

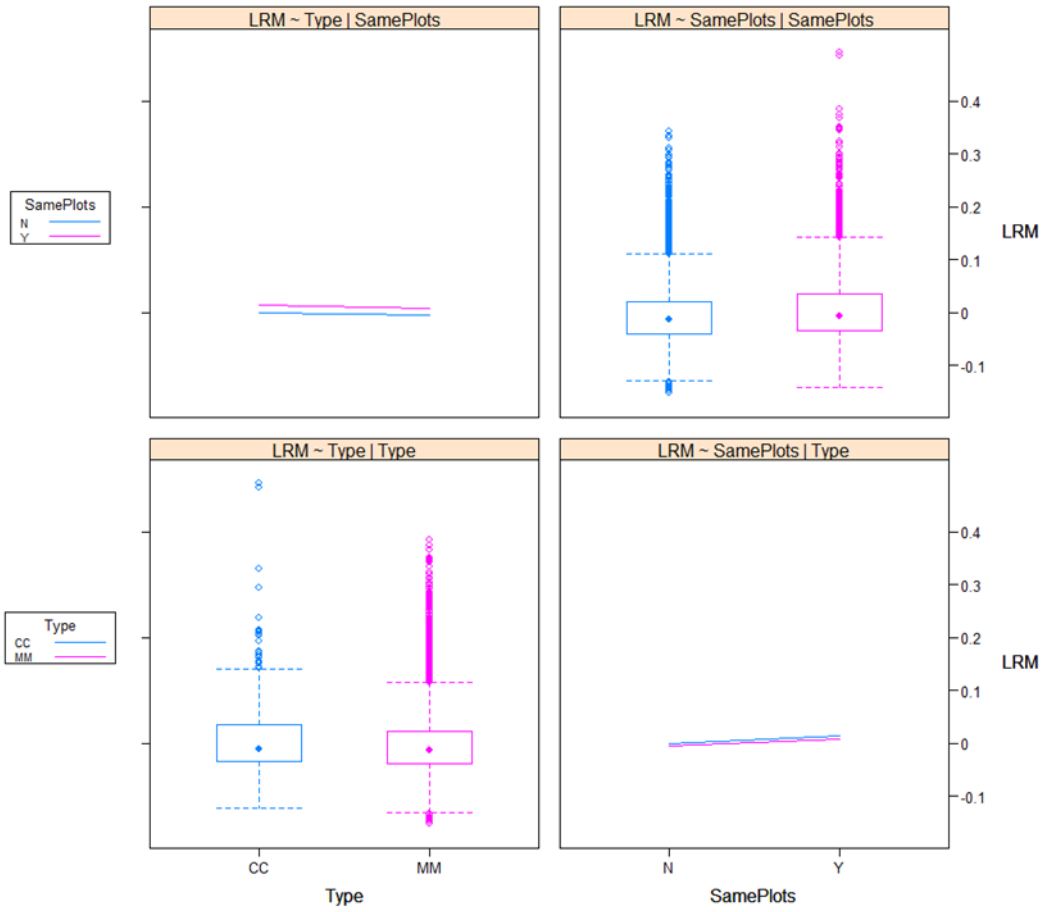


Figure 3.5 Main Effects & Two-way Interactions of Treatment & Plot Origin on LRM
LRM main effects and 2-way interactions in mean LRM of pairs from the same plots or from two different plots, either in the reference treatment (CC) or managed treatment (MM). Each pair mean measure of genetic relatedness is shown on the y axis and the treatment type is shown on the x axis. N indicates that they are not of the same plot and Y indicates that the pair is in the same plot.

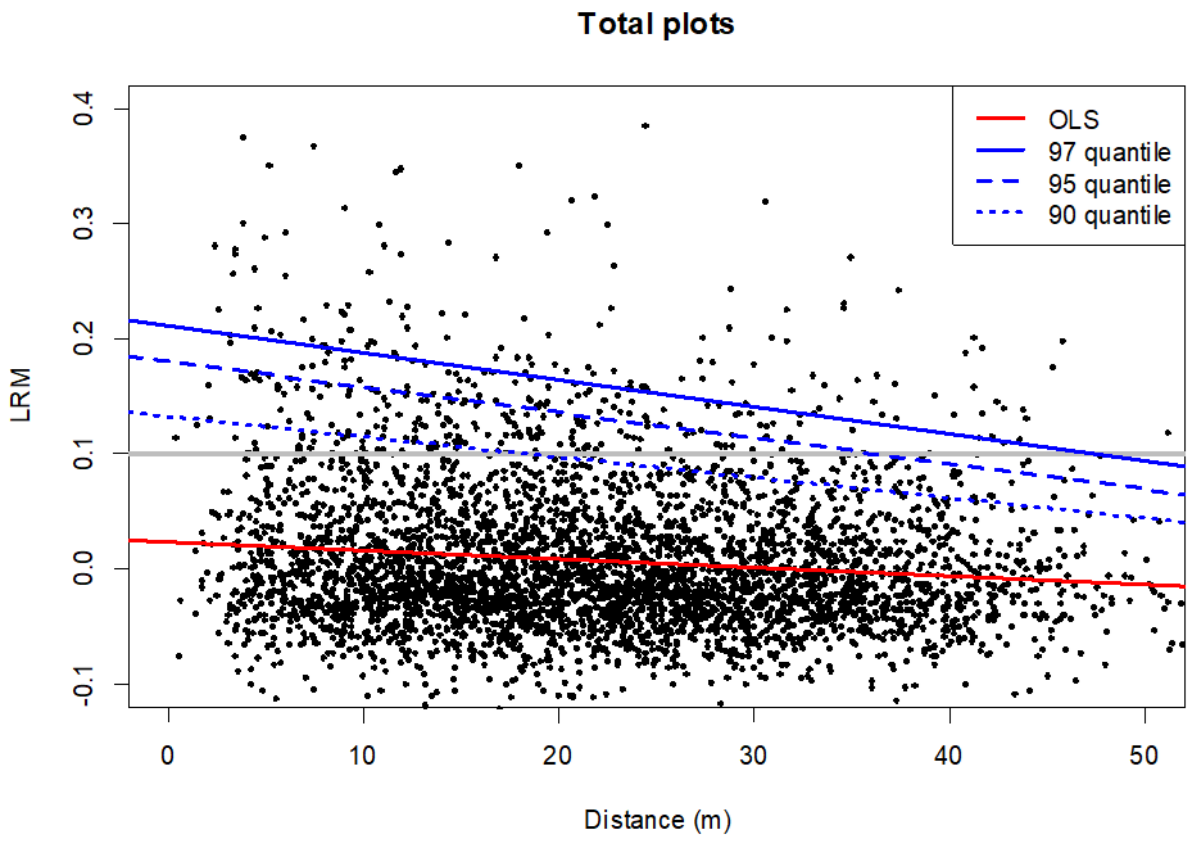


Figure 3.6 Quantile Regression of LRM as a Function of Euclidean Distance
Quantile regression scatter plot for pairwise genetic relatedness across all managed plots. The LRM for each pairwise genetic relatedness between white oak trees across all managed plots shown in a scatter plot with a 97%, 95% and 90% quantile regression. Each pairs measure of genetic relatedness is shown on the y axis and the distance (m) between the 2 individuals in a pair is on the x axis. An LRM value of above 0.1 is considered significant for relatedness between two trees. An increase in the density of LRM is expected around 0 on the y axis for comparisons of individuals from the same species, as they all share a baseline of relatedness. The solid grey line was added at the LRM 0.1 to signify a cut off for consideration of relatives.

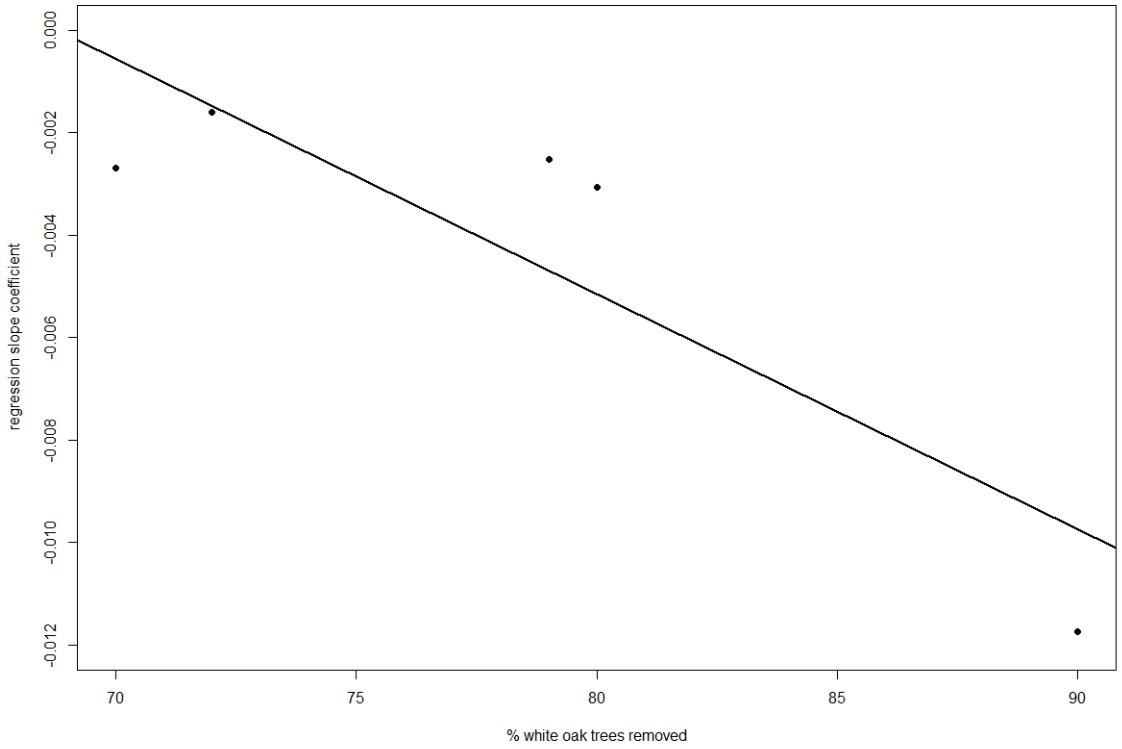


Figure 3.7 Scatter Plot 97% Quantile Regression Slope

Scatter plot of 97% quantile regression slope over percentage of white oak trees removed for each of the five managed plots. Solid line is the fitted trend line of the corresponding univariate linear model.

