Assessment of Genetic Variation of *Acer rubrum* L. and *Liriodendron tulipifera* L. Populations in Unmanaged Forests of the Southeast United States

Katherine E. Kovach

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Committee Members:

Shepard M. Zedaker
E.M. Ulrika Egertsdotter
M. Javed Iqbal

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ABSTRACT

Acer rubrum L. and *Liriodendron tulipifera* L. are prolific throughout their ranges in the Southeastern U.S. and also have increasingly important roles in forestry and wood products in this region. The relatively low density and intermediate strength of the wood makes them versatile for use in many different wood products. Exploring the genetic structure of these species could provide a foundation for further genetic and breeding exploration with these economically important trees. This study utilizes amplified fragment length polymorphism to determine the level of genetic diversity of these species in contrasting physiographic provinces. AFLP was performed using five primer combinations on samples collected from six unmanaged populations of each species in the Mountains and Coastal Plain of the Southeastern U.S. Wood density was determined using an X-ray densitometer. *A. rubrum* lacked strong genetic structure while *L. tulipifera* showed differentiation between physiographic provinces. Genetic diversity of *A. rubrum* was lower within the Mountain populations (H_e: 0.327) than the Coastal Plain populations (H_e: 0.365). The average wood density for *A. rubrum* is lower in the Mountains (539.00 kg/m^3) than in the Coastal Plain (575.43 kg/m^3). Genetic diversity of *L. tulipifera* was higher overall (H_e: 0.289) than within the Mountain populations (H_e: 0.281) or the Coastal Plain populations (H_e: 0.271). The average wood density for *L. tulipifera* is greater in the Mountains (445.45 kg/m^3) than in the Coastal Plain (441.67 kg/m^3).

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Attribution

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Chapter 1: Introduction and Overview of Research

Introduction

Acer rubrum L. (red maple) and *Liriodendron tulipifera* L. (yellow poplar) are prolific throughout the Southeast U.S., and play important roles in forestry and ecology of this region. These species are important for their versatility in wood products, which is increasing due to their abundance and quick regeneration in the Southeast U.S. Finding genetic markers that would correlate to different qualities of these species growth habit such as wood density or branching angle could allow for a better understanding of how the plants respond to different ecological conditions and perhaps give insight into ways these species may be better managed and utilized for production. *A. rubrum* wood with high density and large volumes of lower density wood can be used in furniture making (Walters et al., 1990, Forest-Service, 1974). *L. tulipifera* has high commercial value for lumber, plywood, pulpwood, and veneer, and is competitive with softwoods such as pine species in these markets (Beck et al., 1990).

For forested regions that have in recent history been dominated by oak forests, it is key to study the shifts in species composition to more shade intolerant regeneration that are occurring. *A. rubrum* is a more dominant species in the Southeast U.S. accounting for approximately 3.3% of the volume of saw timber on timberland in the Southeast (Forest-Service, August 2008), due in part to its rapid regeneration, fast initial stem growth, and unspecific growth requirements (Whitney, 1984, Larsen, 1953, Hibbs, 1983). It is thought that *A. rubrum* populations could dominate the overstory of forests across the Southeast U.S. in the near future due to their ability to grow in many environmental conditions and site qualities (Abrams, 1998). *L. tulipifera* accounts for as much as 12.7% of the total volume of saw timber on timberland in the Southeast U.S. (Forest-Service, August 2008). *L. tulipifera* grows best on high quality sites with well drained soil and moderate rainfall (Beck, 1990). Regeneration of other species such as oaks has become limited on such higher quality sites by *L. tulipifera*'s ability to sprout and become established quickly (Loftis, 1990). Realizing that forests are not static communities but continually changing ecosystems, it is important to investigate current

dynamics and species shifts in the forests and how these systems can be more effectively and sustainably managed.

As wood processing and molecular marker technologies continue to advance, integrating tools used in different scientific fields is becoming progressively more important. This has been shown by new medical and molecular marker techniques such as AFLP being applied to disciplines like forestry (Krutovsky, 2006). This study assessed the genetic variations in *L. tulipifera* and *A. rubrum* populations in the Southeast U.S. utilizing Amplified Fragment Length Polymorphisms (AFLP). The main objectives of this study were to: (i) evaluate genetic variation within populations of *L. tulipifera* and *A. rubrum*, (ii) evaluate genetic variation of *L. tulipifera* and *A. rubrum* within physiographic provinces, (iii) to evaluate genetic variation of *L. tulipifera* and *A. rubrum* throughout the Southeastern U.S., and (iv) examine the role of genetic polymorphisms in indicating wood density of *A. rubrum* and *L. tulipifera.*

The Sustainable Engineered Materials Institute (SEMI) at Virginia Polytechnic Institute and State University funded this research. Previous SEMI research found *A. rubrum* had significantly higher wood density averages in the Mountains (642kg/m^3) than in the Coastal Plain (597kg/m^3) and that Mountain and Coastal Plain populations of *L. tulipifera* did not have significant differences (Jones et al., 2006). Higher management regimes including fertilization and irrigation yielded lower wood density in *A. rubrum* (537 kg/m^3) and in *L. tulipifera* (450 kg/m^3) across physiographic provinces (Jones et al., 2006). The average wood density for the unmanaged Mountain populations of *A. rubrum* were 592 kg/m^3 and 540 kg/m^3 for the Coastal Plain populations (Jones et al., 2006). The average wood density for the unmanaged Mountain populations of *L. tulipifera* were 443 kg/m^3, and 454 kg/m^3 for the Coastal Plain populations (Jones et al., 2006). Wood density, typically expressed in either pounds per cubic foot or kilograms per cubic meter, is dependent on specific gravity of the wood and moisture content (Simpson, 1993). The present study narrowed the population pool from SEMI's prior work (Spinney, September 9, 2003) to unmanaged stands located throughout the Southeast U.S. that

were characterized into two physiographic provinces, Mountains and Coastal Plain. AFLP techniques were used to analyze genetic structure and variations in wood density and AFLP markers. The strongest genetic differences were expected between the Mountain sites of highest latitude and those of Coastal Plain with lowest latitude, due in part to growing seasons being longer for the Coastal Plain populations than the Mountains (Beck et al., 1990, Walters et al., 1990). AFLP can be used as a tool in breeding programs, and as a foundation for association studies.

Overview of Research

Field Methods

For the purpose of this study sites were classified by physiographic province and management regime. The populations sampled were unmanaged stands in the Mountains and Coastal Plain of the Southeast U.S. Six *A. rubrum* and six *L. tulipifera* sites (Table 1) were sampled for this study; three in the Mountains and three in the Coastal Plain (Figure 1). Within each population, a minimum of three 0.1 acre plots were chosen. GPS points were taken at the center of the plots and PVC pipe was driven into the ground at those points. Each tree's total height, height of first branch, and diameter at breast height (dbh) were measured, and each tree was marked with a metal tag. For each plot the physiographic province, latitude and longitude, date, management intensity, landowner, state, and plot numbers were recorded. The latitude and longitude of each sitewere used to generate a map using Google Earth (Google, 2008). On average, at least ten trees were cored at each site. Eight to nineteen trees had 12 mm bark-to-bark increment cores taken at each site. Two cores were taken from each tree, one stored in 10% bleach in a cooler and the other stored in a portable freezer at approximately -20°C. Field crews sampled SEMI plots every summer from 2003 to 2007, with sampling *of L. tulipifera* and *A. rubrum* beginning in 2004 (Spinney, September 9, 2003). Leaf material used for the marker analyses, was collected in the 2006 and 2007 sampling seasons from cored trees. Pole trimmers were used to sample branches up to 10ft high and a 12-gauge shotgun with #4 copper coated shot used to

sample higher branches. Leaf samples were stored in a cooler with dry ice in the field, and a power freezer near -20°C was used for transport of leaves.

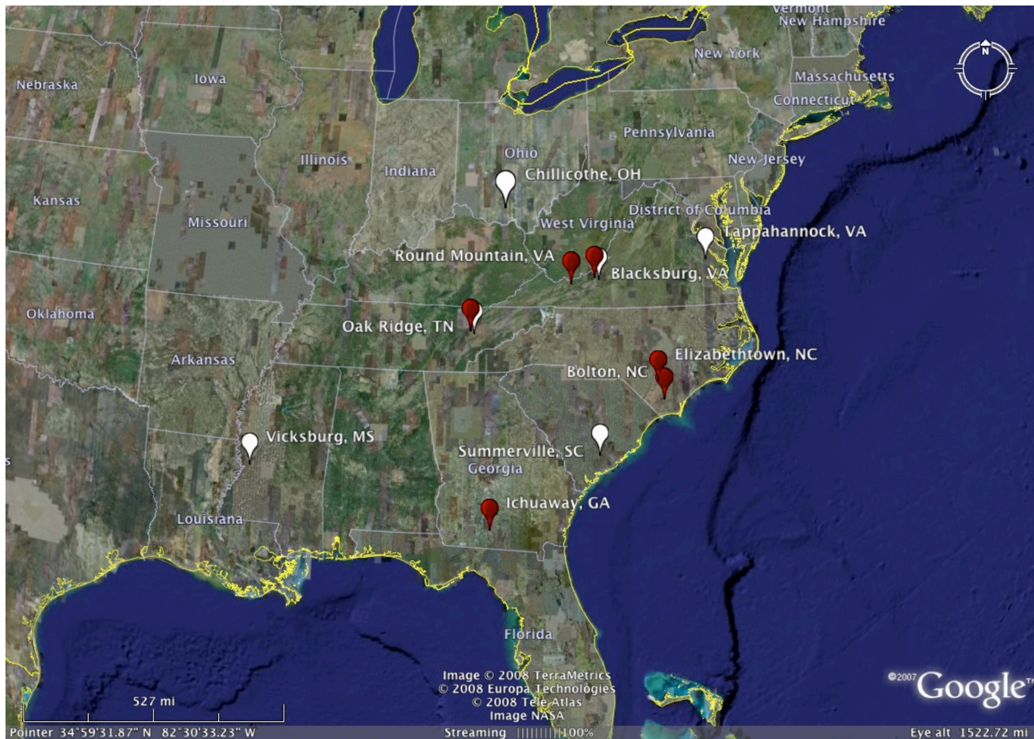

Figure 1 Map of six *L. tulipifera* (white balloons) and six *A. rubrum* (maroon balloons) sites used in this study. Map generated using Google Earth (Google, 2008).

Table 1 Unmanaged *L. tulipifera* and *Acer rubrum* sites used in this study. Includes the population name, physiographic province, latitude, longitude, and number of trees sampled.

Species	Population Location	Physiographic Province	Sample Size	Latitude (N)	Longitude (W)
L. tulipifera	Blacksburg, VA	Mountains	12	37°18'04.02"N	80°26'27.72"
L. tulipifera	Chillicothe, OH	Mountains	17	39°02'45.66"N	83°11'40.98"
L. tulipifera	Oak Ridge, TN	Mountains	16	36°00'09.00"N	84°13'01.00"
L. tulipifera	Summerville, SC	Coastal Plain	18	32°56'46.68"N	80°22'41.40"
L. tulipifera	Tappahannock, VA	Coastal Plain	18	37°40'43.26"N	76°56'16.44"
L. tulipifera	Vicksburg, MS	Coastal Plain	8	32°25'40.52"N	90°43'08.80"
		Total	89		
A. rubrum	Blacksburg, VA	Mountains	11	37°18'04.02"N	80°26'27.72"W
A. rubrum	Round Mountain, VA	Mountains	18	37°10'16.82"N	81° 9'35.78"W
A. rubrum	Oak Ridge, TN	Mountains	16	35°56'11.59"N	84° 6'18.09"W
A. rubrum	Bolton, NC	Coastal Plain	19	34°16'19.41"N	78°25'55.48"W
A. rubrum	Bladen Lakes, NC	Coastal Plain	19	34°42'10.00"N	78°35'53.00"W
A. rubrum	Ichauway, GA	Coastal Plain	16	31° 6'23.95"N	83°34'48.77"W
		Total	99		

Laboratory Methods

Amplified fragment length polymorphism (AFLP) technique was utilized to study genetic polymorphisms. AFLP was chosen because previous studies have shown it to be highly reproducible and a powerful tool for species whose genomes have not been mapped (Cottrell et al., 2003, Gaudeul et al., 2004). This process was split into two major phases, DNA isolation and AFLP marker analysis. DNA isolation was performed largely in Latham Hall, Department of Forestry, Virginia Polytechnic Institute and State University, Blacksburg, VA while the AFLP marker analysis was performed in Danville, VA at the Institute for Advanced Learning and Research. The AFLP protocol followed, including modified DNAzol DNA isolation protocol, is included in Appendix A (Chang et al., 2009). DNA was isolated from the leaf samples during 2006 and 2007 in Latham Hall, Department of Forestry, Virginia Polytechnic Institute and State University, Blacksburg, VA. Concentrations were diluted to approximately 125ng/µl and quality was verified on a 0.6% low EEO agarose gel. AFLP protocol was performed at the Institute of Advanced Learning and Research, Danville, VA. Work in Danville, VA was performed primarily in 2007 and 2008. Restriction digestion of DNA samples was done simultaneously with two restriction enzymes: *Eco*RI Restriction Enzyme Recognition site GAATTG, from New England BioLabs, and *Mse*I Restriction Enzyme Recognition site TTAA, from New England BioLabs. Adapter ligation was performed following the restriction digestion of samples. Quality of products from the Restriction/Ligation step was checked on a 0.6% low EEO agarose gel. Pre-selective amplification was performed on these products, followed by selective amplification with five primer combinations. The five different primer combinations were used on each species and were chosen from 10 initial primer combinations applied to the Tappahannock, VA (*L. tulipifera*) and the Blacksburg, VA (*A. rubrum*) populations (Appendices 5 and 6). The five primer combinations that yielded the most similar results based on Dice similarity calculations were chosen. Each primer combination consisted of one *Eco*RI labeled primer and one MseI un-labeled primer. The same *Eco*RI primer was used in each combination for each species. After the fragments were amplified they were

run on the Beckman Coulter CEQ 8800, capillary electrophoresis system. Electropherogram output from the CEQ 8800 allows for manual checking of AFLP data by comparing electropherogram peaks to binary data to confirm accuracy of results. The CEQ 2000XL Version 4.3 Software enabled viewing of polymorphisms in the DNA samples. AFLP resultant data management began with this software and provided a way to count the number of polymorphisms present and export files used with statistical software.

Environmental Methods

Environmental data including mean annual precipitation, mean annual temperature, and mean annual growing season length were gathered via Daily Surface Data ASCII files from the National Oceanic and Atmospheric Administration (N.O.A.A., 2008). Data was retrieved for years 1958 through 2008, the anticipated average age of stands sampled. Mean annual growing season was determined by calculating the average number of days between the last frost in the spring and the first frost in the winter months from 1958 to 2008.

Data Analysis

NTSYSpc Numerical Taxonomy and Multivariate Analysis System Version 2.2, developed by James Rohlf, was used to explore the degree of genetic diversity of individuals across the landscape (Rohlf, 2005). The Dice coefficient was used with NTSYSpc Ver.2.2 to assess genetic similarity, and a UPGMA clustered dendrogram was created using this similarity index for all samples of both species. PopGene v.3.2 was used to calculate genetic variation statistics for all loci (Yeh and Boyle, 1997). PopGene v.3.2 was also used to calculate Nei's analysis of genetic diversity in subdivided populations, and Nei's original measure of genetic diversity and genetic distance, UPGMA dendrograms based on these diversity indices, percentage of polymorphic loci at a $p \leq 0.05$ significance level (Nei and Feldman, 1972, Yeh and Boyle, 1997). UPGMA dendrogram based on Nei's unbiased genetic distance matrix over all populations was produced using PopGene v.3.2 (Yeh and Boyle, 1997). Analysis of molecular variance (AMOVA) was performed with Arlequin Version 2.0, which established the degree

of genetic variation between populations and between populations grouped by physiographic province (Schneider et al., 2000). An analysis of variance (ANOVA) was performed on the wood density data, along with averages and ranges for each individual population (SAS, 2007).

Chapter 2: Literature Review

Liriodendron tulipifera L.

Life History

Liriodendron tulipifera L.*,* common names yellow-poplar, tuliptree, tulip-poplar, white-poplar, and whitewood, is a member of the Magnoliaceae (Magnolia) family. *L. tulipifera* flowers are orange and white, first developing from age 15-20 years, and continuing to flower for the remainder of the tree's life (Beck et al., 1990). *L. tulipifera* is one of the latest flowering trees in the Southeast U.S., that's flowering period begins in late spring to summer, lasts two to six weeks, and increases with the age and size of the tree (Beck et al., 1990). Insects including flies, beetles, honey bees, and bumble bees (Boyce et al. 1961), pollinate the flowers on or near the day of blooming since the stigmas can dry out soon after blooming (Beck et al., 1990). Mode and duration of pollination can be important when assessing pollination ranges and genetic diversity of a species. Self-pollination also occurs, resulting in fewer viable seed being produced (Boyce and Kaeiser, 1961). It seems reasonable to suspect that the insect pollination of *L. tulipifera* limits its breeding range, though seeds are dispersed by wind. Flowering time as well as seed and pollen dispersal patterns can direct effects on genetic diversity of species and their mating patterns.

L. tulipifera is a shade intolerant species, and given adequate rainfall and sunlight over a long growing season, maximum development will occur (Beck et al., 1990). *L. tulipifera* requires full sunlight for regeneration, but as little as three to ten percent to survive (Olson, 1969, Phillips, 1966). *L. tulipifera* is a highly successful reproducer by seed and stump sprouts. *L. tulipifera* seeds are arranged in an aggregate of winged carpels, that disperse by wind. Annual seed production is estimated at 741,000 – 1,482,000 seeds per hectare in portions of the Southeastern U.S. (Beck et al., 1990, Phillips, 1966, Renshaw and Doolittle, 1958). This is unlike other tree species that have high and low mast years. Along with production, viability of seed remains relatively stable each year, five to twenty percent, and for up to 7 years (Beck et al., 1990, Clark and Boyce, 1964). Before seeds can germinate

they must be exposed to soil and freezing conditions for about three months (Beck et al., 1990). Seedlings can develop at high densities, thinning is needed as they mature. Seedling success is particularly high on clearcuts, burned areas, and other cuts and areas with full sunlight and exposed soil (McGee, 1975). Regeneration of *L. tulipifera* is typically straightforward; requiring exposed soil, open canopy, seed source, and sufficient sunlight. *L. tulipifera* is a flexible rooter, typically forming a tap root (Beck et al., 1990).

Ecology

Due to its reproductive success and growth requirements, *L. tulipifera* is a highly competitive species and has a range covering most of the eastern U.S. It is more site specific than *A. rubrum*, requiring higher levels of light and more water throughout the growing season (Beck et al., 1990). Higher light requirements also cause decreased levels of growth when *A. Rubrum* is overtopped by other tree species. *L. tulipifera*'s degree of dominance creates differentiation between the ecological communities (Zhang et al., 1999). This ecological importance is reflected across the Southeast U.S. In the Southern U.S. *L. tulipifera* is a dominant species in yellow poplar, yellow poplar-white oak-northern red oak, yellow poplar-eastern hemlock, and sweetgum-yellow poplar forest types, and a minor species in 11 forest types (Eyre, 1980). Pure stands of *L. tulipifera* rarely occur (Beck et al., 1990). The dominance of *L. tulipifera* in the Appalachians increases with increased site quality (Loftis, 1990). Due to the fast growing nature of the species, up to 46cm in height and 6.4mm in diameter annually (Hicks, 1998), and its shade intolerance, it is often seen as a successional species in old fields (Beck et al., 1990).

L. tulipifera exhibits phenotypic and genetic variation across its range (Sewell et al., 1996a). One instance of phenotypic variation has been observed in degree of leaf variability becoming greater on a north bound gradient through the range (Kellison, 1968, Parks et al., 1994). Pleistocene glaciations are believed to have caused southern and northern migrations of many species in the U.S., including *L. tulipifera* (Sewell et al., 1996b). Along with these migrations many bottlenecks and isolated

populations are believed to have occurred in many species (Sewell et al., 1996b). Northern and southern (populations in Northern Florida) U.S. haplotypes of *L. tulipifera* are identified by five potential cpDNA changes which have evolved independently, seeming to confirm the suggested migration patterns and occurrence of these bottlenecks and isolated populations (Sewell et al., 1996b). Focusing on the northern haplotype of *L. tulipifera* in the Southeastern U.S. it was found that the genomic DNA of populations sampled in this region was highly similar, though some significant differences were present. This similarity is also supported by the overall lack of geographic boundaries to migration in this region and the capability of this species to have migrated and bred throughout the majority of its distribution, despite being largely insect pollinated. Phenotypic differences including wood density, branching angle, seed dormancy, and leaf characteristics, have been documented throughout the range of *L. tulipifera* and through provenance tests (Kellison, 1968, Sluder, 1972, Farmer et al., 1967, Orwig and Abrams, 1994, Olson, 1969).

L. tulipifera is also ecologically significant due to the wildlife species that utilize it as a food source. In the Appalachians, white-tailed deer are known to forage heavily on flowers of juvenile *L. tulipifera* (Campbell et al., 2002). Livestock, squirrels, some bird species, mice, and rabbits also graze on *L. tulipifera* seedling leaves, buds and twigs (Beck et al., 1990).

L. tulipifera uses large amounts of water and lives in areas with rainfall levels ranging from 760 mm (30 in) to greater than 2030 mm (80 in), with local topography having a large effect on species survival (Beck et al., 1990). Aspect is an important component of local topography with north and east aspects on lower and sheltered slopes such as coves being favorable for *L. tulipifera* growth (Beck et al., 1990). Sampling for the current study required dominant stands of *L. tulipifera,* which often coincided with these site conditions. The sites sampled in the current study were gentle slopes, some of which in coves such as the Chillicothe, OH population. *L. tulipifera* can be found in coves, valleys, stream bottoms and at varying elevations from sea level to mountains. *L. tulipifera* lives in highly

contrasting climates, some with average temperatures as low as -7.2°C(19°F) and others as high as 27.2°C (81°F) (Beck et al., 1990).

L. tulipifera has a widespread range spanning the Eastern U.S. through cold climates of the New England States to warm climates of Florida. According to the forest inventory analysis (Forest-Service, August 2008), the total saw log volume of *L. tulipifera* on timberland in the Southeast U.S. as surveyed from 2000-2007 was 2,886,975,454 cubic ft (81,750,041 cubic meters) with 1,411,809,803 cubic ft (39 978 001.6 cubic meters) on private land (Forest-Service, August 2008). In 2002 the Southeastern U.S. had 89.2% of the *L. tulipifera* volume in the U.S.(Forest-Service, August 2008). The total volume of sawtimber on timberland in the Southeast U.S. is 7.7e11 cubic ft (Forest-Service, August 2008).

L. tulipifera grows on a variety of soil types, primarily Ultisols and Inceptisols, and performs better in soils with loose texture, good moisture and drainage (Beck et al., 1990). Appropriate effective rooting depth and moisture-supplying capacity are important soil characteristics for *L. tulipifera* growth (Della-Bianca and Jr., 1961, Ike and Huppuch, 1968, McCarthy, 1933, Smalley, 1964, Phillips, 1966). Nutrient poor soils, especially in nitrogen, phosphorous and potassium, generally do not enhance growth of *L. tulipifera,* though survival is likely (Beck et al., 1990, Zeleznik and Skousen, 1996). This is evident in mine reclamation studies by Zeleznik et al. in which *L. tulipifera* was outperformed on poor quality mine sites by white ash, but a reasonable level of survival was achieved (Zeleznik and Skousen, 1996).

L. tulipifera grows faster than *A. rubrum* and *Quercus rubra* L. seedlings under a variety of silvicultural understory treatments including vegetation removal, trenching and fertilization, and in unmanaged stands (Beckage and Clark, 2003). Higher growth is exhibited on northeast topographical aspects (Fekedulegn et al., 2003). *L. tulipifera* is more productive on north and east aspects in the Appalachians (Desta et al., 2004). A study in West Virginia showed that *L. tulipifera* experienced up to 40% less growth in drought years according to the Palmer drought severity index and grows best on northwest aspects (Fekedulegn et al., 2003).

There are few natural predators and damaging agents affecting *L. tulipifera,* which makes it a favorable species to manage. Four species of insect are known to negatively impact *L. tulipifera.* The tuliptree scale (*Toumeyella liriodendri*), Tulip poplar weevil (*Odontopus calceatus*), and the Rootcollar borer (*Euzophera ostricolorella*), all decrease vigor of *L. tulipifera* and allow for pathogens to degrade the health of the tree (Beck et al., 1990). The Columbian timber beetle (*Corthylus columbianus*) degrades wood color (Beck et al., 1990). Fire also negatively impacts seedlings, though regeneration from stump sprouts is prolific. Vines such as honeysuckle and other invasive species can damage the form of the tree and eventually kill the tree. The growth rate of *L. tulipifera* roots and above ground biomass has been shown to decrease as temperatures increase, which could be detrimental if temperatures rise quickly for extended periods of time (Constable and Retzlaff, 2000).

Wood Properties and Uses

The wood of *L. tulipifera* has small pores, and the heartwood is light to dark brown, yellowish in color, and only slightly resistant to decay (Forest-Service, 1974). Drying the wood causes an average radial shrinkage of 4.6%, tangential shrinkage of 8.2%, and volume shrinkage of 12.7% (Forest-Service, 1974). Specific gravity of *L. tulipifera* increases from 0.4 to 0.42 when dried and slower grown wood has low bending strength and weight (Forest-Service, 1974). Specific gravity varies significantly within populations of *L. tulipifera* (Kellison, 1968, Taylor, 1968, Thorbjornsen, 1961), and other wood properties also differ between trees in the same population under the same environmental constraints (Zobel and Talbert, 1984). These findings also conclude that it is difficult to attribute wood properties to site index (Thorbjornsen, 1961) or to geographic locales (Taylor, 1977, Thor and Core, 1977). Other studies have however shown decreases in specific gravity as site index increases (Barefoot, 1963, Sluder, 1972). The specific gravity of mature wood is not related to the growth rate of the mature wood of a species, and properties of young wood, including wood density, are not always consistent with the properties present in mature wood of the same tree (Core et al., 1975, Zobel and Sprague, 1998). Poisson's ratio decreases with increasing load with *L. tulipifera* (Zink et al., 1997). Silvicultural

nitrogen applications ranging from 40 to 640 lb/acre all had negative effects on the density of *L. tulipifera* wood in TN (Ross et al., 1979).

L. tulipifera is used in a variety of forest products and is especially important in the Southeast U.S. due to the volume present, ease of management, rapid growth, good form, and high quality wood (Vick, 1985). *L. tulipifera* is used as raw material for oriented strand board (OSB), laminated veneer lumber, millwork (Luppold et al., 2002), and construction lumber (Johnson et al., 1997). *L. tulipifera* lumber is widely used for furniture making, musical instruments, paneling, and veneer (Beck et al., 1990, Forest-Service, 1974). Lower grade material is used for plywood, pulpwood, fairly weak connector joints, pallets, and boxes (Forest-Service, 1974). *L. tulipifera* is also admired as an ornamental species and its nectar contributes to honey production (McCarthy, 1933).

Genetics

Like many species, *L. tulipifera* exhibits phenotypic differences throughout is range and within local populations. Studying these differences and their causal agents is interesting to forest managers and those interested in particular wood properties such as specific gravity and straightness of stem (Sluder, 1972, Beck et al., 1990, Salick, 1995). Some traits have been actively managed for in *L. tulipifera,* with varying degrees of success.

Provenance tests have been performed on *L. tulipifera* showing that seedlings of northern origin did not grow well in longer day lengths of 18 hours (Vaartaja, 1961). Dormancy relationships have also shown to be dependant on seed origin, with more northerly sources going dormant earlier and breaking dormancy later (Beck et al., 1990). More work is needed on wood property relationships to seed sources from different regions of *L. tulipifera* range. The strongest genetic differences could be expected between Mountain populations of high latitude and Coastal Plain populations of low latitude due in part to growing seasons being longer in the Coastal Plain areas (Beck et al., 1990, Walters et al., 1990), and also historic divergence of chloroplast DNA (Qiu and Parks, 1994, Sewell et al., 1996a). A study on the variation of *L. tulipifera* chloroplast DNA in the U.S. showed that northern and southern

populations of *L. tulipifera* were evolutionarily distinct (Parks and Wendel, 1990) and that populations in northern Florida were a different cpDNA haplotype than all other sampled North American populations (Sewell et al., 1996b, Parks and Wendel, 1990).

Somatic embryogenesis systems for *L. tulipifera* are of increasing importance to breeding and propagation of successful genotypes. Embryogenesis systems for *L. tulipifera* and *L. tulipifera* x L. Chinese hybrids have been successfully developed, yielding more than 50% viable somatic embryos (Dai et al., 2004). The development of successful embryogenesis systems makes propagating individuals with desirable phenotypes more readily attainable. The wide commercial usefulness of *L. tulipifera* makes successful breeding of the species with desirable traits particularly valuable. Identifying DNA fragments associated with desirable traits is critical to the development of breeding programs. At the University of Georgia, molecular marker techniques have been applied to *L. tulipifera* for a variety of applications (Dai et al., 2004, Clayton et al., 1998, Wilde et al., 1992). Also, foreign genes have been expressed in *L. tulipifera* and modified plants to have the ability for uptake and phytoremediation of mercury (Clayton et al., 1998).