CHAPTER 4. SYNOPSIS

4.1 Summary

During this research project, we aimed to identify the effects that forest stand thinnings had on the genetic diversity, genetic structure and relatedness of white oak stands. Specifically, if varying levels of artificial selection plots would have significant effects on the observed heterozygosity compared to plots experiencing natural selection. A research study formerly implemented by the USDA Forest Service on the Daniel Boone National Forest in 1961, served as the sampling site and basis for our study. A total of 275 white oak foliage samples were collected at the study site. DNA extractions were successfully completed on 225 foliage samples, while samples from one plot failed to yield useable amounts of DNA. We tested 84 predicted microsatellite marker PCR primer pairs (sequences provided by the Hardwood Genomics Project) on three white oak DNA samples that we used as reference samples for allele calling. After applying a series of screening filters, we identified an implementation set of 16 polymorphic gSSR markers and recommend their use in future conservation genetic studies of white oak. Using 12 of the most consistent and clearly amplifiable microsatellite markers, we evaluated the degree of genetic variation differing between unthinned reference plots and thinned managed plots in white oak stands on the study site. We then analyzed the differences in genetic diversity between number of subsequent thinnings, between differing residual thinning basal areas and between varying levels of thinning intensities. Lastly, we examined the relationship of genetic relatedness between pairs of white oak trees within and across plots and treatments. In summary, for 12 gSSR markers, we studied allele frequency and heterozygosity at each marker and pairwise relatedness between trees over markers across 225 trees sampled from a population of white oak subject to varying types and intensities of selection.

4.2 Significant Results

Based on the results of our analysis, we feel that there are three main research highlights that are the most insightful and significant to this research project. Our first

significant result is covered in chapter 2, where we successfully identified 16 highly polymorphic microsatellite markers for white oak (*Quercus alba* L.). By doing so we reached our first objective of identifying polymorphic microsatellite markers for assessing genetic diversity of white oak stands and populations. To our knowledge, no polymorphic microsatellite markers previously existed for *Q. alba* prior to this study. Our second significant result is covered in chapter 3, where it can be concluded that heterozygosity was generally reduced relative to expected (i.e., assuming Hardy Weinberg Equilibrium) in the artificial selection, or thinned plots, compared to the reference plots (i.e., natural selection only). From our data it can also be concluded that the number of subsequent thinnings generally produce a higher number of loci experiencing a significant reduction in heterozygosity relative to expected. Our third result, which was also covered in chapter 3, shows us that the mean genetic relatedness between pairs of white oak trees is generally reduced in the thinned plots compared to the non-thinned (i.e., reference) plots. In addition, we observed general trends for the genetic relatedness between pairs of trees to decrease as distance between trees increased. The noteworthy results of this study indicate that thinning and the number of subsequent thinnings, jointly decrease the heterozygosity relative to expected and genetic relatedness of white oak stands. Further analysis is needed to determine the patterns of the absolute changes in heterozygosity as well as basing the comparison on the proportion of white oaks removed (via thinning) rather than the intended thinning treatments. In conclusion, we were able to successfully identify 16 polymorphic microsatellite markers and use 12 of them to observe an overall reduction of their heterozygosity relative to expected and a decrease in genetic relatedness between pairs of trees in thinned stands compared to non-thinned stands of white oak. Our results show in general that natural selection (i.e., reference plots) in white oak stands tend to produce identifiable related groups of trees potentially possessing more fitness due to higher levels of heterozygosity relative to expected compared to stands that experienced artificial selection (i.e., thinned plots).

4.3 Future Research

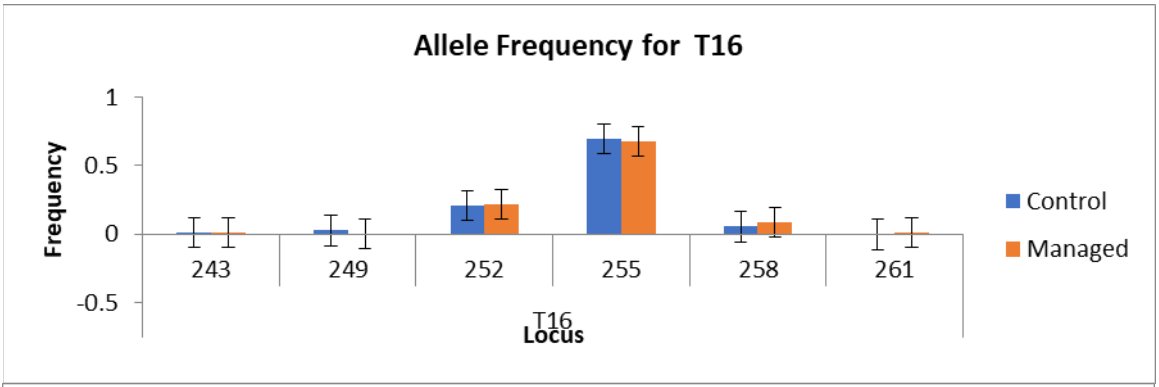
During the extent of the research project, we had to continuously adapt and remain flexible while adhering to our original objectives. Although we believe that the project

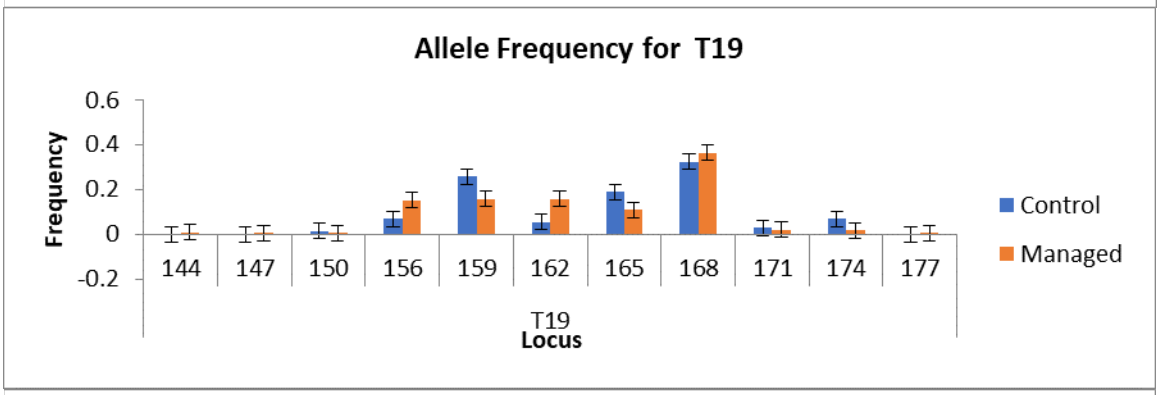
was successful, there are a few insights and suggestions worth noting here. Firstly, the original USDA Forest Service Study provided us with a foundation and opportunity to sample a thinned white oak population in an already implemented experiment with pre-existing long-term residual tree data. Without these pioneering researchers and this long-term study, we could have not built or been inspired to ask the questions that we addressed in this thesis. The long-term thinning study provided us with a basis to formulate questions concerning silvicultural thinnings and their impact on the genetic diversity for a valuable forest tree species. However, the absence of reference plots (i.e., control plots) from the original 1961 implementation left us with a predicament of not having long term phenotypic data for unthinned or natural selection plots to compare in our study. We would suggest locating additional stands (i.e., beyond our two ad hoc reference plots) of unthinned white oak since at least 1961, for a more balanced comparison. Secondly, the extent of our research effectively examined white oak of dominant, co-dominant and intermediate canopy classes, not white oak seedlings. Thus, our sample trees did not allow us to observe the genetic diversity of the white oak progeny or across multiple generations. We would suggest sampling seedlings in the plots, via a nested plot layout, to get a multigenerational snapshot of the population. By doing so, we would be able to see if the reduction in heterozygosity relative to expected that we observed have an impact on the genetic diversity and population structure (including levels of inbreeding and gene flow) the new generation of seedlings. Thirdly, we suggest adding another study population to the McKee data for a more comprehensive and significant evaluation of the impact of thinning on genetic diversity. The Baldrock study site, also on the Daniel Boone National Forest, has plots with matching treatments and plot design. It is our belief that adding this second population would strengthen the study and add statistical significance by providing more sample individuals, while also providing a more comprehensive study on the effects of silvicultural thinnings across populations. Fourthly, we encourage studies throughout the range of white oak. On a large spatial scale, we would be able to observe species-wide diversity and population structure and determine if the collective genetic resilience is enough to combat varying strategies and intensities of artificial selection. The application of landscape-scale genomics could become critical for the long-term sustainability, conservation and

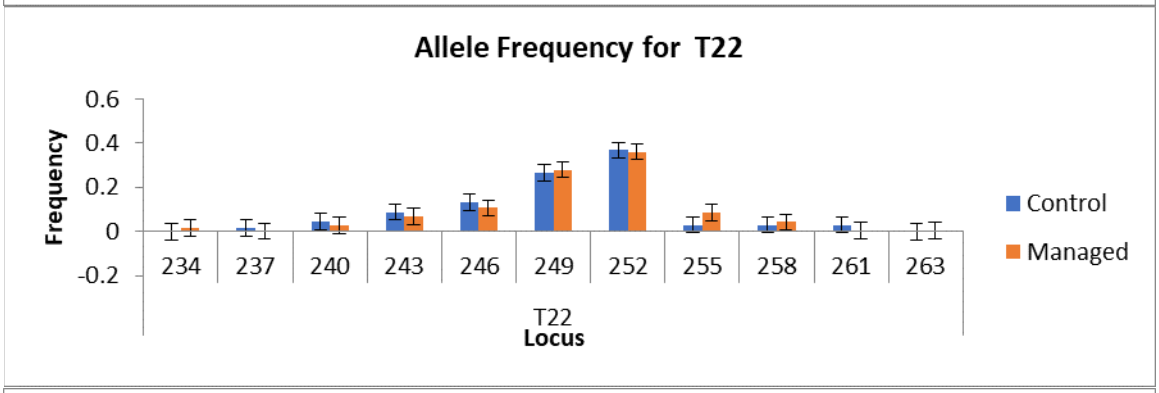
preservation of forest tree species, especially those with high ecologic and economic value such as white oak.