***Acer rubrum* L.**

Life History

Acer rubrum L., common names of red maple, scarlet maple, swamp maple, soft maple, Carolina red maple, Drummond red maple, and water maple, is a member of the *Aceraceae* (Maple) family. Along with *L. tulipifera, Acer rubrum* is one of the most abundant tree species in the Eastern U.S. It's native range spans from Southeastern Canada, as far south as Florida, and as far west as the Mississippi river (Little, 1979).

A. rubrum is a polygamo-dioecious plant, whose breeding capacity is expanded by having some male plants, some female plants, and some plants with flowers of both sexes. *A. rubrum* flowers occur high in the trees, and emerge long before *L. tulipifera,* between March and May throughout its range and as early as 4 years old (Walters et al., 1990). The fruit of *A. rubrum* are double samaras produced

in abundance and disseminated through June, with all seeds falling within a two-week period dispersed by the wind (Walters et al., 1990). Once in contact with soil, it takes a week or less for *A. rubrum* seeds to germinate, with germination success as high as 91% (Walters et al., 1990). Seeds can remain viable for at least 2 years on the forest floor, and germination requirements are few with sunlight availability being an important factor (Marquis, 1975). *A. rubrum* is a shade tolerant species, but seedlings have a higher level of tolerance, which promotes regeneration success (Walters et al., 1990). Regeneration can also occur through vigorous sprouting after cutting or disturbance by fire, with multiple sprouts from individual stumps.

Ecology

A. rubrum is becoming an increasingly dominant part of the forests throughout its range, reaching what some would call invasiveness in some areas (Abrams, 1998). According to the Society of American Foresters *A. rubrum* is a dominant component of 56 of the 88 forest cover classifications in the Eastern U.S. (Eyre, 1980). As is evident from its broad distribution, *A. rubrum* is able to withstand a variety of climate, soil, and topography conditions. *A. rubrum* can withstand temperatures as low as -40°C (Dansereau, 1957). *A. rubrum* is a moderately shade tolerant pioneer species, often found in old fields and disturbed areas, with the ability to survive in higher levels of shade than *L. tulipifera*. *A. rubrum* does not show significant aspect preference and is able to grow well in both moist and dry conditions from ridges to swamps (Desta et al., 2004, Stephenson, 1974). Further north *A. rubrum* grows more dominantly on ridges, and further south in swamps (Walters et al., 1990). *A. rubrum* grows in soils of varying parent materials of the following soil types: Entisols, Inceptisols, Ultisols, Alfisols, Spodosols, and Histosols (Walters et al., 1990).

Along with its broad range, *A. rubrum* can grow in a variety of site conditions. The taproot is only a few inches deep with remaining roots extending horizontally and up to 10 inches (25cm) deep into the soil (Walters et al., 1990). Aeration is important to *A. rubrum* roots, as is evident by its ability to withstand flooding, but not silt deposits that are left behind (Broadfoot and Williston, 1973). *A. rubrum*

has diffuse porous wood. Despite being abundant throughout its range, *A. rubrum* plays limited ecological roles. One such role is being a quality food source for deer and rabbits (Walters et al., 1990).

A. rubrum responds differently to varying types of competition. *A. rubrum* is shade tolerant, but grows much better as it ages with increased levels of sunlight (Walters et al., 1990). Shade tolerance as a seedling is advantageous when there is an overstory present, and is favorable in shelterwood management regimes. The ability to sprout quickly and respond positively to increased light levels gives *A. rubrum* regeneration advantages after major disturbances that drastically alter the overstory.

Though *A. rubrum* grows prolifically, it is susceptible to numerous damaging agents. The form of *A. rubrum* trees is often poor due to wounding from mixed sources. It has also been found that *A. rubrum* is highly resistant to herbicide by injection (Kossuth et al., 1980). Many insect infestations reduced tree vigor. *A. rubrum* is vulnerable to boring insects and scale insects, which largely attack the leaves of the trees. Boring insects include the gall making maple borer (*Xylotreehus aceris*), the maple callus borer (*Synanthedon acerni*), and the Columbian timber beetle (*Corthylus columbianus*) (Walters et al., 1990). The most common scale insects and moths affecting *A. rubrum* are cottony maple scale (*Pulvinaria vitis*), the maple leaf scale (*P. acericola*), the oystershell scale (*Lepidosaphes ulmi*), the gypsy moth (*Lymantria dispar*), the linden looper *(Erannis tiliaria*), the elm spanworm (*Ennomos subsignaria*), and the red maple spanworm (*Itame pustularia*) (Walters et al., 1990). Due to *A. rubrum* increased dominance in much of its range as a result of some other dominant species being drastically reduced in numbers due to insect and fungi infestations, the gypsy moth has become of particular concern. Fire also causes mild to severe damage to *A. rubrum* which responds to fire by sprouting from the root collar, though intense fires are likely to kill the above-ground portions of the tree (Walters et al., 1990). In addition to insects, fungi also attack wounds on *A. rubrum*, and some canker forming diseases attack the stems (Stephenson, 1974).

Wood Properties and Uses

A. rubrum lumber is commonly used for wood products in the Southeast U.S., due in part to is abundance. *A. rubrum* is a considered a soft maple, having wood that is not as strong as hard maples and heartwood of lighter color (Forest-Service, 1974). Common markets for this wood are container and shipping materials such as pallets, crates and boxes, to furniture and sometimes veneer with distinct rings (Forest-Service, 1974, Walters et al., 1990). *A. rubrum* has also been shown to be equivalent and sometimes superior to bigtooth aspen (*Populus grandidentata michx.*) as a flakeboard construction material (Kuklewski et al., 1985). When dried, the wood volume shrinks 12.6%, more than half of that of hard maples, and the wood color is a light red/brown (Forest-Service, 1974). The average specific gravity of *A. rubrum* before drying is approximately 0.49 increasing to 0.54 at 12% moisture content (Forest-Service, 1974). *A. rubrum* is also an aesthetically pleasing species, often planted as an ornamental in cities and elsewhere, due in part to fall foliage colors. Like other maples, *A. rubrum* is sometimes used to produce syrup.

Genetics

Like *L. tulipifera, A. rubrum* has phenotypic differences throughout its range, largely noticeable along with changes in physiographic province and latitude. In more northern and mountainous areas there tends to be more cold hardiness, more robust samaras, and earlier reproductive development (Walters et al., 1990, Townsend, 1972, Winstead et al., 1977). *A. rubrum* is capable of hybridizing with silver maple (Walters et al., 1990).

A. rubrum has great genetic potential due to its broad range and suitable site conditions (Townsend et al., 1979). Successful varieties of *A. rubrum* have been developed to combat air pollution, limited water, and vericillium wilt, for ornamentals often used in cities (Townsend et al., 1979). Studies have been done addressing *A. rubrum* genetics without using marker techniques or other genetic analysis tools. In a study on genetic correlation to seed dormancy break, it was determined that *A. rubrum* seeds were much more dependent on temperature than genetics (Perry and

Wu, 1960). Other maples, such as sugar maple has had active breeding programs due to its commercial value in the sugaring industry (Cornell, 2007).

Wood Density

There are different wood properties that are of interest in forestry and forest products such as fibril angle, width, length, and cellular chemistry. Each is of interest in the production of different products and are interrelated with wood density or specific gravity (Zobel and Buijtenen, 1989). Growth of a tree affects wood density, which is in turn an important factor for wood products, fiber products, and energy yield. Wood density, typically expressed in either pounds per cubic foot or kilograms per cubic meter, is dependant on specific gravity and moisture content of the wood (Simpson, 1993). The specific gravity of wood is the ratio of the wood density to the density of water in the wood, and is often used as a standard between species (Forest-Service, 1974). Specific gravity has been found to vary significantly within populations of *L. tulipifera* (Kellison, 1968, Taylor, 1968, Thorbjornsen, 1961), and other wood properties also differ between trees in the same population under the same environmental constraints (Zobel and Talbert, 1984). Wood density varies within and between species usually within 320 to 720 kg/m^3 (20-45 lb/ft^3) (Forest-Service, 1974).

Studies on the genetic association of wood properties in different species have been performed successfully in a few tree species. A study of over 2000 tree species in South and Central America found that wood density decreased with rising altitude and that 74% of wood density variation was explained by genus (Chave et al., 2006). According to the study, this provides a means of calculating biomass at the genus level, when specific species densities are not available. The issue of biomass calculations and carbon emissions is of increasing interest in areas of South and Central America that are experiencing rapid forest harvesting. Due to global warming carbon sequestration and greenhouse gas emissions are of increasing interest. Biomass of an area can be determined if the volume of wood is known, and can be more accurately determined if wood density of the dry weight is also known (Chave et al., 2006, Fearnside, 1997). Two Eucalyptus provenances had consistent wood densities and pulp

yields when grown in cold and warm climates (Clarke, 2000). Wood Density's dependence on the environment and biology of a tree makes it the most informative wood property (Chave et al., 2006).

Research identifying candidate genes associated with wood property traits via quantitative trait loci (QTL) have been successful and suggest promise for further advances in breeding and genetically identifying desirable wood property traits (Brown, 2003). Studies in tree species, and non-woody plants such as *Arabidopsis thaliana,* and *Zinnia elegans* have shown that gene expression involved in cell differentiation and wood formation is directed by specific transcription factors (Demura and Fukuda, 2007). It has also been found that tensile growth stress can be generated by decreasing lignin and increasing cellulose microfibrils parallel to fiber axes in the secondary wall (Yoshida et al., 2002).

Genetics

Molecular Marker Techniques

Molecular marker techniques are rapidly advancing versatile in their application. For instance, AFLP was developed in the medical field (Vos et al. 1995) and is now applied to many disciplines including forestry and plant biology. Generally speaking, genomics is the study of complete DNA sequences of species, which enables gene mapping at a fine scale. Genomics studies the entire genome while marker mapping focuses on particular areas or genes in the genome. In this sense, marker mapping is a much narrower approach to studying genomes. As integrating the tools and ideas from different disciplines continues, it is evident that pooling the strengths of genomics and gene mapping for future population genetics studies will be beneficial, especially in forest research targeting association mapping studies (Krutovsky, 2006). Different population genetics techniques including AFLP, SSR, and SNPs are currently available in forestry and are becoming more applicable to population genomics and association mapping studies (Krutovsky, 2006).

Genetic markers are DNA sequences or genes that can be identified or located in the genome. Markers can be associated with a particular phenotype and used as breeding and management tools in forestry for achieving desirable phenotypes (Ridley, 2003). Markers can be easily identifiable, such as

AFLP using gel electrophoresis (Vos et al., 1995), and aid in genetic association studies and attaining breeding objectives. There are many different marker techniques including but not limited to microsatellites (SSR), single nucleotide polymorphism (SNP), and amplified fragment length polymorphism (AFLP). Each marker technique has advantages and disadvantages with respect to research objectives and limitations.

AFLP is beneficial when the genome of a species has not been mapped, while SSR would be more informative on species whose genomes have been mapped and which genetic information had previously been gathered (Quellera et al., 1993, Jump and Penuelas, 2007). Microsatellites are short nucleotide sequences that are repeated many times (Quellera et al., 1993). Microsatellite markers (SSRs) are generally more informative than AFLP markers when the genome of the study organism is known, which has been highlighted in previous research (Jump and Penuelas, 2007). SSRs are co-dominant markers, able to detect heterozygosity at a locus of interest, providing information about individual alleles. Single nucleotide polymorphisms are variations or changes in individual nucleotides in the DNA of an individual. This is a much more time consuming process and requires that the specific region of DNA where the polymorphism is occurring, already be known. An advantage to this methodology, in addition to being widely used, there are multiple free access databases online of SNPs (NCBI, 2008).

In the research project at hand, AFLP was used to assess genetic variations across the landscape and associations with wood density. AFLP is a highly reproducible marker technique (Vos et al., 1995). AFLP is a dominant marker technique that cannot distinguish between heterozygous and dominant. As its name suggests, amplified fragment length polymorphism analyzes DNA fragment lengths, and variations in those lengths between individuals indicates variation in the genetic makeup. AFLP is particularly useful in studying genetic diversity across the landscape, and robust because it requires much smaller sample sizes than SSR (Jump and Penuelas, 2007). Currently SSR is more commonly used, partly because it is more informative for species whose genomes have been

sequenced. Another advantage of AFLP is that it does not require that the genome be sequenced for the organism being studied. In a comparison of AFLP to microsatellite markers, AFLP markers were found to be more robust for genetic differentiation on small spatial scales, in determining the relatedness of nearby populations, and determining populations of origin (Gaudeul et al., 2004). These findings have been supported by others, including studies utilizing limited primer combinations to assess genetic diversity in accessions (Pang et al., 2006).

Molecular Marker Applications in Plants

Many molecular marker techniques including AFLP originated in human genetic studies (Vos et al, 1995). These marker techniques have been applied to genomic studies of agricultural crops and non-agricultural products and have expedited the sequencing of multiple plant genomes (Smallwood, 2006). This interest has also been pursued in forestry practices, most recently notable in the sequencing of the genome of *Populus trichocarpa* (black cottonwood) (Tuskan G.A., 2006).

Linkage disequilibrium studies have been utilized extensively in human genetics research (Pritchard and Przeworski, 2001, Goldstein and Weale, 2001). Linkage disequilibrium studies have more recently been applied to plants, largely focusing on agricultural species. Linkage disequilibrium studies yield strong results in unmanaged populations because they can provide information on relatedness and historical recombination events in a population.

The first study to utilize linkage disequilibrium mapping in a plant was in 2001 and applied linkage disequilibrium mapping to natural populations of sea beets (Hansen et al., 2001). AFLP has been utilized to determine if short distances spanned by linkage disequilibrium enable the identification of markers that are very tightly linked to a target gene (Hansen et al., 2001). The main target was to look for a gene in the genome that was associated with the gene for annual growth habit (B gene) determining whether the beets need a period at low temperatures before bolting, which should be identifiably associated with an AFLP marker (Hansen et al., 2001).

When examining linkage disequilibrium, dominant genotypes are typically of interest, which is appropriate in the usage of AFLP. There may be multiple fragments of the same length, which are not homogeneous, which could produce false positives when determining genetic linkage (Hansen et al., 2001). Another disadvantage of AFLP is that the co-dominant and recessive genotypes are not examined (Vos et al., 1995). Linkage between tightly linked loci can be detected by screening for linkage disequilibrium (Hansen et al., 2001). Future studies in linkage disequilibrium could be applied to other species, ecosystems, and across different landscapes.

Since initial studies of sea beets (Hansen et al., 2001) other agricultural crops have been explored including soybean (Kopisch-Obuch and Diers, 2006). Much research has been done in pursuit of developing soybean crops that are resistant to the soybean cyst nematode (SCN), as this is a major pest to this crop in the U.S. (Kopisch-Obuch and Diers, 2006). Segregation distortion against the SCN resistance allele occurs in most breeding efforts aimed at selecting for the SCN resistant allele (Kopisch-Obuch and Diers, 2006). Utilizing AFLP to test for segregation distortion with the SCN resistant locus and seedling emergence revealed a dominant relationship between the SCN-resistance allele and seedling emergence (Kopisch-Obuch and Diers, 2006).

Many association studies have been performed in trees, largely in conifer species (Neale and Savolainen., 2004). A multi-gene association genetics study involving single nucleotide polymorphisms and various evolutionarily important and desirable commercial wood qualities of loblolly pine, *Pinus taeda* L., found strong association between a-tubulin and earlywood microfibril angle, and supported the effectiveness of dissecting complex adaptive traits while using genes involved in key pathways such as the formation of microtubules (Gonzalez-Martinez et al., 2006b). Such studies could have implications for the future dealing with improved commercial value of timber species through providing a means of producing beneficial genetically engineered crops. This first published multi-gene association study in conifer trees yielded useful methodology for others to pursue this approach (Gonzalez-Martinez et al., 2006b). Candidate genes from previous studies prove beneficial

additions to association genetics studies, saving the researchers time and effort that would have been spent arriving at results that had already been well documented (Gonzalez-Martinez, 2006). Being able to incorporate outcome from other research in this way shows strong continuity in the field and promotes cooperation between researchers and the integration and utilization of various research techniques.

Association genetics studies can use quantitative trait loci to study complex gene and trait associations involving multiple gene effects on traits rather than only single gene effects on traits. Quantitative Trait Loci (QTL) are segments of DNA that affect and are linked to quantitative traits such as growth rates, with many QTL being associated with individual traits. QTLs can be molecularly identified (for example, with PCR or AFLP) to help map regions of the genome that contain genes involved in specific quantitative traits. This can be an early step in identifying and sequencing these genes, but most importantly with associating genes to traits of interest. Mapping of quantitative traits has much promise because it is the only current mapping technique that has the ability to analyze adaptive traits (Krutovsky, 2006). It has high requirements however, including a mapped genome of the species of interest. These methods have been utilized in conifer species. Another criticism of QTLs is for tree species with low levels of linkage disequilibrium and an unmapped genome it is not possible to utilize association mapping (Krutovsky, 2006). Association genetics is particularly well suited for conifers like *P. taeda* because it has high levels of nucleotide diversity and low linkage disequilibrium (Brown et al., 2004, Gonzalez-Martinez et al., 2006b), uncomplicated propagation allows large populations that are easily maintained, and it retains most of its natural variability almost regardless of population size and hundreds of generations removed from the wild type (Gonzalez-Martinez et al., 2006b). Large populations are needed for association genetic studies and higher frequencies of false positives of associations are more common than with QTL studies.

While QTL studies were quite effective in identifying effects, location and number of individual QTL, these studies did not have the capability of evaluating multiple QTL for the same trait until recent

years and are restricted to a single lineage (Gonzalez-Martinez et al., 2006b). Loblolly pine is a conifer and thereby has a large genome, making finding single gene-trait relationships challenging and uncommon (Gonzalez-Martinez et al., 2006a). Working with species with smaller genomes could increase likelihoods of informative association genetics studies. Strong genetic association has been found between α-*tubulin* and earlywood microfibril angle (Gonzalez-Martinez et al., 2006a).

Association genetics ideas have been introduced that could help develop useful tools in forest biotechnology (Gonzalez-Martinez et al., 2006b). It is thereby important that QTL, co-location and other studies are continued so that candidate genes for economically and evolutionarily significant traits can be identified. Identification of these genes will allow for further association genetics studies to address not only single gene effects on traits but also multiple-gene effects. Current work in association genetics emphasizes the need for cooperation between researchers at various stages of genetic exploration and the integration of research techniques into a broad range of application which is supported by use of previously determined SNPs (Gonzalez-Martinez et al., 2006b).

Molecular Marker Applications in Forestry

The integration of genetics into forestry has been beneficial to the advancement of both sciences as a new specialty, and produced many practical applications of genetics in forestry. The core of genetics' role in forestry is dependent on the economic viability of the advances made through genetics. The demands and capabilities of genetics in forestry are being influenced by the advancement of the science and rising social components (Burley, 2001). The potential for genetics applications in forestry is continuing to rise and anticipated to benefit not only the science, but also economies that are directly dependent on forestry, particularly tree improvement programs in the Southeast U.S. (Byram et al., 2005a). Successful breeding programs and complex trait studies have been implemented in conifer species, which are more often favored for these studies due to random mating patterns and nucleotide diversity, among other things (Neale and Savolainen., 2004).

The economic viability of breeding programs and genetic applications in forestry is often questioned, but supported by the efficiency and accuracy that these techniques provide. Genetic advancement has attributed for much of the increase in forest productivity in the southeast U.S. as increased breeding efficiency and highly successful phenotypes (Byram et al., 2005a). Breeding for particular traits or using markers to breed for certain wood properties can be beneficial even if the trait gains are modest. A cost-benefit analysis found economic gains from using marker based selection for wood density and diameter at breast height traits in *Pinus radiata* Donn. ex D. Don. despite small loci effects (Wilcox et al., 2001).

Tree breeding, population studies, trait association studies, and clonal plantations are some aspects of forestry to which genetics continue to contribute. Time is a major advantage of advanced breeding methods over traditional methods. It takes less time to breed plants for certain desirable traits with newer technologies than waiting for generations of the species to become mature enough to regenerate. Associating desirable traits to genetic markers through association studies is becoming increasingly advantageous and popular. Integrating genomics and marker techniques in studying forest populations is of the utmost importance (Krutovsky, 2006). The precision and relative ease of identifying and quantifying genetic markers as opposed to measuring phenotype can be of further benefit to forestry studies. Tests on silvicultural and genetic treatment effects on production capacity in *P. taeda* (loblolly pine) and *Pinus elliottii* Engelm. *var. elliotttii* (slash pine) have shown economically important outcome for breeding programs (Roth et al., 2007). It has been determined that genotype and silvicultural treatment had the greatest positive effect on production and that genotype, silvicultural treatment, and location did not interact at a significant level (Roth et al., 2007).

Breeding programs have shown much success in forestry, with some bred genotypes capable of maintaining consistently desirable phenotypes across climatic and site differences. *Pinus taeda* has been bred in the Southern U.S. and showed that genotype by environment interactions are not currently the highest concern for breeding programs (McKeand et al., 2006a). Plantation forestry is becoming

increasingly time, cost, productivity, and space efficient. Plantations are gaining economic viability, an important tool in genetic conservation, and are facilitated by advances in forest biotechnology and silvicultural methods that are advantageous to breeding and propagating trees (Nehra et al., 2005).

Association mapping studies could be beneficial to breeding and plantation forestry efficiency, and builds heavily upon previous research. One such successful multi-gene association genetics study involved single nucleotide polymorphisms and wood property traits for *P. taeda*, and was performed as a expansion of prior QTL and co-location studies (Gonzalez-Martinez et al., 2006a). *P. taeda* has had associations drawn between microfibril angle and a specific gene (Gonzalez-Martinez et al., 2006a). Association mapping studies utilizing AFLP can perform a bulk segregate analysis in which two pools of DNA are screened for markers, one pool of samples with the desired trait, and one pool of samples that do not have the desired trait. If certain markers occur in the samples with the desired phenotypic trait and not in the individuals without the desired phenotypic traits, those markers would be further examined for association with the desired phenotypic trait. In this thesis project, the phenotypic trait of interest is wood density that exists in a gradient. Therefore, systematic ranges of desirable wood densities would be identified for pooling of samples performed. In order to make associations between an anonymous AFLP marker and a trait like wood density it would be useful to have a highly saturated linkage map (Paterson et al., 1991). Many polymorphisms identified may prove to be extremely variable when tested in the larger population which is unfolding in DNA fingerprinting of woody species including apple and blackberries (Wagner, 1992).

Forestry is not only important to local economies, but also the global economy that promotes collaboration between business and scientific communities (Byram et al., 2005a). The Western Gulf Forest Tree Improvement Program (WGFTIP) has focused on breeding for high volume timber due to timber volume based compensation (Byram et al., 2005b). Breeding for multiple characteristics if often complicated and increasingly unsuccessful as traits become compounded. Viability of a breeding program is recognized by the importance of desirable traits and is dependant on the grower and buyer

needs, and economic competitiveness. WGFTIP breeding programs and compensation are beginning to rely more on the quality and traits of timber rather than solely volume of timber, thereby promoting breeding programs focused on wood properties other than production capacity (Byram et al., 2005b).

Molecular Ecology

Molecular ecology is a fairly recently diverged branch of science which incorporates the tools of genetics into the study of ecological systems, ranging from applied science to theoretical (Beebee and Rowe, 2004). Parallels are drawn between the development of biochemistry as a science and the development of molecular ecology (Beebee and Rowe, 2004). The field was more formally declared in the early 1980s and now holds two prominent scientific journals: Molecular Ecology, and Molecular Ecology Notes.

One of the most fascinating and promising aspects of molecular ecology is not only the integration of techniques from various scientific fields, but the success that it has had in declaring itself as a separate entity. Integration of techniques is important to the advancement of scientific discovery, and maintaining a somewhat clearly defined degree of separation between fields lends to the development of a more cohesive area of science. Molecular ecology focuses on the genetics of conserving species, gene flow in natural populations, implications in not only flora but fauna populations as well, and more (Beebee and Rowe, 2004).

Genetics has the potential to be extremely important in species and biodiversity conservation. Not only does understanding what genetic diversity is in a population but also the importance of that diversity can be vital to conservation issues. Importance of genetic diversity can range from the economical value of a species or population, to maintaining a viable breeding population to ensure healthy levels of fitness. Genetic diversity is considered an important aspect of species survival in the central role it plays in adaptation and reproduction success. Genetic diversity can therefore give insight into the health of a population and its potential to maintain itself reproductively. This is especially important in the conservation of rare and endangered species.

The goal of gene conservation is not only to save desirable qualities of species that may be economically important, but also to maintain a large enough gene pool to allow a species to sustain itself for years to come (Helms, 1998). Genetic uniformity, sometimes the result of bottlenecks, can drastically weaken populations and threaten chance of recovery (Ledig, 1996). In the event of rare and endangered species where management plans are being implemented, it is important to take into account gene flow in the planning of reserve size and shape (Given, 1986). Understanding genetic composition and bottlenecks that populations have been through is also paramount to determining conservation strategies and likelihoods.

Molecular ecology expands beyond causes for and appropriate strategies for gene and species conservation to understanding how all species interact in much the same way that ecology approaches this subject. Molecular ecology takes that approach one step further to include and focus on genetic relationships involved in ecological processes. This opens the vantage point of the discipline to studies seeking to address a more hereditary and molecular understanding of ecological mechanisms and processes.

Chapter 3: Assessment of genetic variation of *Liriodendron tulipifera* L. populations in unmanaged forests of the Southeastern United States

Katherine E. Kovach[1]
Muhammad Javed Iqbal[2]
E.M. Ulrika Egertsdotter[3]
Shepard M. Zedaker[1]

[1]Department of Forestry, Virginia Polytechnic Institute and State University, 228 Cheatham Hall, Mail Code 0324, Blacksburg, VA 24061
[2] Institute for Sustainable and Renewable Resources (ISRR) at the Institute for Advanced Learning and Research (IALR), 150 Slayton Ave., Danville, VA 24540
[3]School of Biology, Georgia Institute of Technology, 500 Tenth Street, NW, Atlanta, GA 30332-0620

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Corresponding Author: Katherine E. Kovach, Department of Forestry, Virginia Polytechnic Institute and State University, 228 Cheatham Hall, Mail Code 0324, Blacksburg, VA 24061, kekovach@vt.edu

Abstract

Liriodendron tulipifera L. is prolific throughout its range in the Southeastern U.S. and is playing increasingly important roles in forestry and wood products in this region. The relatively low density and intermediate strength of the *L. tulipifera* wood makes it versatile for use in wood products. Changes in forestry practices, land management, and ecological shifts have resulted in increased numbers of *L. tulipifera* in this region's forests. Therefore, it is important to analyze changes in the genetic diversity of populations within this region in order to monitor and predict future health of these populations and their potential role in local economies. In this study, amplified fragment length polymorphism (AFLP) was utilized to determine the level of genetic diversity of *L. tulipifera* in contrasting physiographic provinces. AFLP analysis was performed on samples collected from six unmanaged populations in the Mountains (Virginia, Tennessee, and Ohio) and Coastal Plain (Virginia, South Carolina, and Mississippi) of the Southeastern U.S. Wood density was determined using an X-ray densitometer. Highest genetic diversity (H_e: 0.281) was observed within Mountain populations and the lowest genetic diversity (H_e: 0.271) was observed within Costal Plain populations. This was despite the fact that the Coastal Plain sites had the greatest geographic distances between them. AMOVA results indicate that the populations were more genetically similar within than between physiographic provinces. Mean annual precipitation, growing season, and temperature were more similar for the Coastal Plain populations than the Mountain populations. The average wood density for *L. tulipifera* was greater in the Mountains (445.45 kg/m^3) than in the Coastal Plain (441.67 kg/m^3), though not significantly different. Ecological factors seem to have some effect on the population structures revealed by the AFLP analysis. The patterns of genetic diversity observed could also provide a solid foundation for further genetic gains by breeding of this economically and ecologically important tree.

Introduction

Liriodendron tulipifera L. is a deciduous tree with a wide range that extends east of the Mississippi river from northern Florida to the New England area in the U.S. *L. tulipifera* is one of the most common soft-hardwood species in its range and has increasingly important roles in forestry and wood products. The tree has a distinctive conical crown and large showy flowers that bloom in late spring and are pollinated by insects (Wilcox and Taft, 1969). *L. tulipifera* has high commercial value for lumber, plywood, pulpwood, and veneer, and is rapidly replacing softwoods in these markets (Beck et al., 1990).

With such a widespread range, *L. tulipifera* can survive in many different ecological scenarios. Changes in management, including shifts in landownership to smaller lots and individual owners in Southeastern U.S. forests are causing *L. tulipifera* to become more common, making understanding its biology and population structure of great interest. Many of the forests of this region have recently been dominated by oak hardwood forest types, but due to ecologic and management shifts species composition has also shifted (Abrams, 2003). *L. tulipifera* grows in a wide range of environmental conditions, and due to its rapid initial growth rate can respond quickly to increases in light to the forest floor. *L. tulipifera* grows well on higher quality sites with adequate light levels, making regeneration of oaks increasingly challenging due to the added competition (Loftis, 1990). Realizing that forests are not static communities but continually changing ecosystems, it is important to know more about the forests and how these systems can be more effectively and sustainably managed. It is important to examine the changes in species composition that are occurring and to note that *L. tulipifera* is one of the species becoming increasingly dominant (Abrams and Downs, 1990, Dyer, 2001, Orwig and Abrams, 1994).