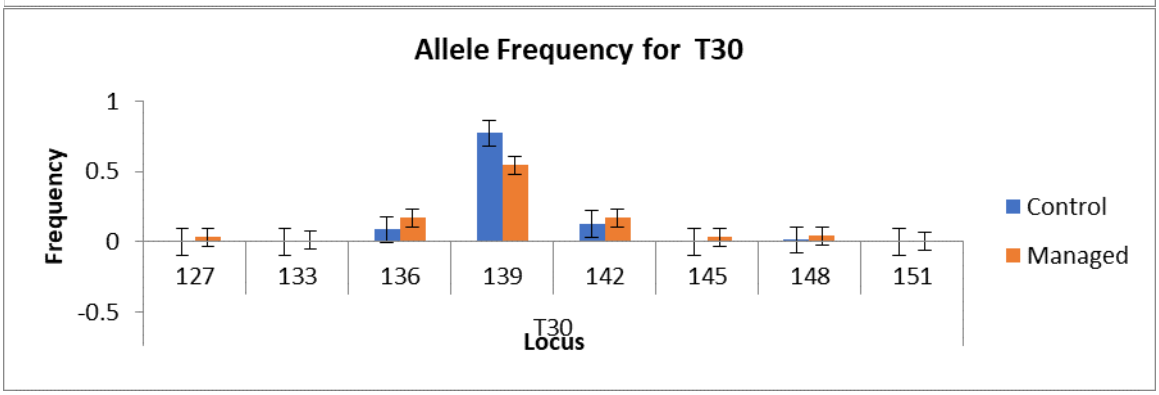
APPENDICES

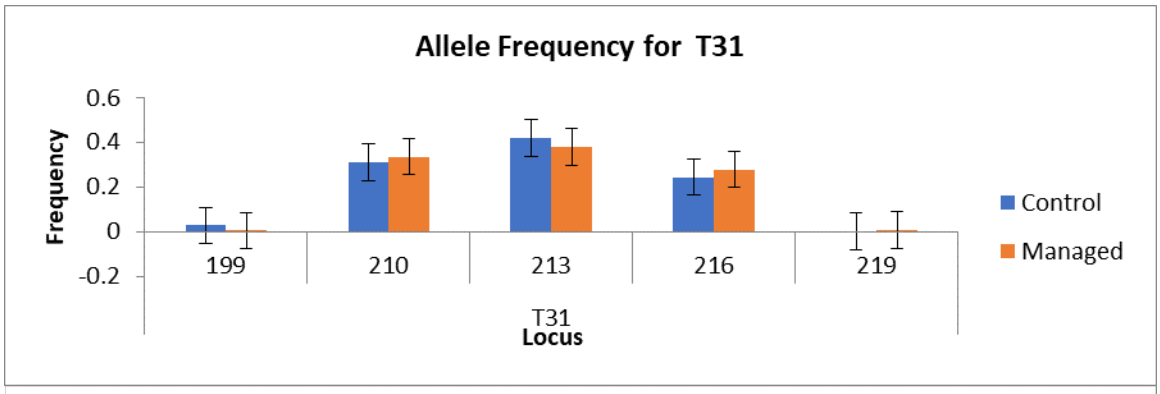
APPENDIX A. [ALLELE FREQUENCIES BETWEEN REFERENCE AND MANAGED PLOTS]

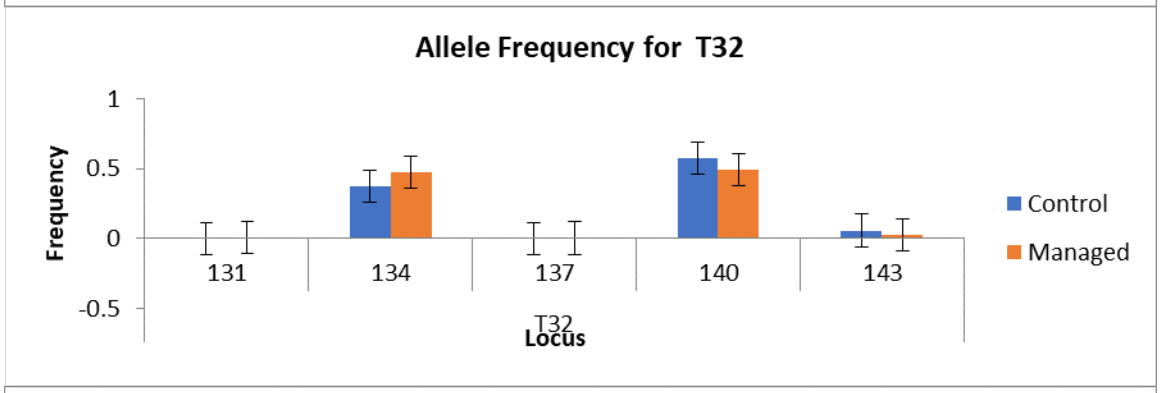


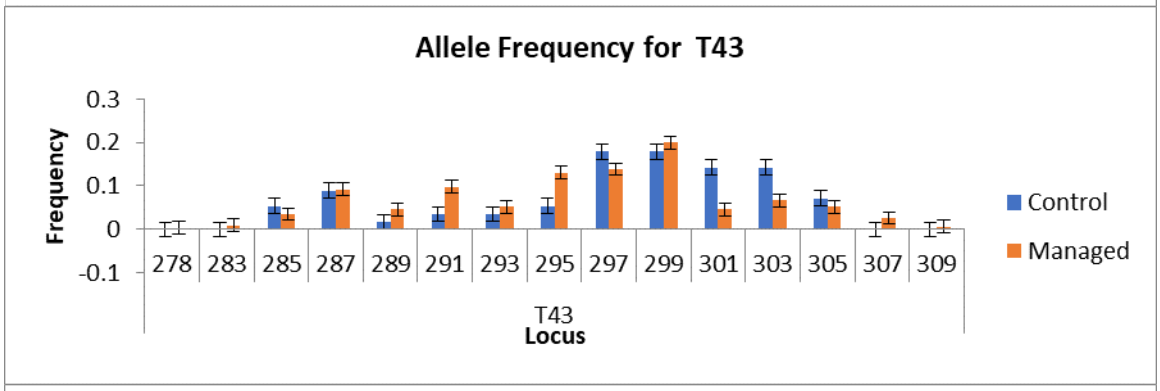


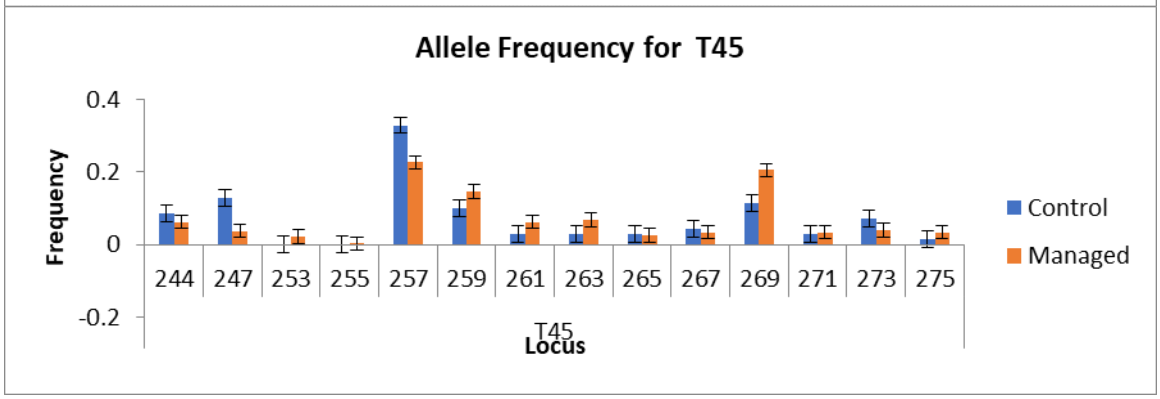


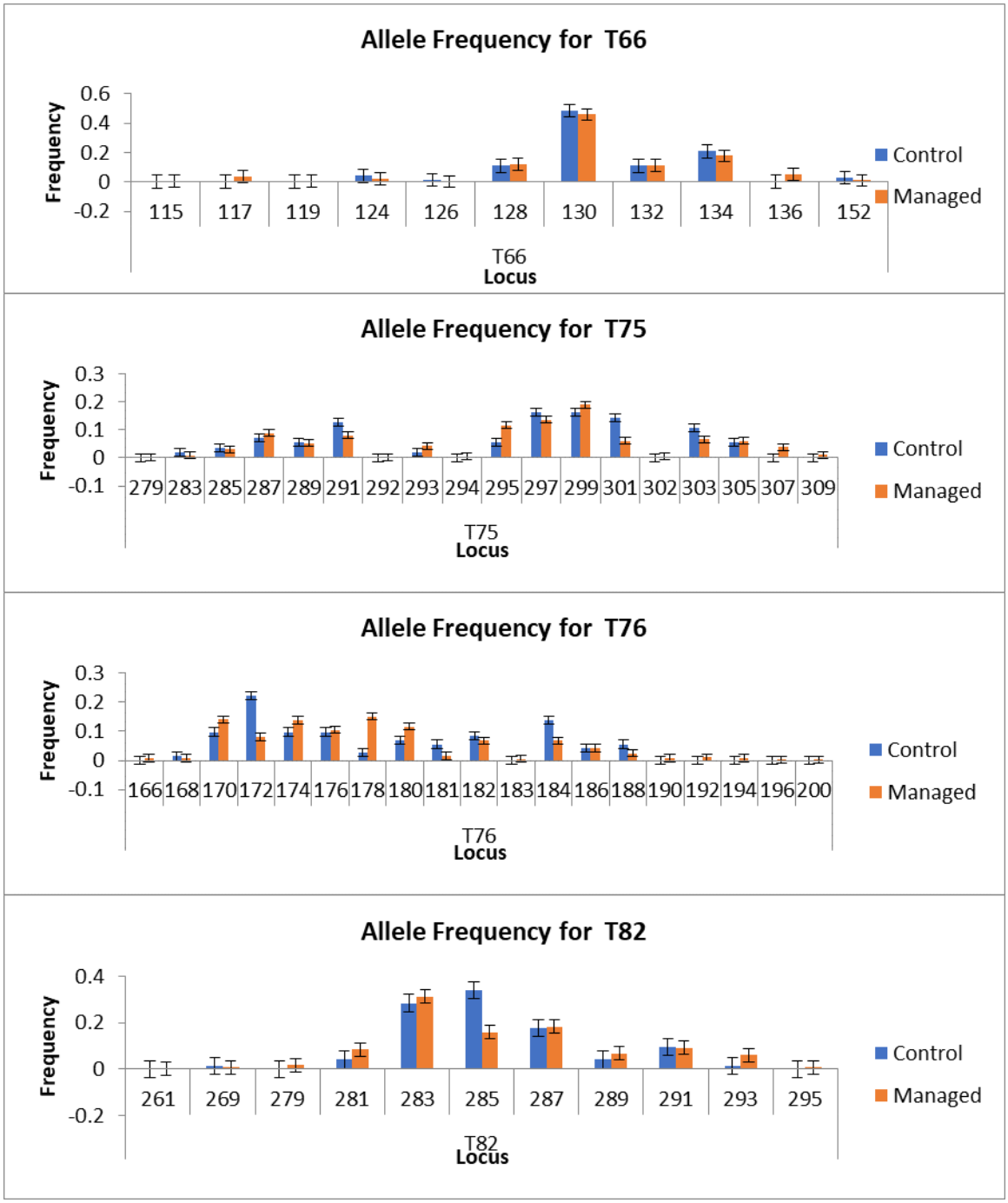



Allele Frequency for T31
Frequency
0.6
0.4
0.2
0
-0.2
199
210
213
216
219
T31
Locus
Control
Managed


Allele Frequency for T32
Frequency
1
0.5
0
-0.5
131
134
137
140
143
T32
Locus
Control
Managed