Molecular markers are useful in the study of genetic variation and as breeding tools. Molecular markers can be used in association studies, where regions of DNA are correlated to particular phenotypic traits, and in breeding programs where associated traits are put to use, as well as other

pursuits. With advances in technology, the integration of molecular marker techniques into forestry and ecology studies is increasing, especially with successful breeding in conifer species (Neale and Savolainen., 2004). *Pinus taeda* is a popular plantation tree in the Southern U.S. that has had many provenance studies and successful breeding events that are now being furthered by molecular markers (McKeand et al., 2006b). Studies of natural populations of *P. taeda* has shown that phenotypic variations such as mean annual increment for volume throughout species range, can be attributed to genetic differences (Sierra-Lucero et al., 2002). Findings such as these suggest that selective breeding for optimal phenotypes and genotypes can be done successfully with fairly young trees with emphasis being placed on populations with high expression of desirable traits (Sierra-Lucero et al., 2002).

This study assesses the genetic diversity in *L. tulipifera* populations in the Southeastern U.S. utilizing amplified fragment length polymorphism (AFLP). AFLP is an anonymous marker technique selected for this study because it is highly reproducible and a powerful tool for species whose genomes are not known (Gaudeul et al., 2004, Cottrell et al., 2003, Jones et al., 1997). AFLP was developed in the mid 1990s (Vos et al., 1995) and applied effectively to mapping disease resistance in *Populus* spp. shortly thereafter (Cervera et al., 1996). Studies have been done in many species of plants comparing the benefits and effectiveness of AFLP to different marker techniques. Gaudeul et al. (2004) compared the effectiveness of AFLP versus microsatellite markers for determining relatedness of 12 populations of *Eryngium alpinum* L. in France, using a sample size of 24 individual plants from each population. (Cottrell et al., 2003) used microsatellite markers to determine differences in genetic structures of 3 oak populations with large sample size and highly varied management regimes. Different marker techniques have different advantages, and AFLP is often desirable because it does not require a sequenced genome of the species of interest.

L. tulipifera does exhibit phenotypic and genetic variation across its range (Sewell et al., 1996a). Phenotypic variation has been observed by increasing degree of leaf variability as we move north through the range (Kellison, 1968, Parks et al., 1994). The strongest genetic differences could be

expected between Mountain populations of high latitude and Coastal Plain populations of low latitude due in part to differences in growing seasons (Walters et al., 1990) and also historic divergence of chloroplast DNA (Qiu and Parks, 1994, Sewell et al., 1996a). Variation of *L. tulipifera* chloroplast DNA in the U.S. showed that northern and southern populations of *L. tulipifera* were evolutionarily distinct (Parks and Wendel, 1990) and that populations in northern Florida were a different cpDNA haplotype than all other sampled North American populations (Sewell et al., 1996b, Parks and Wendel, 1990).

Further exploration of the genetic diversity of *L. tulipifera* and how it varies throughout its range could be useful in determining more effective management tools if desired phenotypes are associated with environmental or genetic conditions. Previous research at SEMI (Sustainable Engineered Materials Institute at Virginia Polytechnic Institute and State University) found that *L. tulipifera* did not have significant wood density differences between physiographic provinces. However, the management regime including fertilization and irrigation yielded lower wood density in *L. tulipifera* (Jones et al., 2006). When analyzing all management intensities collectively, unmanaged to irrigated and fertilized, it was observed that *L. tulipifera* had higher wood densities in the Piedmont and similar wood densities in the Mountains and Coastal Plain (Jones et al., 2006). The physiographic provinces used were Mountains, Piedmont, and Coastal Plain and latitude classes were above 36º, between 36º and 32º, and below 32º. The present study focused on a subset of the larger SEMI data pool while expanding the current data pool to include molecular marker information and an additional field site. The sites used in this study were unmanaged populations of *L. tulipifera* in Mountains and Coastal Plain provinces of the Southeastern U.S.

The objectives of this study were: (i) evaluate genetic variation within populations of *L. tulipifera*, (ii) evaluate genetic variation of *L. tulipifera* within physiographic provinces, and (iii) to evaluate genetic variation of *L. tulipifera* throughout the Southeastern U.S. Attempts were also made to assess potential correlations between wood density and genetic variation and ecological data.

Materials and Methods

Sites and Sampling

Six unmanaged populations were sampled on private and public lands in the Mountains and the Coastal Plain of the Southeast U.S. (Table 2). A map of the field sites was generated from the latitude and longitude of each site using Google Earth (Google, 2008). A minimum of three 0.1acre (0.04 ha) plots were established in each population. The total height, height to live crown and diameter at breast height (dbh) were measured for each tree within the plots. Sampled trees represented the diameter class distribution of the plot. For each plot the physiographic province, latitude/longitude, management intensity, landowner, site, and plot numbers were recorded. Each site had 9-19 trees sampled with 12 mm bark-to-bark increment cores taken from breast height. In order to minimize invasion by insects or pathogens, trees were plugged with wooden plugs. Cores were placed in labeled plastic bags in a portable freezer at approximately -20°C.

Leaf material for the marker analysis was collected with pole trimmers on branches less than 15ft high. A 12-gauge shotgun with #4 copper coated shot was used to sample leaves higher than 15ft. Sampled leaves were placed in plastic bags and immediately stored in a cooler with dry ice, and transported in a portable freezer at -20°C.

Environmental Analysis

Environmental data including mean annual precipitation, mean annual temperature, and mean annual growing season length were gathered via Daily Surface Data ASCII files from the National Oceanic and Atmospheric Administration (N.O.A.A., 2008). Data was retrieved for years 1958 through 2008, the anticipated average age of stands sampled. Mean annual growing season was determined by calculating the average number of days between the last frost in the spring and the first frost in the winter months from 1958 to 2008.

DNA isolation

Leaf tissue was stored at -20°C until DNA isolations. Leaf tissue was ground in a mortar and pestle with liquid nitrogen and a SPEX SamplePrep Model 2000 Geno/Grinder laboratory mill (SPEX CertiPrep®, Inc., Metuchen, NJ). A slightly modified version of the Plant DNAzol (Molecular Research Center, Inc. Cincinnati, OH) protocol was used to isolate the DNA from the leaf tissue (Ausubel et al., 1990, Wilfinger et al., 1997, Chang et al., 2009). The quality of DNA was verified by running on a 0.8% (w/v) agarose gel at 97 volts for 1.5hours and concentration was measured using a NanoDrop (Thermo Fisher Scientific, Waltham, MA). DNA samples were diluted with 1X TE buffer to approximately 125 ng/µl concentrations.

AFLP Analysis

AFLP was performed with a modified version of the Vos et al. (1995). The AFLP process includes restriction digestion and adapter ligation of DNA, pre-selective amplification, selective amplification, and electrophoresis (Vos et al., 1995). The restriction digestion and adapter ligations were performed on genomic DNA (500 ng) with *Eco*RI and *Mse*I restriction enzymes and adapters (New England BioLabs, Ipswich, MA). The final restriction digestion and adapter ligation volume (11 µL) contained 0.05 M NaCl, 0.045 mg/mL BSA, 1 µM *Eco*RI adapter, 5 µM *Mse*I adapter, 5 U *Eco*RI (NEB), 5 U *Mse*I (NEB) and 1 U T4 DNA ligase (NEB), 1× T4 ligase buffer (50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP) (New England BioLabs, Ipswich, MA) and was incubated for 6 hours at 37 °C. Products from the restriction digestion and adapter ligation step were diluted 10 times with 0.1 x TE buffer (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA).

Diluted restriction-ligation product (3 µL) was used in pre-selective amplification in a MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA) which was programmed for 72 °C for 2 min followed by 20 cycles of 94 °C for 20 s, 56 °C for 30 s, and 72 °C for 2 min, and a final steps of 72 °C for 2 min and 60 °C for 30 min. Pre-selective amplification reactions (13µl) contained 1× PCR buffer

(100 mM Tris-HCl (pH 8.3), 500 mM KCl), 2.0 mM $MgCl_2$, 0.2 mM dNTP, 10 µM *Eco*RI and *Mse*I pre-selective primers, and 0.25 U of JumpStart *Taq* DNA polymerase (Sigma-Aldrich, St. Louis, MO). Pre-selective amplification products were diluted 10 times with 0.1× TE buffer and 2 µL of diluted product were used in selective amplification.

Selective amplification PCR (8 µL) contained 1× PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.625 µM of dye labeled *Eco*RI primer, 0.625 µM *Mse*I primer, and 0.2 U of JumpStart *Taq* DNA polymerase (Sigma-Aldrich, St. Louis, MO). The PCR amplification consisted of an initial denaturation step of 94 °C for 2 min, followed by the first cycle of 94 °C for 20 s, 66 °C for 30 s, 72 °C for 2 min and one degree decrease in annealing temp in each of the next nine cycles. This was followed by 25 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 3 min. The reactions were then incubated at 60 °C for 30 min. Primer combinations used in selective amplification were selected after performing selective amplifications on one complete population with 10 primer combinations (one *Eco*RI dye labeled and 10 *Mse*I primers). The 10 combinations were narrowed to five primer combinations that were used with all samples from all populations. A Sigma Genosys *Eco*RI -S1 primer was used with five different *Mse*I primers with base extensions CG, CC, GG, GA, and AG (S1, S4, S5, S8, and S10).

Selective amplification products from each sample (1.0 µl) were combined with 0.3 µl of the DNA size standard – 600 (Beckman-Coulter Inc., Fullerton, CA) and 39.7µl of sample loading solution (Beckman-Coulter Inc., Fullerton, CA). Capillary electrophoresis was performed using CEQ 8800 Genetic Analysis System (Beckman-Coulter Inc., Fullerton, CA). The Frag-4 analysis parameters of the CEQ software were applied to the samples and size standard (Beckman-Coulter Inc., Fullerton, CA). All AFLP fragments were scored as binary data (1, peak present; 0, peak absent) along with their sizes, which were verified with the electropherograms. The data set was trimmed to fragment lengths from 95-300bp, the most reliable range for the CEQ 8800 (Saunders et al., 2001). Data from the CEQ 8800 software was then exported into Excel for analysis.

Wood Density Analysis

Increment cores were dried to approximately 12% moisture content before being processed. Cores were glued into wooden holders and sawn lengthwise to approximately 1.65mm thickness. The slices were scanned with a Quintek QTRS-01X Tree Ring Scanner (Knoxville, TN, USA) to acquire average wood density measurements. Core density outputs were visually verified with scanned core image to ensure proper density measurements.

Statistical Analysis

NTSYSpc (Numerical Taxonomy and Multivariate Analysis System) Version 2.2 (Rohlf, 2005) was used to calculate genetic distance and similarity. The unweighted pair group method with arithmetic mean (UPGMA) dendrogram of the Dice similarity index were calculated with NTSYSpc (Dice, 1945, Nei and Li, 1979). Nei's original diversity index (Nei, 1973) was calculated using PopGene v.3.2 (Yeh and Boyle, 1997). UPGMA dendrogram based on Nei's original genetic distance matrix across all populations was created using PopGene v.3.2 (Yeh and Boyle, 1997). Shannon's diversity index, Gst values ($G_{st}= D_{st}/ H_t$) (Nei, 1987), gene flow (N_m) (McDermott and McDonald, 1993), and other population statistics were also calculated. Analysis of molecular variance (AMOVA) between populations, within populations and between physiographic provinces was calculated using Arlequin v.3.11 (Excoffier et al., 2005). Average and range statistics were calculated for the wood density measurement, including an ANOVA of the average wood density values (SAS, 2007).

Results

Overall Genetic Variability

Each amplified fragment counted during the AFLP analysis is considered a restriction fragment (Vos et al., 1995). The 5 AFLP primer combinations generated a total of 954 DNA fragments from 88 samples ranging in size from 95 to 300 bp. The average number of polymorphic DNA fragments was 811, over 100 fewer than the total number. The overall percent polymorphic loci (PPL) was 84.92%. The estimates of overall genetic diversity were low (H_e = 0.29 and I = 0.44). The levels of marker

polymorphisms were higher overall (954) than for either of the physiographic provinces (Mountains: 933, Coastal Plain: 949). Higher values of the Nei gene diversity H_e (Nei, 1973) are indicative of higher diversity (Nei, 1978). The H_e was higher (0.29) overall, compared to the Mountains (0.28), or Coastal Plain (0.27) sites (Table 3). The Shannon's Information Index (*I)* (Lewontin, 1972) which is another measure of gene diversity, was also higher (0.44) overall, compared to the Mountains (0.43), or the Coastal Plain (0.42). The overall average of wood density was 443.56 kg/m^3 (Table 4). Mean annual precipitation was 46.93 inches (Figure 3) and mean annual growing season was 210.60 days (Figure 4). The mean annual temperature for all these sites between 1958 and 2008 was 58.38°F (Figure 5).

Within Populations

There was a high degree of genetic similarity within populations (Table 3), and also a significant level of molecular variance (Table 5). The levels of H_e within each population ranged from 0.23 in Chillicothe, OH to 0.28 in Oak Ridge, TN (Table 3). The levels of *I* range from 0.34 in Chillicothe, OH to 0.43 in Oak Ridge, TN (Table 3). The number of polymorphic loci also varied depending on the population (Table 3).

The genetic distance between each site is shown in the lower diagonal of Table 6, and was used to create a dendrogram by applying UPGMA. These genetic distances were a result of the Nei's original measure of genetic identity (Nei, 1973). Each population was on average more similar to itself than to other populations as can be seen by how each population typically clusters together in Figure 6. The level of molecular variance within populations was significant (Table 5).

The populations with the biggest contrast in average wood density (Oak Ridge, TN: 471.69 kg/m^3; Vicksburg, MS: 387.58kg/m^3) differ by 89.47 (kg/m^3) (Table 4). The smallest range of wood densities, 77.28 kg/m^3, and average wood density, 387.58 kg/m^3, were found in the Coastal Plain population of Vicksburg, MS, which was also the smallest sample size of this study. The largest range of wood densities, 180.09 (kg/m^3) and average wood density, 477.05 (kg/m^3), were found in the

Coastal Plain population of Tappahannock, VA. Differences in wood density at these populations appear to be less dependent on physiographic province and more dependent on specific site characteristics like mean annual precipitation and growing season. Vicksburg, MS (257 days) had the longest mean annual growing season, while Blacksburg, VA had the shortest (166 days) (Figure 4). Vicksburg, MS also had the highest mean annual precipitation (55.8 inches) and the highest mean annual temperature (65.25°F) (Figure 5). Blacksburg, VA had the lowest mean annual temperature (51.80°F) and the lowest mean annual precipitation occurred in Chillicothe, OH (38.14 inches) (Figure 3).

Between Populations

The degree of genetic and marker variation between populations varied. The relative magnitude of genetic differentiation among populations, Gst, was higher for the Mountains (0.075) than for the Coastal Plain (0.0667) or the overall differentiation (0.0446). The AMOVA and pair wise Fst showed that there were significant differences between each of the populations studied except for Vicksburg, MS and Summerville, SC (Table 7). The marker differences between the Mountains and Coastal Plain were significant (Table 7). Genetic variation was also significant between populations. The genetic distances between populations were generally higher between physiographic provinces (Table 6). The number of polymorphic loci ranged from 699 in Chillicothe, OH to 889 in Summerville, SC (Table 3). The PPL ranged from 73.19% in Chillicothe, OH to 93.09% in Summerville, SC (Table 3). There was similarity with the number of polymorphic loci and the PPL, as well as I and H_e of the different populations (Table 3).

The average wood densities of the unmanaged populations sampled differed more on an individual population level than at the physiographic province level, though there was no significant amount of variation between any of the sites. The biggest difference between mean wood densities of populations was between Vicksburg, MS (387.58 kg/m^3) and Tappahannock, VA (477.05 kg/m^3). The greatest difference in mean annual precipitation was between Chillicothe, OH (38.14 inches) and

Vicksburg, MS (55.80 inches) (Figure 3). The difference in mean annual growing season was greatest between Blacksburg, VA (166 days) and Vicksburg, MS (257 days) (Figure 4). The greatest difference in mean annual temperature was between Blacksburg, VA (51.80°F) and Vicksburg, MS (65.25°F) (Figure 5). The distances between sites ranged from 309.49 km between Blacksburg, VA and Chillicothe, OH to 1386.79 km between Tappahannock, VA and Vicksburg, MS (Figure 6).

Between Physiographic Provinces

Sites were more genetically similar within physiographic provinces than between. The degree of marker similarity within each physiographic province was higher than the degree of similarity between them (Figure 7). There were lower levels of marker polymorphisms in the Mountain populations 81.74% than the Coastal Plain populations 88.1%, however measures of genetic variation typically indicated higher levels in the Mountains. Overall, the Coastal Plain sites were more genetically similar to each other than the Mountain sites (Figure 8). The overall Gst value was 0.045, while the Mountain populations were 0.075 and the Coastal Plain populations were 0.067. I was higher for the Mountain populations, 0.43, than the Coastal Plain, 0.42, indicating that the total gene diversity of the Mountain populations was higher than the Coastal Plain. The I was higher for the Mountains, 0.43, than the Coastal Plain, 0.42. The Mountain populations also had a greater range and std. dev. for H_e and I. The levels of H_e were slightly higher for the Mountains, 0.28, than the Coastal Plain, 0.27 (Table 3). The total genetic diversity Ht of the Mountains, 0.283, was also higher than the Coastal Plain, 0.271, but lower than the overall diversity, 0.290.

The average wood densities for the Mountain populations, 445.45kg/m^3, and the Coastal Plain populations, 441.67kg/m^3, were very similar, with the Coastal Plain being slightly lower (Table 4). The average range of wood density values obtained was larger in the Coastal Plain, 136.7 kg/m^3, than in the Mountains, 131.6 kg/m^3. The mean annual precipitation was higher for the Coastal Plain (50.84 inches) than for the Mountains (43.01 inches) (Figure 3). Mean Annual growing season was lower for the Mountains (185 days) than the Coastal Plains (237 days) (Figure 4). Mean Annual temperature was

lower for the Mountains (54.27°F) than the Coastal Plain (62.50°F) (Figure 5). The Mountain populations were on average geographically closer together, 341.16 km, than the average distance between Coastal Plain populations, 980.95 km (Figure 7).

Discussion

The overall genetic diversity of *L. tulipifera* was fairly low and there was a high degree of genetic similarity within populations. The overall level of genetic differentiation was generally higher than for either of the physiographic provinces. The highest levels of inter site genetic differences were expected between the Mountain sites of highest latitude and Coastal Plain sites of lowest latitude, but this pattern did not appear in the data. Higher levels of genetic diversity were found between Mountain populations than Coastal Plain populations despite the Coastal Plain populations having greater geographic distances between them. Populations were more genetically similar within physiographic provinces than between, which correlated with higher levels of similarity in the mean annual temperature and growing season lengths in the Coastal Plain. The mean annual growing season, precipitation, and temperature were generally more similar within physiographic provinces, which could attribute to some of the genetic similarities within each province. Similarity within physiographic province could also be attributed to seed and environment interactions. *L. tulipifera* reproduces by stump sprouts and wind dispersed seeds. In the Southeast U.S. annual seed production is estimated at 741,000 – 1,482,000 seeds per hectare consistently from year to year (Beck et al., 1990, Phillips, 1966, Renshaw and Doolittle, 1958, Olson, 1969). Along with production, viability of seed remains relatively stable each year (5-20%) for up to 7 years, and three to ten percent of seedlings survive (Beck et al., 1990, Clark and Boyce, 1964). With environmental conditions being similar within physiographic provinces, seeds with certain markers could be more likely to germinate and thrive than other seeds. This combination could be responsible for high degree of similarity within physiographic provinces.

Variation in a species' range can often be attributed in part to evolution and migration of the species. Pleistocene glaciations are believed to have caused southern and northern migrations of many

species in the U.S., including *L. tulipifera* (Sewell et al., 1996b). Along with these migrations many bottlenecks and isolated populations are believed to have occurred in many species (Sewell et al., 1996b). Northern and southern (populations in northern Florida) U.S. haplotypes of *L. tulipifera* have been identified by five potential cpDNA changes which have evolved independently, seeming to confirm the suggested migration patterns and occurrence of bottlenecks and isolated populations (Sewell et al., 1996b). Focusing on the northern haplotype of *L. tulipifera* in the Southeastern U.S. it was found that the DNA of populations sampled in this region was highly similar though statistically significant differences were present. This similarity is also supported by the overall lack of geographic boundaries to migration in this region and the capability of this species to have migrated and bred throughout the majority of its distribution, despite being largely insect pollinated. Fewer natural barriers to migration exist in the Coastal Plain which could account for the higher degrees of similarity found in those populations as opposed to the Mountain populations in contrast to the Mountain populations being approximately half the distance from one another as the Coastal Plain populations. This consistency may change in the future as human induced changes on the landscape continue to increase and forests in this region become fragmented. Though genetic differences were low, they were significant between all sites except for two of the Coastal Plain populations. Phenotypic differences including wood properties, seed dormancy, and leaf characteristics, have been documented throughout the range of *L. tulipifera* and through provenance tests (Kellison, 1968, Sluder, 1972, Farmer et al., 1967, Orwig and Abrams, 1994, Olson, 1969).

Spatial genetic structure involves genetic variation and diversity for a species across a specified landscape. Spatial genetic structure studies in trees have generally shown high genetic diversity within populations and low diversity between populations, which could be due in par to poor sample sizes and sampling procedures (Jump and Penuelas, 2007). *L. tulipifera* findings support the diversity patterns suggested by Jump and Penuelas (2007). Jump and Penuelas (2007) looked at variation in patterns of spatial genetic structure (SGS) by using SSR and AFLP analysis performed on 210 samples of *Fagus*

sylvatica, a Spanish wind-pollinated tree. It was confirmed that genetic diversity was not homogenous across the landscape (I: 0.380, H_e: 0.244) and that AFLP had stronger results than SSR when working with smaller sample sizes (Jump and Penuelas, 2007). There are many studies on intraspecific genetic and phenotypic variation in temperate forests, and now increasingly in tropical forest species. AFLP studies on the widely distributed Caribbean tree *Pterocarpus officinalis* (Jacq.) found that approximately half of its genetic diversity lies within populations and geographic patterns of diversity are also evident (Rivera-Ocasio et al., 2002). *P. officinalis* is an insect pollinated tree (Little and Wadsworth, 1964) and this study involved continental South and Central American populations as well as Caribbean island populations (Rivera-Ocasio et al., 2002). Unlike *L. tulipifera, P. officinalis* can distribute via ocean water transport (Rivera-Ocasio et al., 2002). The overall percent polymorphism for the primer combinations used in the tropical study (68%) (Rivera-Ocasio et al., 2002) was much lower than that of the *L. tulipifera* in the present study. A study on intra and inter-population AFLP variations of the dominant and economically important Indonesian trees *Shorea leprosula and S. parvifolia* showed the most genetic variation within populations, 70.2% and 66.2% respectively (Cao et al., 2006). Both *S. leprosula and S. parvifolia* have percentage of polymorphic loci (PPL) near 50% and expected heterozygosity (H_{ep}) of 0.16 and 0.14 respectively (Cao et al., 2006).

Wood density ranges differed between Mountains and Coastal Plain populations, with the range of average wood density for *L. tulipifera* being greater in the Mountains than in the Coastal Plain. These differences however were not significant at the physiographic province scale. There is potential for correlation between wood traits of interest and DNA markers. One successful association genetics study combined previous QTL and co-location results with single nucleotide polymorphisms and wood property data for *Pinus taeda.* For *P. taeda,* α-tubulin was successfully associated with a specific gene (Gonzalez-Martinez et al., 2006a). One approach to these types of associations is with a bulk segregate analysis in which DNA from samples with the desired trait are pooled and DNA from samples without the desired trait are pooled and both pooled samples are screened for markers. Systematic ranges of

favorable wood densities would have to be identified in order for pooling of samples to be successful. In order to make associations between this type of anonymous marker and a quantitative trait like wood density it would help to have a highly saturated linkage map (Paterson et al., 1991). Many polymorphisms identified in this study may prove to be extremely variable when tested in larger populations, which is being observed in DNA fingerprinting of other woody species including apple and blackberries (Wagner, 1992).

Acknowledgements

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Figure 2 Map of *L. tulipifera* study populations (blue balloons) generated with Google Earth (Google, 2008).

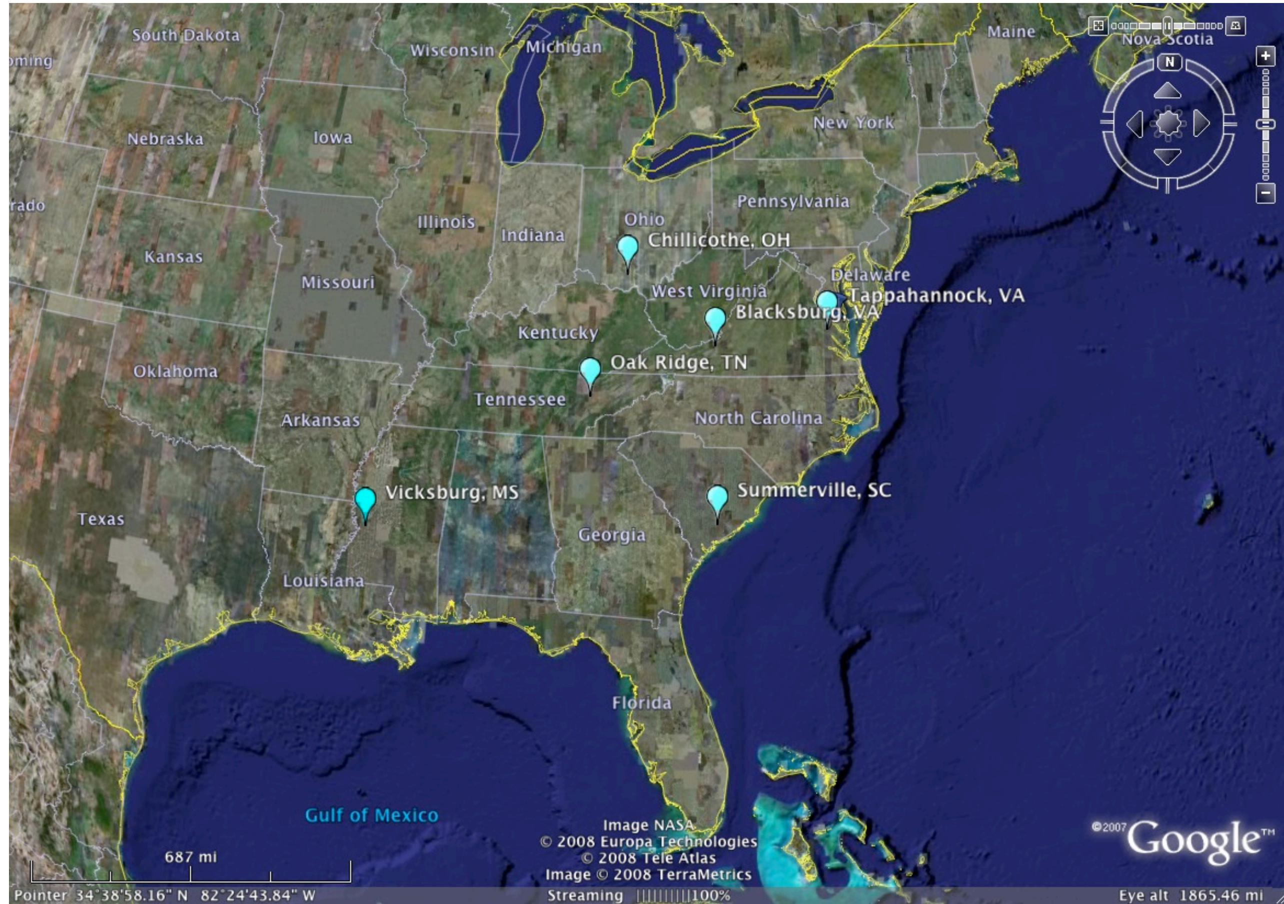


Table 2 Location and sample information for *L. tulipifera* sites used in this research. Latitude and longitude were acquired at the 1st plot from each location.

Population Location	Physiographic Province	Sample Size	Latitude (N)	Longitude (W)
Blacksburg, VA	Mountains	12	37°18'04.02"	80°26'27.72"
Chillicothe, OH	Mountains	17	39°02'45.66"	83°11'40.98"
Oak Ridge, TN	Mountains	16	36°00'09.00"	84°13'01.00"
Summerville, SC	Coastal Plain	18	32°56'46.68"	80°22'41.40"
Tappahannock, VA	Coastal Plain	18	37°40'43.26"	76°56'16.44"
Vicksburg, MS	Coastal Plain	8	32°25'40.52"	90°43'08.80"
	Total	89		

Table 3 Genetic diversity within individual, Mountain, Coastal Plain, and overall *L. tulipifera* populations used in this study. Statistics were calculated using PopGene v.3.2 (Yeh and Boyle, 1997).