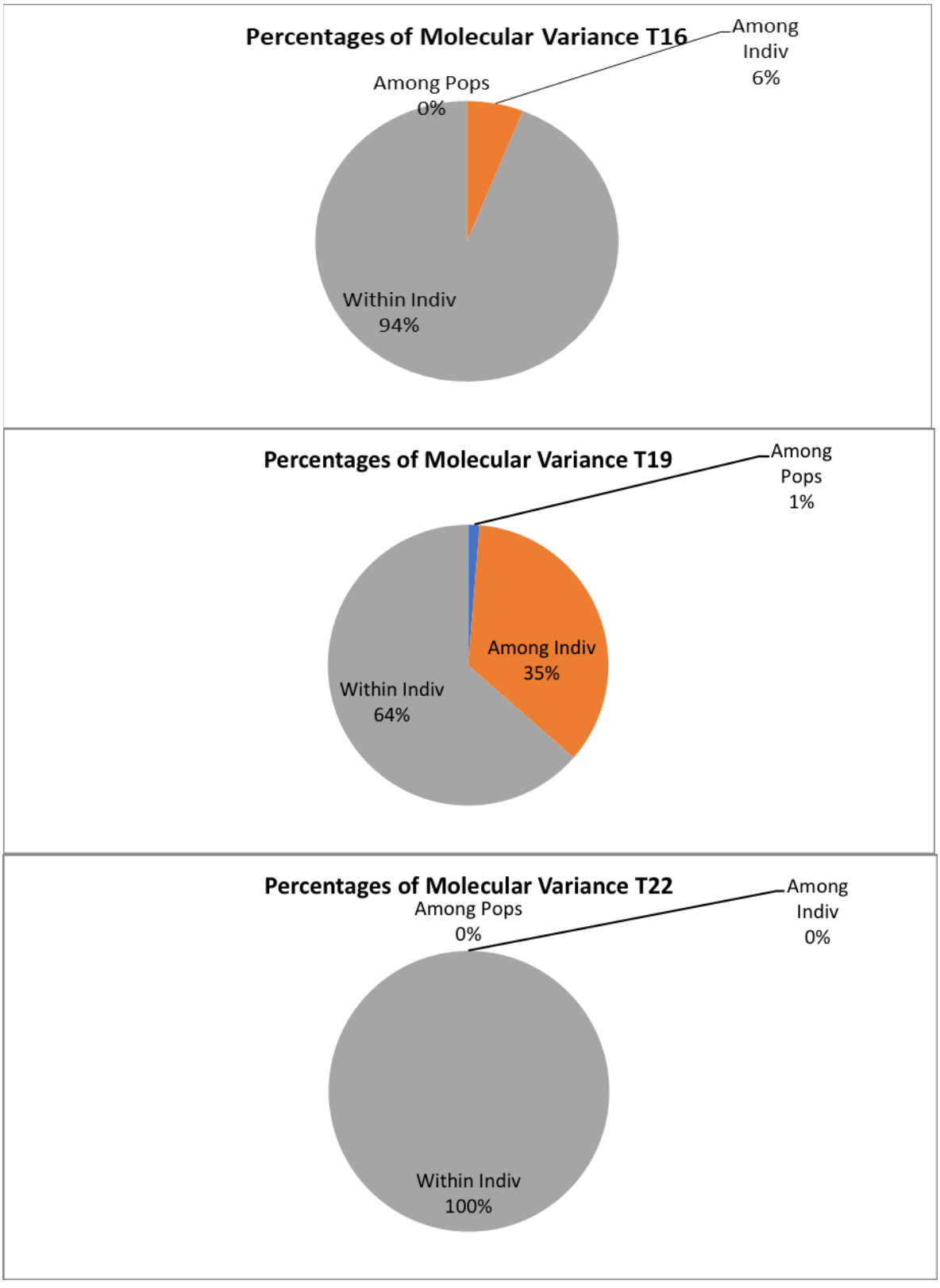

Allele Frequency for T43
Frequency
0.3
0.2
0.1
0
-0.1
278
283
285
287
289
291
293
295
297
299
301
303
305
307
309
T43
Locus
Control
Managed

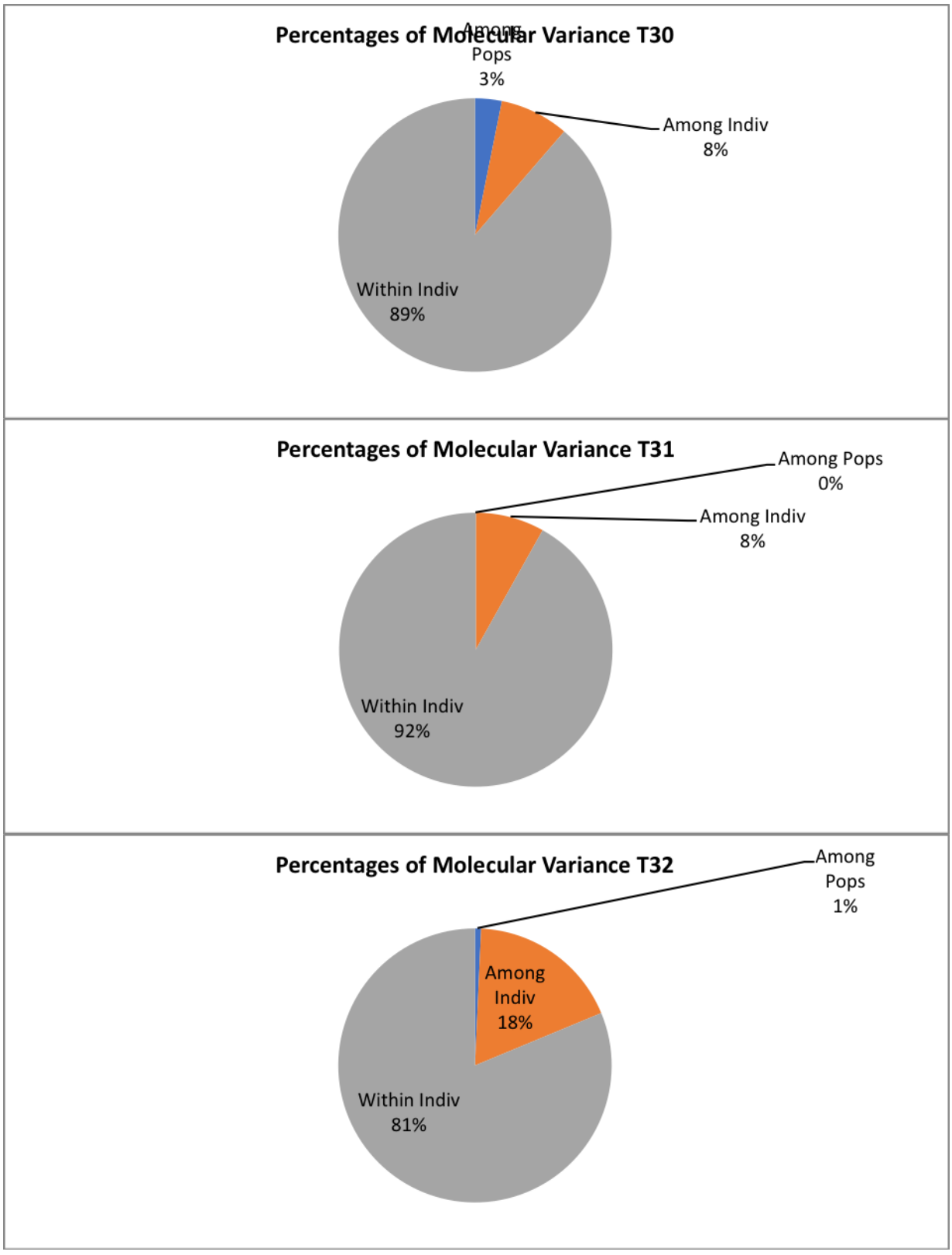

Allele Frequency for T45
Frequency
0.4
0.2
0
-0.2
244
247
253
255
257
259
261
263
265
267
269
271
273
275
T45
Locus
Control
Managed

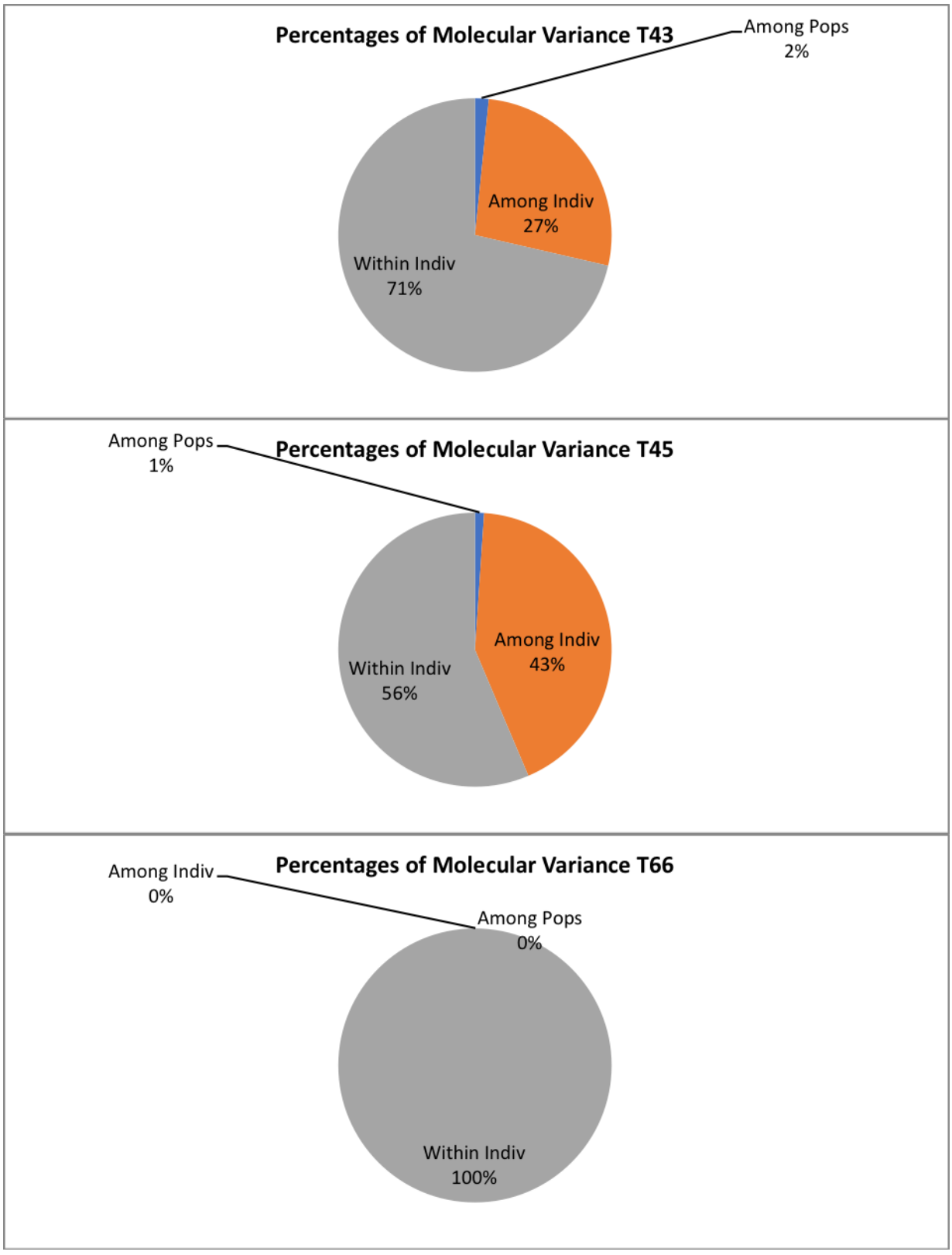


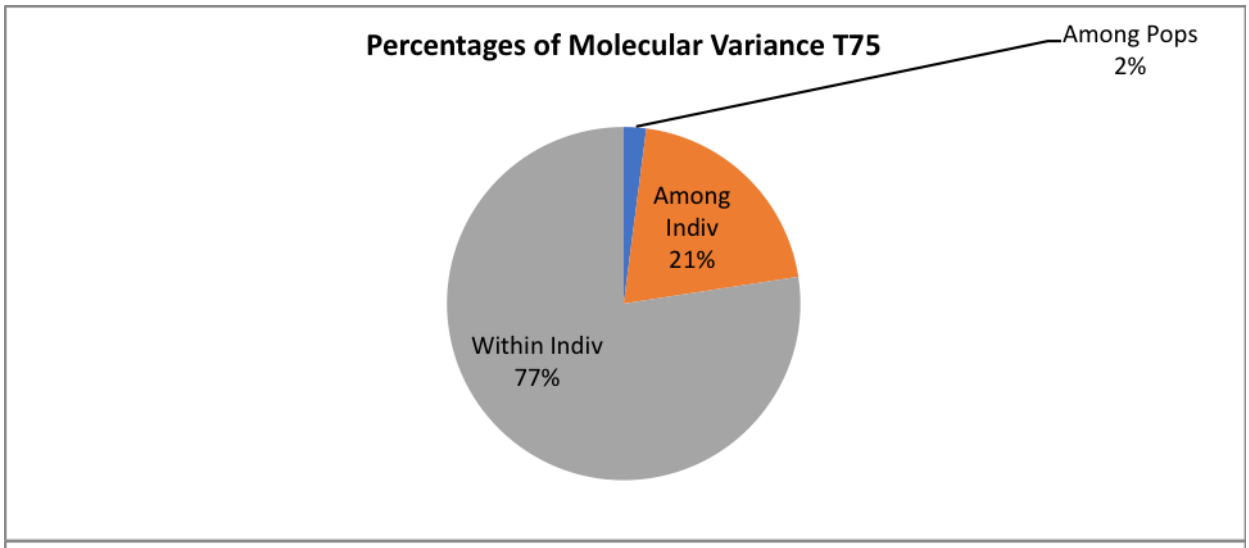
[Appendix A- Allele frequencies per loci between reference and managed plots. Blue bars are the allele frequency means at a specific locus for both reference plots. Orange bars are the allele frequency at a specific locus for all five managed plots. Error bars are provided for easy interpretation of significance.]

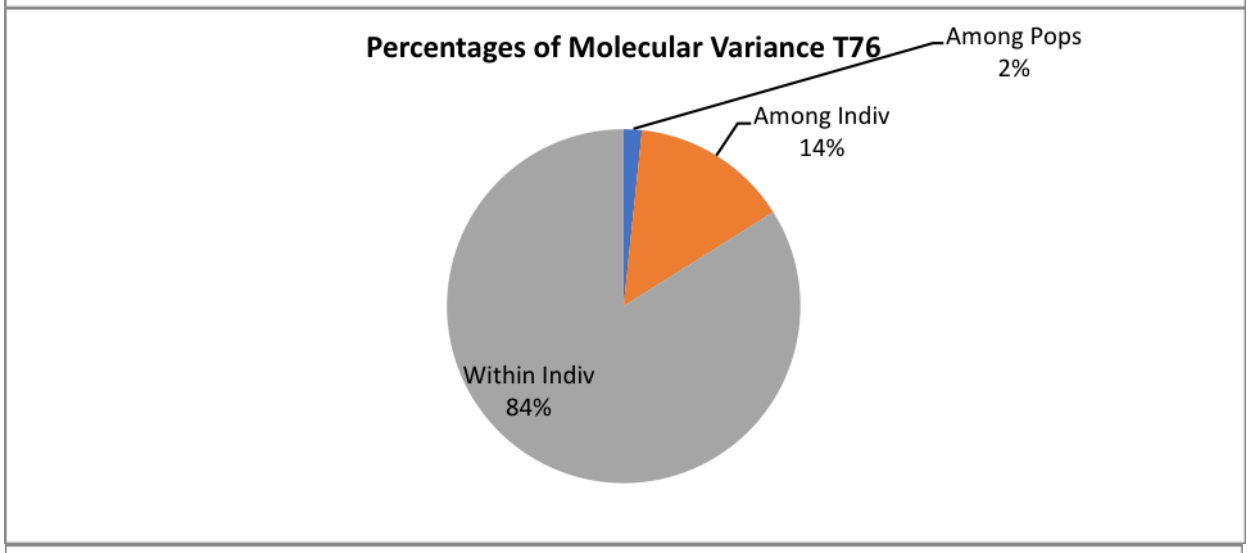
[APPENDIX B. Molecular Variance Attributed to Managed versus Reference]

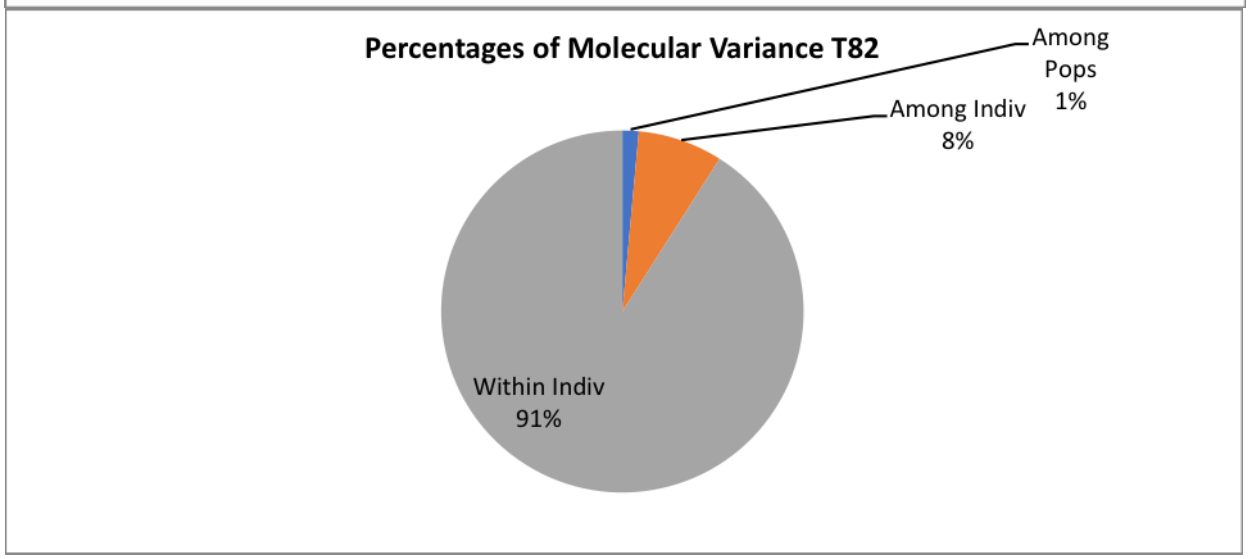



Percentages of Molecular Variance T30
Among Pops 3%
Among Indiv 8%
Within Indiv 89%
Percentages of Molecular Variance T31
Among Pops 0%
Among Indiv 8%
Within Indiv 92%
Percentages of Molecular Variance T32
Among Pops 1%
Among Indiv 18%
Within Indiv 81%


Percentages of Molecular Variance T43
Among Pops
2%
Among Indiv
27%
Within Indiv
71%
Percentages of Molecular Variance T45
Among Pops
1%
Among Indiv
43%
Within Indiv
56%
Percentages of Molecular Variance T66
Among Indiv
0%
Among Pops
0%
Within Indiv
100%


Percentages of Molecular Variance T75
Among Pops
2%
Among
Indiv
21%
Within Indiv
77%


Percentages of Molecular Variance T76
Among Pops
2%
Among Indiv
14%
Within Indiv
84%


Percentages of Molecular Variance T82
Among
Pops
1%
Among Indiv
8%
Within Indiv
91%

[Appendix B- A measure of the molecular variance, showing the percentage attributed to within individuals (grey), among individuals (orange) and among populations (blue) for reference-vs- managed delineation of populations.]

[APPENDIX C. LRM Histogram and Fitted Probability Density Function]