Physiographic Province	Population	Sample Size	Polymorphic loci	PPL (%)	n_a	n_e	H_e	I
Mountains	Blacksburg, VA	12	804	84.2	1.842	1.469	0.277	0.418
Mountains	Chillicothe, OH	17	699	73.2	1.732	1.381	0.226	0.344
Mountains	Oak Ridge, TN	16	839	87.9	1.879	1.486	0.282	0.426
Coastal Plain	Summerville, SC	18	889	93.1	1.931	1.400	0.250	0.392
Coastal Plain	Tappahannock, VA	17	838	87.8	1.878	1.441	0.263	0.402
Coastal Plain	Vicksburg, MS	8	797	83.5	1.835	1.395	0.246	0.383
	Mountains	45	933	81.7	1.977	1.469	0.281	0.432
	Coastal Plain	43	949	88.1	1.994	1.441	0.271	0.423
	Overall	88	954	84.9	1.999	1.484	0.289	0.444

PPL: Percent polymorphic loci
n_a: the number of alleles per locus
n_e: the effective number of alleles per locus (Hartl and Clark, 1989)
H_e: gene diversity (Nei, 1973)
I: Shannon's information index (Lewontin, 1972)

Table 4 Wood density data results for *L. tulipifera* populations.

Physiographic Province	Population	Sample Size	Average (kg/m^3)	Range (kg/m^3)
Mountains	Blacksburg, VA	12	425.77	110.40
	Chillicothe, OH	17	438.89	133.40
	Oak Ridge, TN	16	471.69	151.00
	Average:		445.45	131.60
Coastal Plain	Summerville, SC	18	460.38	152.72
	Tappahannock, VA	17	477.05	180.09
	Vicksburg, MS	8	387.58	77.28
	Average:		441.67	136.70

Figure 3 Mean annual precipitation (inches) of *L. tulipifera* populations from 1958 to 2008.

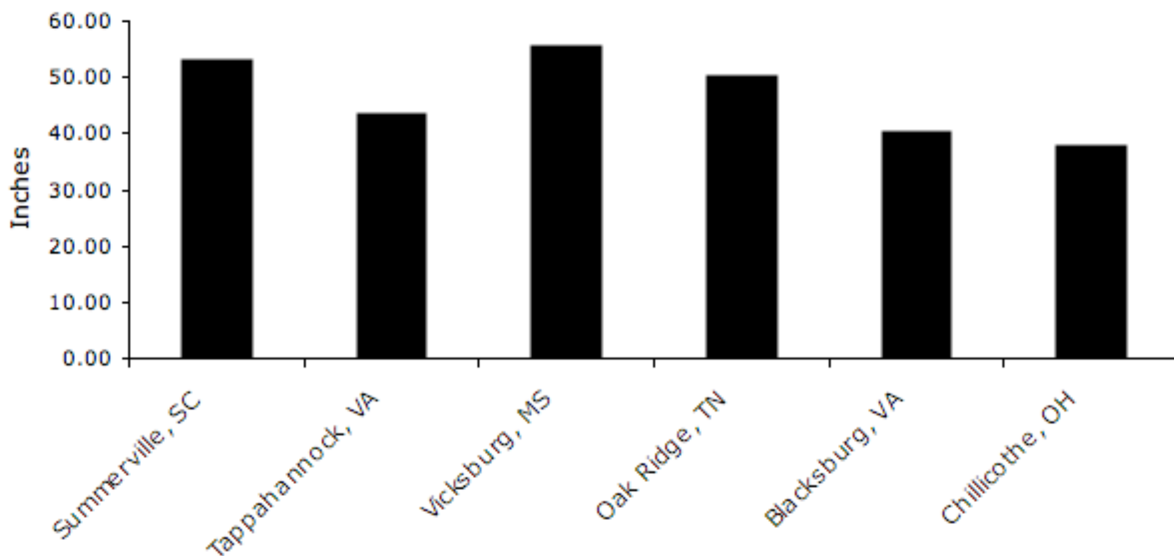


Figure 4 Average growing season (days) of *L. tulipifera* populations from 1958 to 2008.

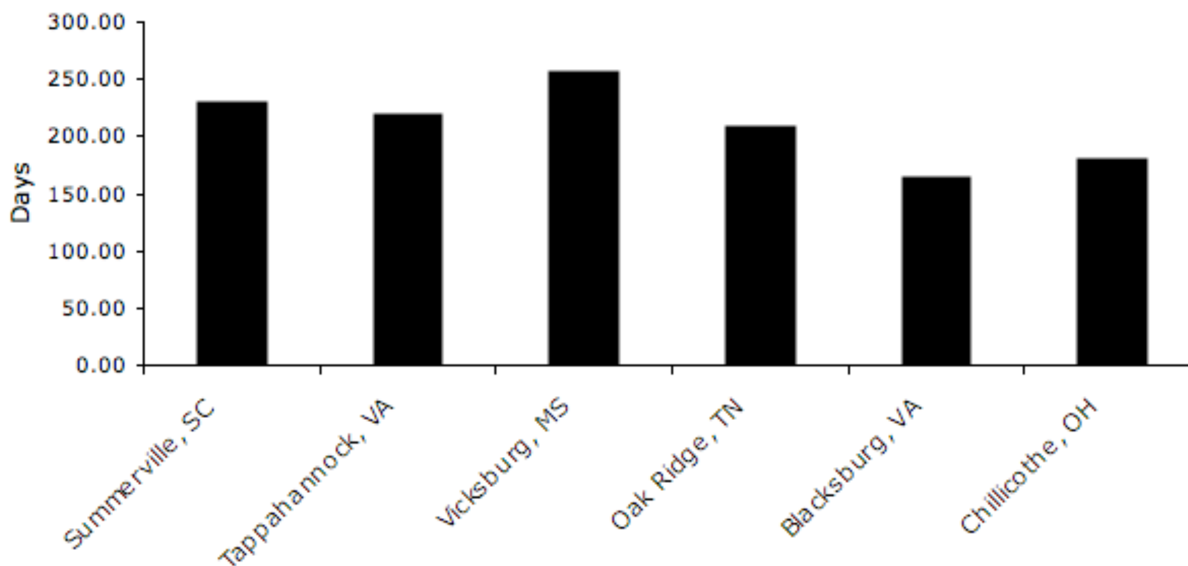


Figure 5 Mean annual temperature of *L. tulipifera* populations from 1958 to 2008.

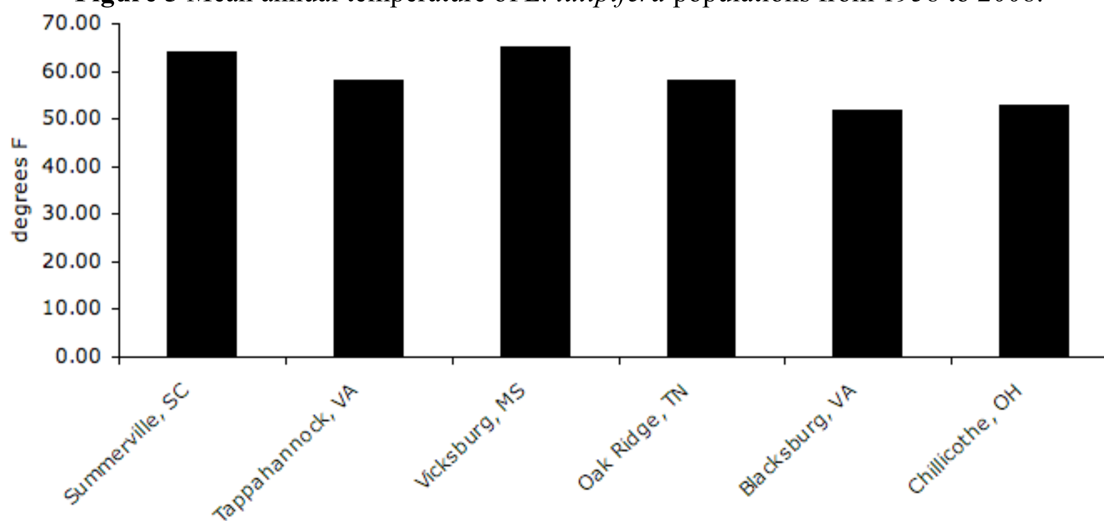


Table 5 Summary of AMOVA for AFLP phenotypes of *L. tulipifera* populations.

Source of Variation	*df*	SSD	Variance Components	Total (%)	*p* value
Between physiographic provinces	1	675	8.7	5.39	0.09677
Within populations	83	11979	144.3	89.02	0.0000
Among populations within physiographic provinces	4	1098	9.1	5.59	0.0000
Total	88	13753	162.1		

df Degree of freedom
SSD sum of squared deviation
p value the probability of value occurring by chance

Table 6 Nei's (Nei and Feldman, 1972) original measures of genetic identity (above diagonal) and genetic distance (below diagonal) for each *L. tulipifera* site. Calculated with NTSYSpc Version 2.20k(Rohlf, 2005).

Population	Blacksburg, VA	Chillicothe, OH	Oak Ridge, TN	Summerville, SC	Tappahannock, VA	Vicksburg, MS
Blacksburg, VA	****	0.951	0.968	0.949	0.943	0.936
Chillicothe, OH	0.050	****	0.952	0.915	0.927	0.902
Oak Ridge, TN	0.033	0.049	****	0.966	0.960	0.954
Summerville, SC	0.052	0.089	0.035	****	0.962	0.976
Tappahannock, VA	0.059	0.076	0.040	0.039	****	0.953
Vicksburg, MS	0.066	0.103	0.047	0.024	0.048	****

Figure 6 Geographic distances (km) between each *L. tulipifera* population.

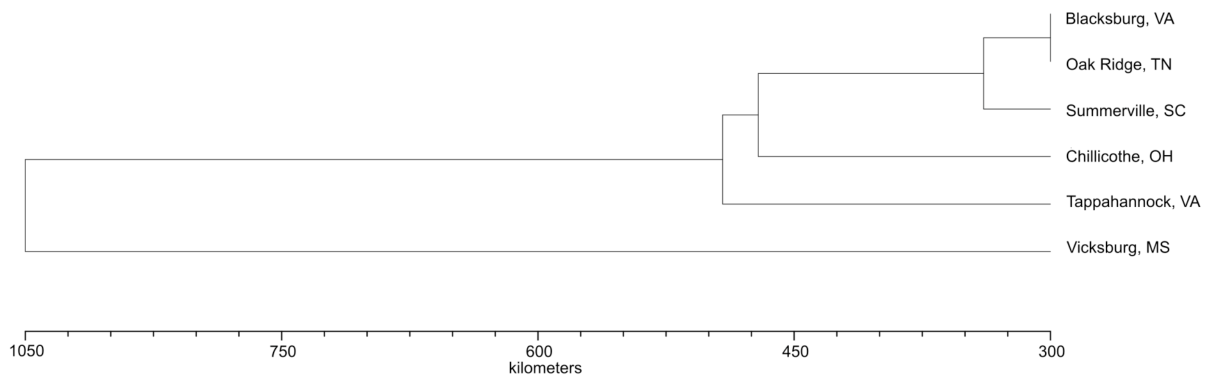


Table 7 Pairwise F_{st} and significant *p* values of *L. tulipifera* populations.

Population	Blacksburg, VA	Chillicothe, OH	Oak Ridge, TN	Summerville, SC	Tappahannock, VA	Vicksburg, MS
Blacksburg, VA	0.000	+	+	+	+	+
Chillicothe, OH	0.046	0.000	+	+	+	+
Oak Ridge, TN	0.027	0.054	0.000	+	+	+
Summerville, SC	0.072	0.143	0.066	0.000	+	-
Tappahannock, VA	0.114	0.163	0.097	0.096	0.000	+
Vicksburg, MS	0.078	0.176	0.070	0.002	0.100	0.000

Significance level of 0.05

Figure 7 UPGMA dendrogram based on Dice's similarity coefficient (Dice, 1945) of genetic distance among samples of *L. tulipifera* from three Coastal Plain and three Mountain sites in Southeastern U.S. Generated with NTSYSpc Version 2.20k (Rohlf, 2005).

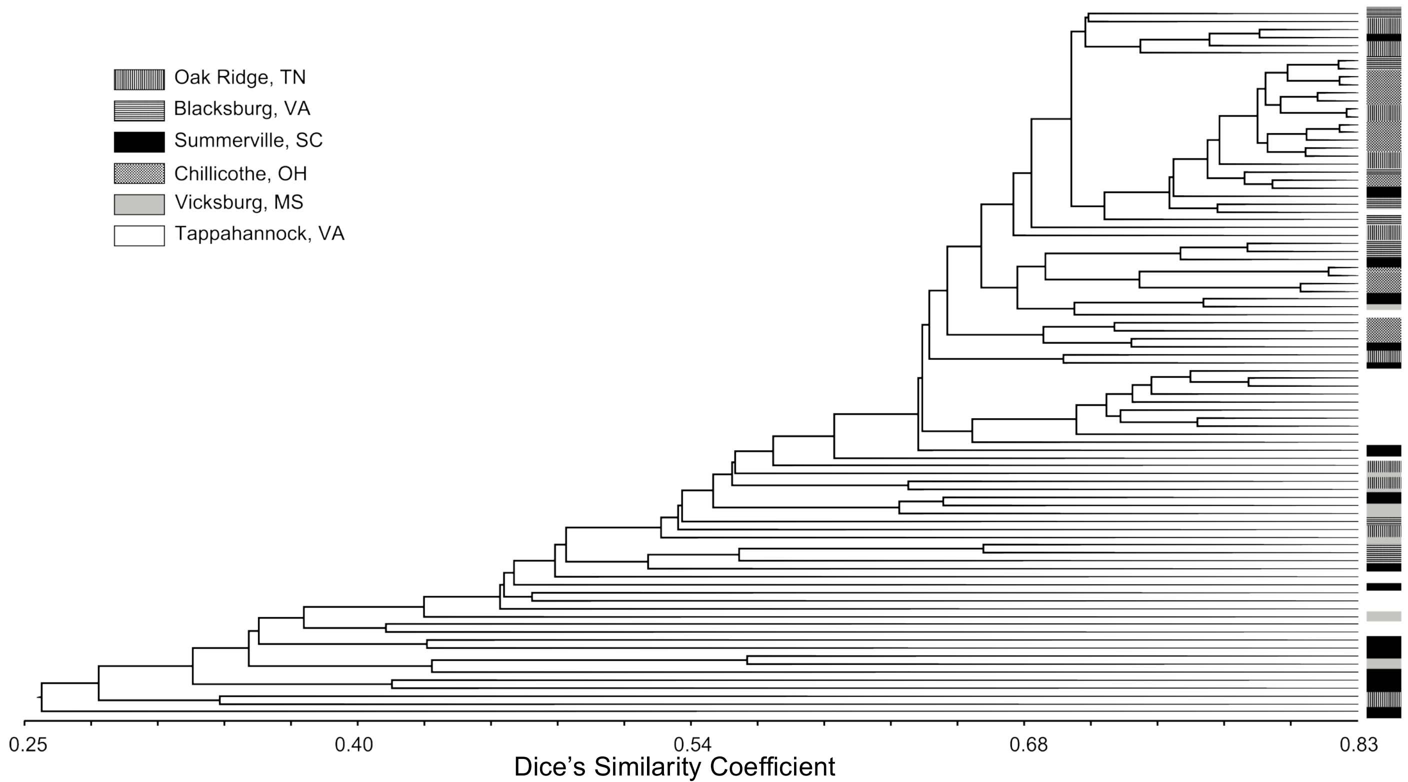
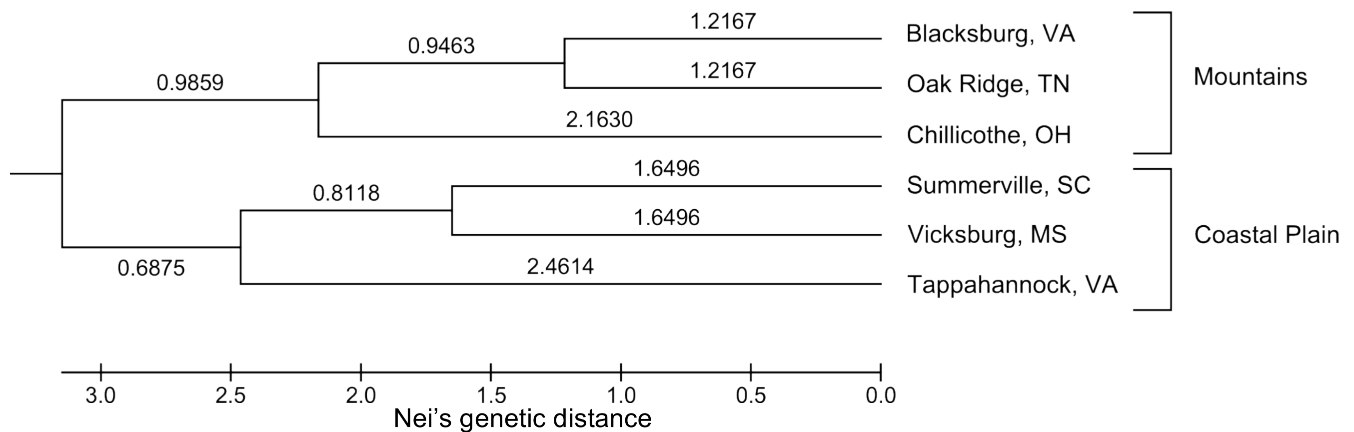