Figure 1- LRM for each pairwise genetic relatedness in Reference Plot 1

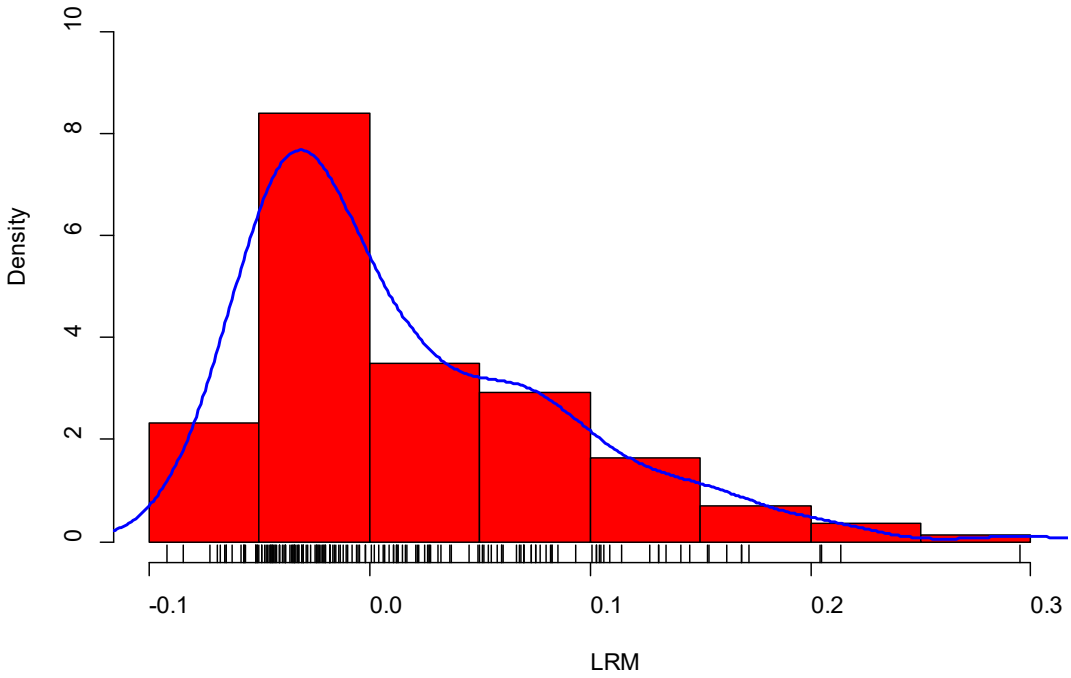


Figure 2- LRM for each pairwise genetic relatedness in Reference Plot 2

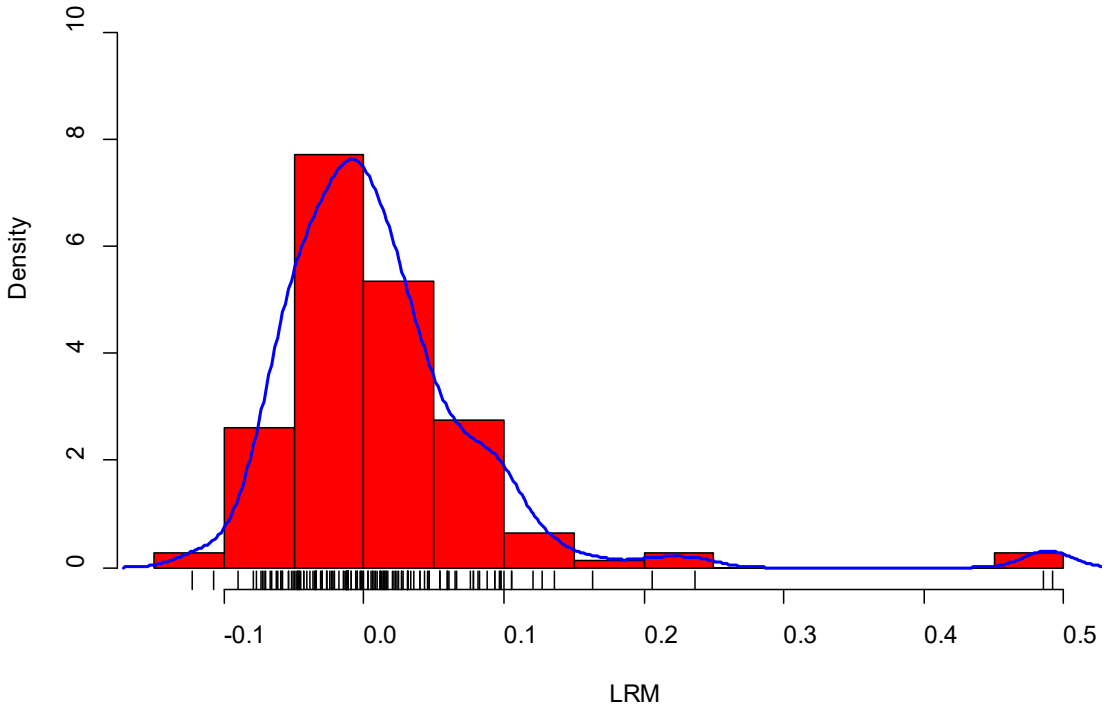


Figure 3- LRM for each pairwise genetic relatedness for Management Plot 3.

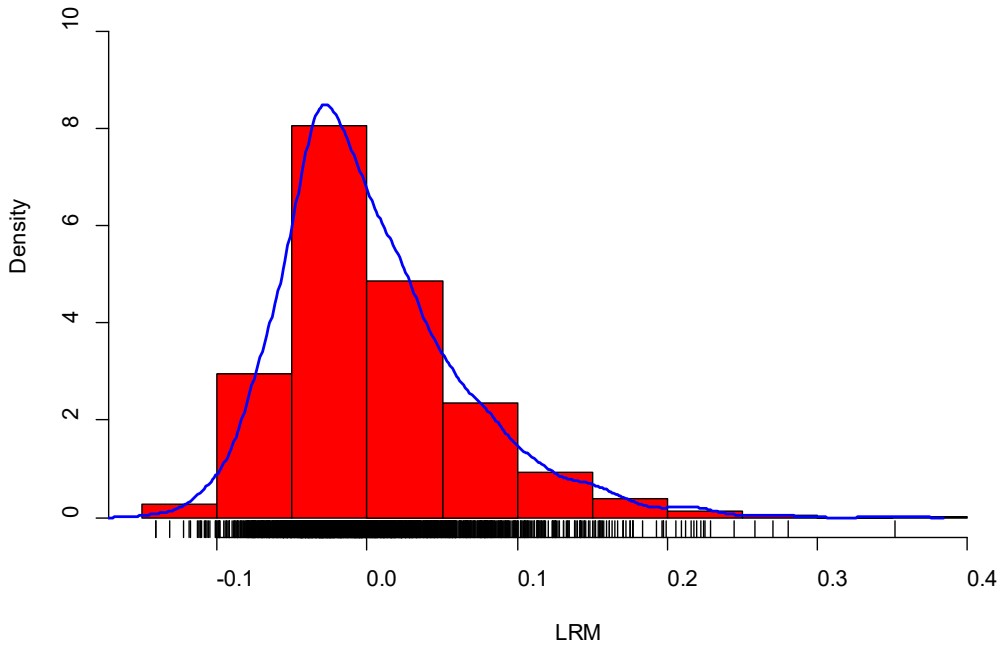


Figure 4- LRM for each pairwise genetic relatedness for Management Plot M4.

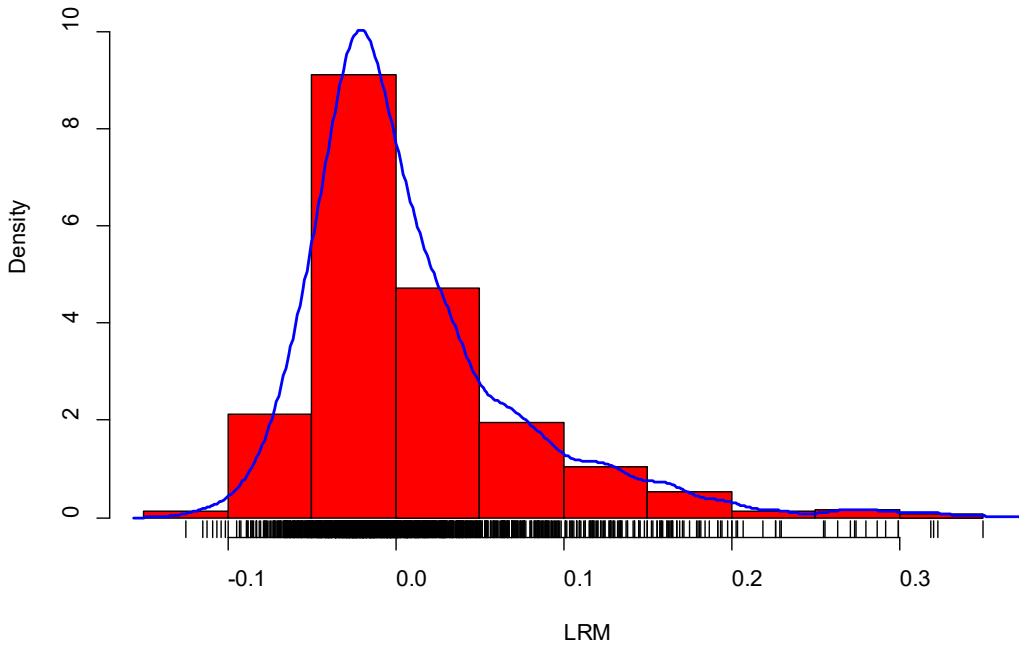


Figure 5- LRM for each pairwise genetic relatedness for Management Plot M10.

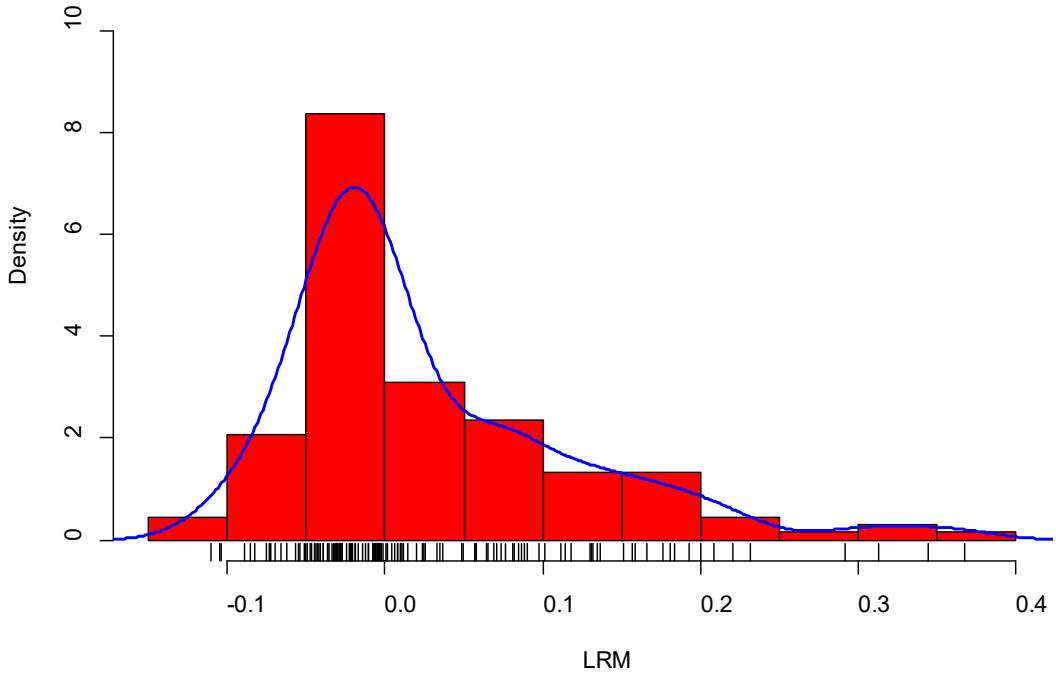


Figure 6- LRM for each pairwise genetic relatedness for Management Plot M11.

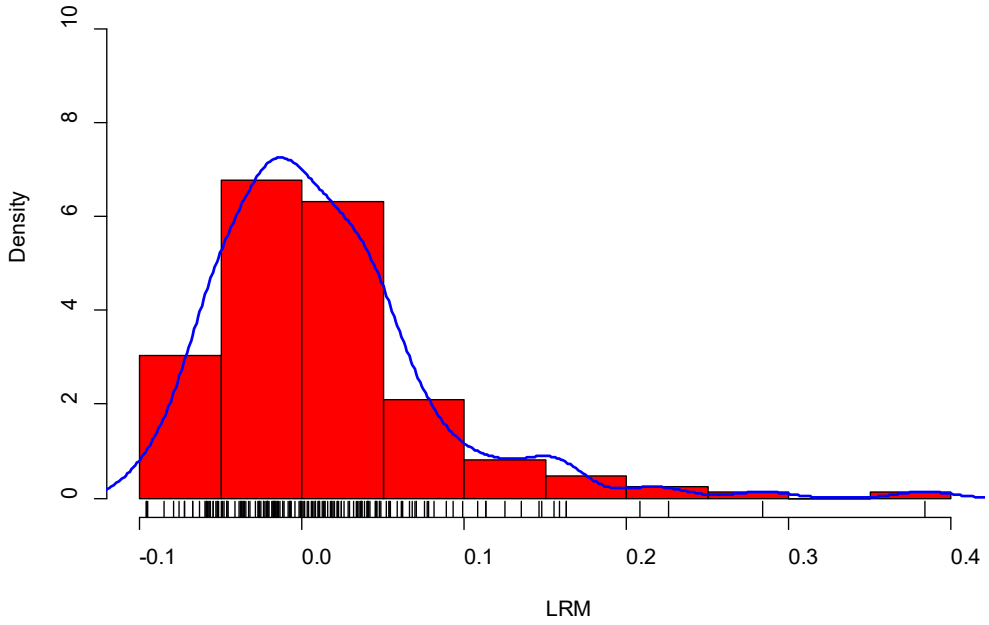
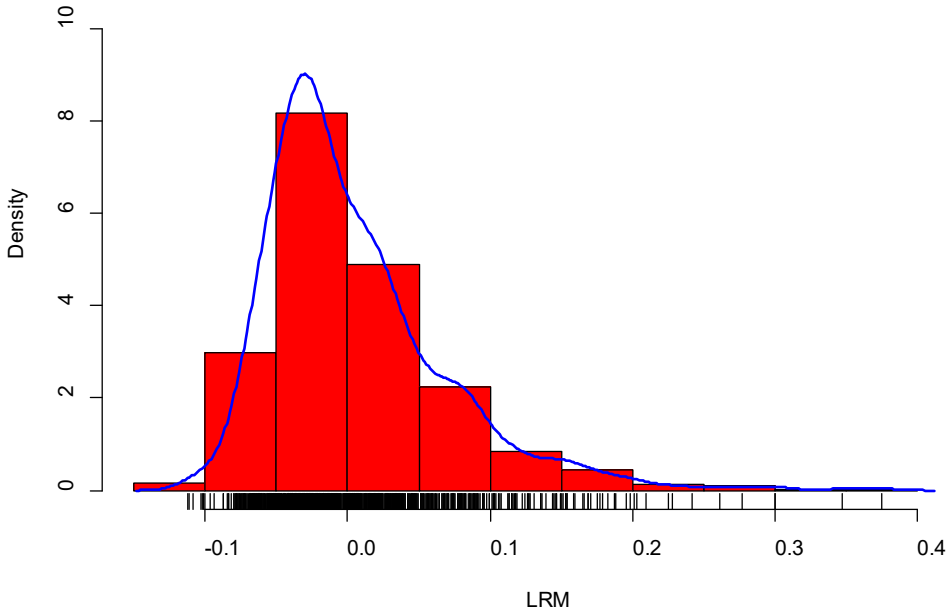


Figure 7- LRM for each pairwise genetic relatedness for Management Plot M18.



[Appendix C- The LRM for each pairwise genetic relatedness between white oak trees within plots. Each pairs measure of genetic relatedness is shown on the x axis and the density at which that measure of genetic relatedness within the plot occurs is shown on the y axis. An LRM value of above 0.1 is considered significant for relatedness between two trees. An increase in the density of LRM is expected around 0 on the x axis for comparisons of individuals from the same species, as they all share a baseline of relatedness.]

[APPENDIX D. Quantile Regression Scatter Plot for LRM in Managed Plots]

Figure 1- Quantile regression scatter plot for pairwise genetic relatedness in Plot M3.

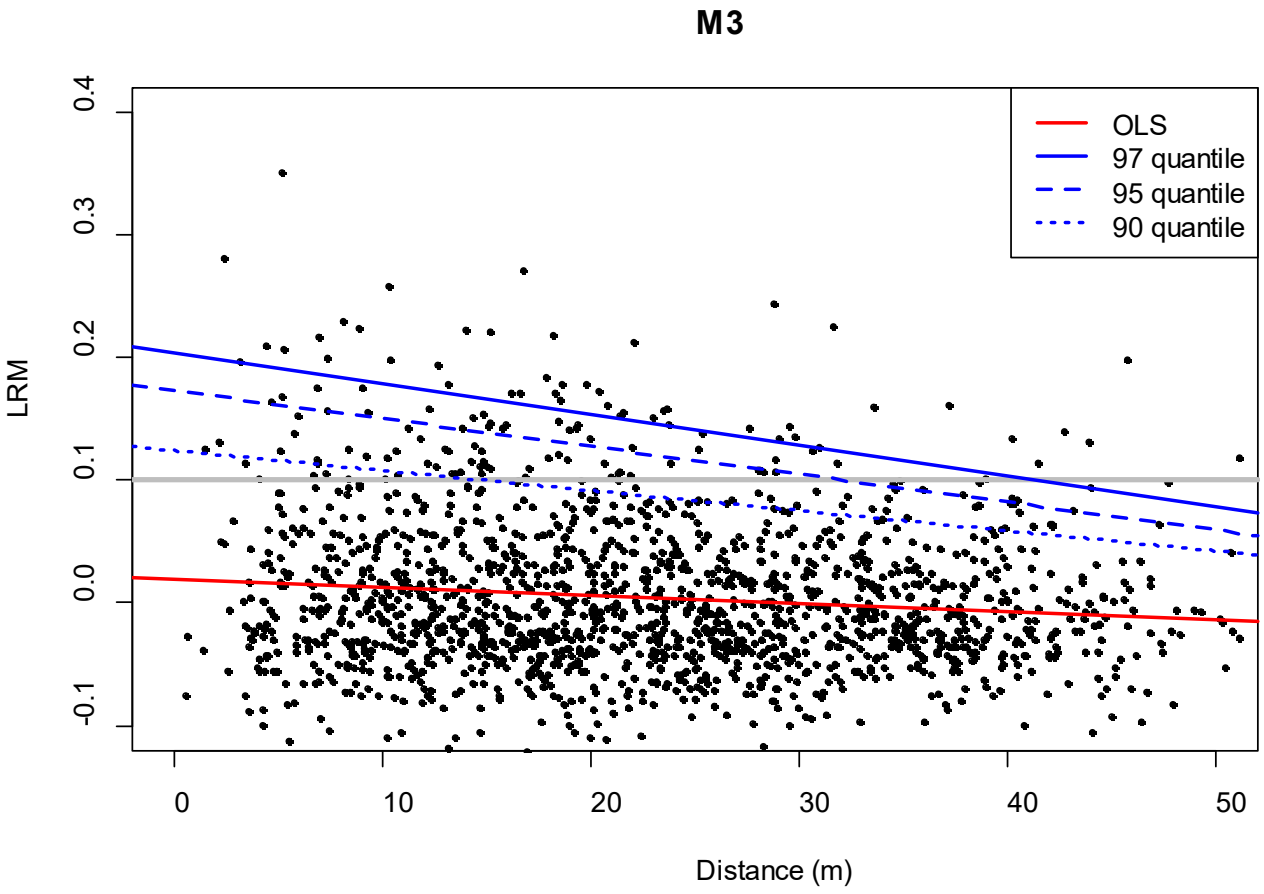


Figure 2- Quantile regression scatter plot for pairwise genetic relatedness in Plot M4.

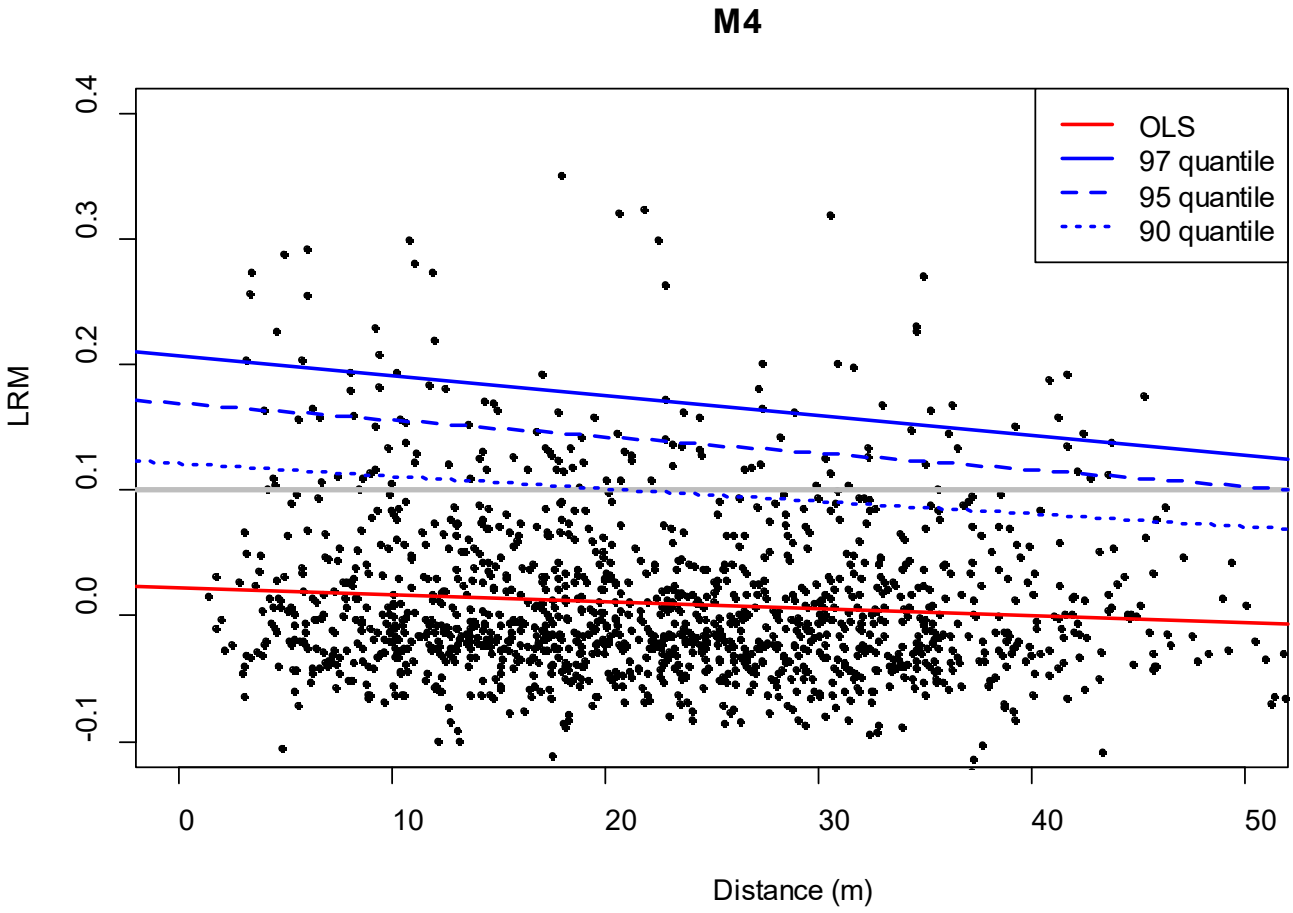


Figure 3- Quantile regression scatter plot for pairwise genetic relatedness in Plot M10.

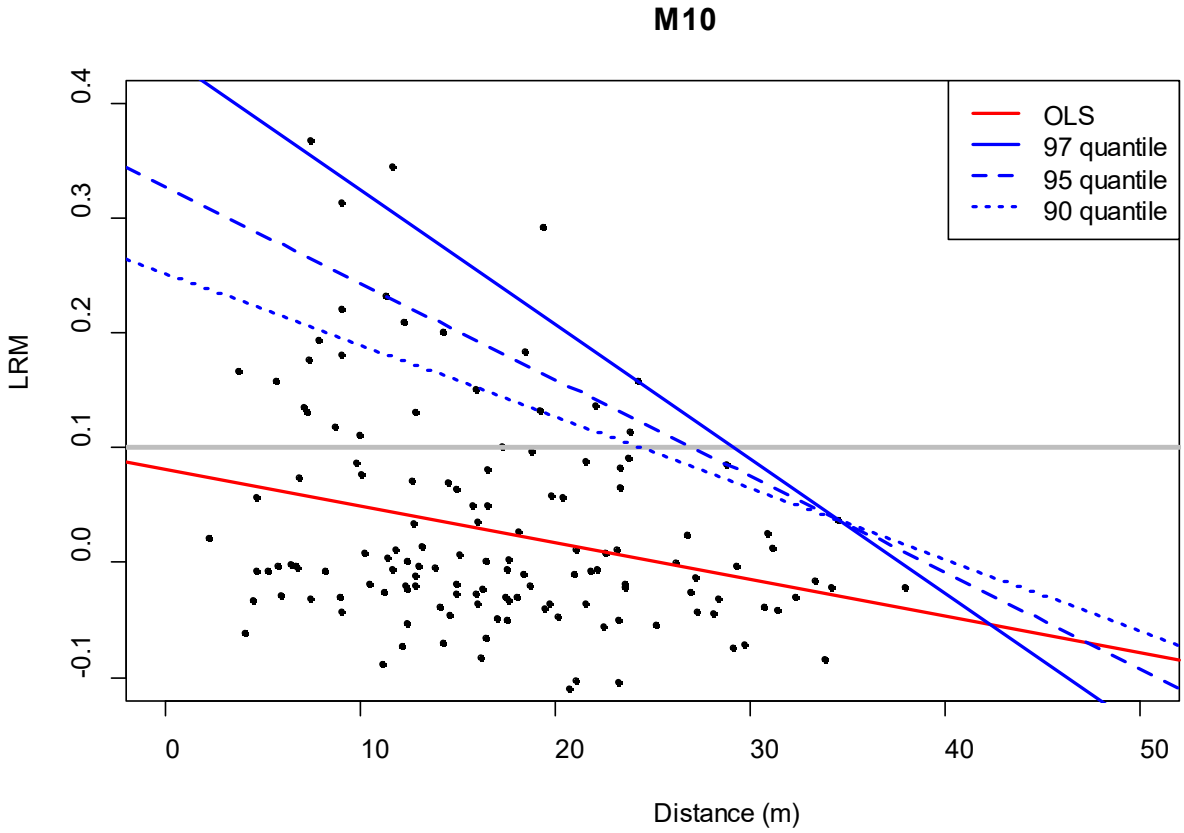


Figure 4- Quantile regression scatter plot for pairwise genetic relatedness in Plot M11.

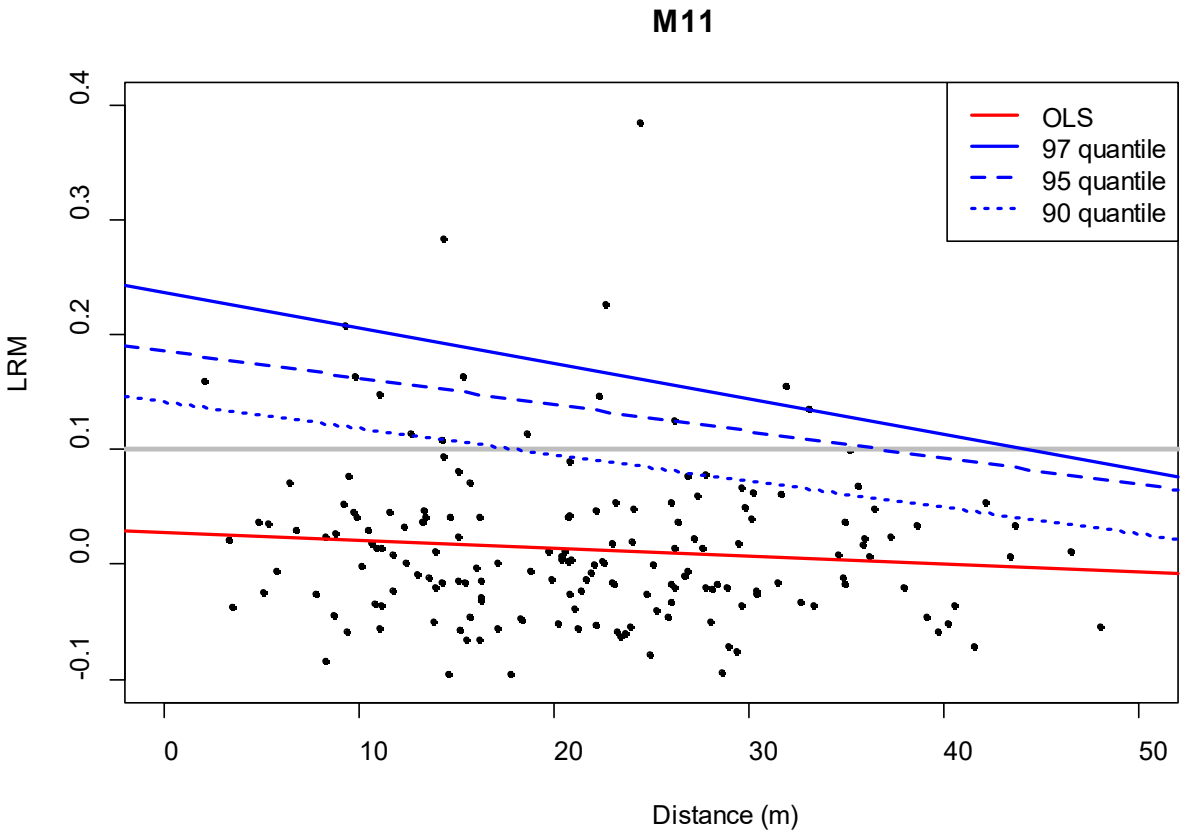
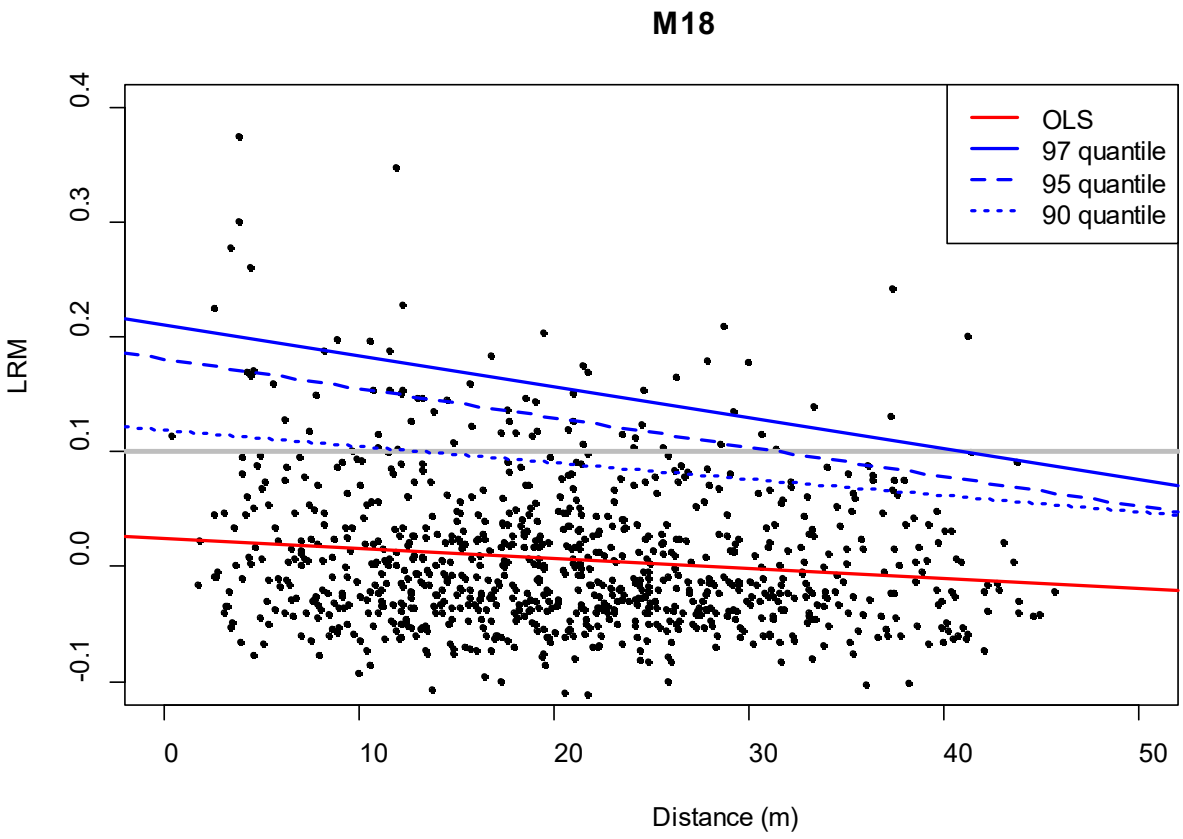


Figure 5- Quantile regression scatter plot for pairwise genetic relatedness in Plot M18.



[Appendix D- The LRM for each pairwise genetic relatedness between white oak trees in managed plots shown in a scatter plot with a 97%, 95% and 90% quantile regression. Each pairs measure of genetic relatedness is shown on the y axis and the distance (m) between the 2 individuals in a pair is on the x axis. An LRM value of above 0.1 is considered significant for relatedness between two trees. An increase in the density of LRM is expected around 0 on the y axis for comparisons of individuals from the same species, as they all share a baseline of relatedness. The solid grey line was added at the LRM 0.1 to signify a cut off for consideration of relatives.]

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VITA

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