Figure 8 UPGMA dendrogram of genetic distance between populations of *L. tulipifera* based on Nei's genetic distance (Nei and Feldman, 1972) generated by NTSYSpc version 2.20 (Rohlf, 2005). Mountain populations: Blacksburg, VA, Oak Ridge, TN, and Chillicothe, OH. Coastal Plain populations: Summerville, SC, Vicksburg, MS, and Tappahannock, VA.



Chapter 4: Assessment of genetic and wood density variation of *Acer rubrum* L. populations in unmanaged forests of the Southeastern United States.

Katherine E. Kovach[1]
Muhammad Javed Iqbal[2]
E.M. Ulrika Egertsdotter[3]
Shepard M. Zedaker[1]

[1]Department of Forestry, Virginia Polytechnic Institute and State University, 228 Cheatham Hall, Mail Code 0324, Blacksburg, VA 24061
[2] Institute for Sustainable and Renewable Resources (ISRR) at the Institute for Advanced Learning and Research (IALR), 150 Slayton Ave., Danville, VA 24540
[3]School of Biology, Georgia Institute of Technology, 500 Tenth Street, NW, Atlanta, GA 30332-0620

Keywords: AFLP, *Acer rubrum* L., genetic diversity, population structure.

Corresponding Author: Katherine E. Kovach, Department of Forestry, Virginia Polytechnic Institute and State University, 228 Cheatham Hall, Mail Code 0324, Blacksburg, VA 24061, kekovach@vt.edu

Abstract

Acer rubrum L. is prolific throughout the Southeastern U.S. and much of its range in the Eastern U.S. *A. rubrum* is an abundant and economically important species for the wood products industry. The relatively low density and intermediate strength of the wood makes *A. rubrum* suitable for use in many composite wood products. Forest and land management shifts have caused increases in the volume of *A. rubrum* in the Southeast U.S. This study utilizes amplified fragment length polymorphism (AFLP) to determine levels of natural genetic variation of *A. rubrum* in Mountain and Coastal Plain populations of the Southeast U.S. AFLP was performed using five primer combinations on samples from six unmanaged populations. Wood density was determined using an X-ray densitometer and environmental data was gathered from NOAA databases. Genetic diversity was higher within the Coastal Plain populations (H_e: 0.365) than within the Mountain populations (H_e: 0.327). UPGMA of genetic distances showed that populations within physiographic provinces were more genetically similar to each other than between physiographic provinces, with the exception of the Ichauway, GA population which was also the furthest geographically from the other populations. The average wood density for *A. rubrum* was lower in the Mountains (539.00 kg/m^3) than in the Coastal Plain (575.43 kg/m^3) though not statistically different. Examining the genetic structure of this species could provide a foundation for further genetic and breeding exploration with this economically and ecologically important tree.

Introduction

Acer rubrum L., red maple, is ubiquitous in the Southeast U.S., and is increasingly important in forest products and forest ecology of this region. It's native range spans from Southeastern Canada, south to Florida, and as far west as the Mississippi river (Little, 1979). *A. rubrum* has become an increasingly dominant species in many parts of the Southeast U.S. due in part to its rapid regeneration and initial stem growth (Whitney, 1984, Larsen, 1953, Hibbs, 1983). It is also thought that *A. rubrum* populations could dominate the overstory of many areas of the Southeast U.S. in the near future (Abrams, 1998). According to the Society of American Foresters, *A. rubrum* is a dominant component of 56 of the 88 forest cover classifications in the Eastern U.S. (Eyre, 1980).

As is evident from its broad distribution, *A. rubrum* is able to withstand a variety of climate, soil, and topographical conditions. *A. rubrum* can withstand temperatures as low as -40°C (Dansereau, 1957). *A. rubrum* is a shade tolerant pioneer species, but grows much better with increased levels of sunlight and is often found in old fields and disturbed areas (Walters et al., 1990). *A. rubrum* does not show significant aspect preference and is able to grow well in both moist and dry conditions from ridges to swamps (Desta et al., 2004, Stephenson, 1974), growing more dominantly on ridges in northern portions of its range, and in swamps in southern parts of its range (Walters et al., 1990). *A. rubrum* flowers emerge between March and May throughout its range as early as 4 years old (Walters et al., 1990). The fruit of *A. rubrum* are abundantly produced, dispersed by wind, and disseminated through June, which is earlier than most wind dispersing tree species in this region (Walters et al., 1990). Once in contact with soil, it takes at a week or less for *A. rubrum* seeds to germinate, with germination success as high as 91% (Walters et al., 1990). Seeds can remain viable for at least 2 years on the forest floor with high light availability being a key germination requirement (Marquis, 1975). *A. rubrum* is a shade tolerant species, but seedlings have a higher level of tolerance, which promotes regeneration success where full sunlight is not available (Walters et al., 1990). Regeneration can also occur through vigorous sprouting after cutting or disturbance by fire, with multiple sprouts from

individual stumps. Shade tolerance as a seedling is advantageous when there is an overstory present. The ability to sprout quickly and respond positively to increased light levels gives *A. rubrum* regeneration advantages after major disturbances that drastically alter the overstory.

Though *A. rubrum* grows prolifically, it is susceptible to numerous damaging agents. Risks associated with a species that is common over a large geographic area where other species have been reduced in part by insect and fungi infestations, are important reasons to study the genetic diversity throughout its range. The form of *A. rubrum* trees is often poor due to wounding of mixed sources, though it is highly resistant to herbicide by injection (Kossuth et al., 1980). Many insect infestations reduce tree vigor including boring insects and scale insects, especially gypsy moth (*Lymantria dispar*), which largely attack the leaves of the trees (Walters et al., 1990). In addition to insects, fungi also attack wounds on *A. rubrum*, and some canker forming diseases attack the stems (Stephenson, 1974). *A. rubrum* responds to fire by sprouting from the root collar, though intense fires are likely to kill the above-ground portions of the tree (Walters et al., 1990).

There is great potential in studying *A. rubrum* due to its broad range of distribution, range of suitable site conditions, and phenotypic differences (Townsend et al., 1979). In more northern and mountainous regions there tends to be more cold hardiness, more robust samaras, and earlier reproductive development (Walters et al., 1990, Townsend, 1972, Winstead et al., 1977). *A. rubrum* is capable of hybridizing with silver maple (Walters et al., 1990). Successful varieties of *A. rubrum* have been developed to combat air pollution, survive with limited water, counteract vericillium wilt, and function as ornamentals often used in cities (Townsend et al., 1979). Genetic studies on rootstock from different *A. rubrum* cultivars have been successful in selecting trees highly suited for growth in cities (Gerhold et al., 1976). Studies have been done addressing *A. rubrum* genetics without using marker techniques or other genetic analysis tools. In a study on genetic correlation to seed dormancy break *A. rubrum* seeds were much more dependent on temperature than genetic traits (Perry and Wu, 1960).

A. rubrum is commonly used for wood products in the Southeast U.S., due in part to its abundance. *A. rubrum* is considered a soft maple, commonly sold for container and shipping materials such as pallets, crates and boxes, furniture and veneer (Forest-Service, 1974, Walters et al., 1990). *A. rubrum* has also been shown to be equivalent and sometimes superior to aspen (*Populus grandidentata michx.*) as a flakeboard construction material (Kuklewski et al., 1985). When dried, the wood volume shrinks 12.6%, more than half of that of hard maples, and the wood color is a light red/brown (Forest-Service, 1974). The average specific gravity of *A. rubrum* before drying is approximately 0.49 increasing to 0.54 at 12% moisture content (Forest-Service, 1974).

Finding levels of genetic diversity of this species in various growth habitats could allow for a better understanding of how the trees respond to different ecological conditions and give insight into ways that this species may be better managed and utilized for production. As *A. rubrum* of high wood density, and high volumes of lower density wood can be used in furniture making (Walters et al., 1990, Forest-Service, 1974), if DNA markers could be developed for these important traits, fast track breeding strategies could then be developed for these and other traits of interest. There are many venues that could be explored after markers for this species are developed, including more effective management regimes. The main objectives of this study are to: (i) evaluate genetic variation within populations of *A. rubrum*, (ii) evaluate genetic variation of *A. rubrum* within physiographic provinces, and (iii) to evaluate genetic variation of *A. rubrum* as related to environmental and wood density data throughout the Southeastern U.S.

Materials and Methods

Field Methods

Field methods were consistent with those on page 34. Six *A. rubrum* populations (Table 8) were sampled; three in the Mountains and three in the Coastal Plain of the Southeast U.S. (Figure 9). Google Earth was used to generate a map of the populations from the latitude and longitude of each site (Google, 2008). Each study area was chosen based on physiographic province and latitudinal

distribution. Two 12mm bark to bark cores and leaf material were sampled from 11-19 trees at each site, and stored in a portable freezer at approximately -20°C. Leaves were sampled with a shotgun and pole trimmers.

Environmental Data Analysis

Environmental data were gathered from Daily Surface Data ASCII files from the database of the National Oceanic and Atmospheric Administration of various field stations near the populations sampled, (N.O.A.A., 2008). Data was obtained for years 1958 through 2008, the approximated average age of populations sampled. Data included mean annual precipitation (inches), mean annual temperature (°F), and mean annual growing season length (days). Mean annual growing season was calculated from the average number of days between the last frost in the spring and the first frost in the winter months for each year of data from 1958 to 2008. Geographic distances between populations were calculated using the GPS coordinates of each sampling location. A distance matrix was then calculated to evaluate geographic distance between each population.

Laboratory Methods

DNA Isolation

Leaf tissue was stored at -20°C prior to DNA isolations. Leaf tissue was ground with a mortar and pestle, liquid nitrogen, and a SPEX SamplePrep Model 2000 Geno/Grinder laboratory mill (SPEX CertiPrep®, Inc., Metuchen, NJ). A modified version of the Plant DNAzol (Molecular Research Center, Inc. Cincinnati, OH) protocol was used to isolate the DNA from the leaf tissue (Ausubel et al., 1990, Wilfinger et al., 1997, Chang et al., 2009). The quality of DNA was verified by running on a 0.8% (w/v) agarose gel at 97 volts for 1.5hours. DNA samples were diluted with 1x TE buffer to 125 ng/µl concentrations.

AFLP Analysis

AFLP was performed with a modified version of the Vos et al. (1995). The AFLP process includes restriction digestion and adapter ligation of DNA, pre-selective amplification, selective

amplification, and electrophoresis (Vos et al., 1995). The restriction digestion and adapter ligations were performed on genomic DNA (500 ng) with *Eco*RI and *Mse*I restriction enzymes and adapters (New England BioLabs, Ipswich, MA). The final restriction digestion and adapter ligation volume (11 µL) contained 0.05 M NaCl, 0.045 mg/mL BSA, 1 µM *Eco*RI adapter, 5 µM *Mse*I adapter, 5 U *Eco*RI (NEB), 5 U *Mse*I (NEB) and 1 U T4 DNA ligase (NEB), 1× T4 ligase buffer (50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP) (New England BioLabs, Ipswich, MA) and was incubated for 6 hours at 37 °C. Products from the restriction digestion and adapter ligation step were diluted 10 times with 0.1 x TE buffer (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA).

Diluted restriction-ligation product (3 µL) was used in pre-selective amplification in a MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA) which was programmed for 72 °C for 2 min followed by 20 cycles of 94 °C for 20 s, 56 °C for 30 s, and 72 °C for 2 min, and a final steps of 72 °C for 2 min and 60 °C for 30 min. Pre-selective amplification reactions (13µl) contained 1× PCR buffer (100 mM Tris-HCl (pH 8.3), 500 mM KCl), 2.0 mM $MgCl_2$, 0.2 mM dNTP, 10 µM *Eco*RI and *Mse*I pre-selective primers, and 0.25 U of JumpStart *Taq* DNA polymerase (Sigma-Aldrich, St. Louis, MO). Pre-selective amplification products were diluted 10 times with 0.1× TE buffer and 2 µL of diluted product were used in selective amplification.

Selective amplification PCR (8 µL) contained 1× PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.625 µM of dye labeled *Eco*RI primer, 0.625 µM *Mse*I primer, and 0.2 U of JumpStart *Taq* DNA polymerase (Sigma-Aldrich, St. Louis, MO). The PCR amplification consisted of an initial denaturation step of 94 °C for 2 min, followed by the first cycle of 94 °C for 20 s, 66 °C for 30 s, 72 °C for 2 min and one degree decrease in annealing temp in each of the next nine cycles. This was followed by 25 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 3 min. The reactions were then incubated at 60 °C for 30 min. Primer combinations used in selective amplification were selected after performing selective amplifications on one complete population with 10 primer combinations (one *Eco*RI dye labeled and 10 *Mse*I primers). The 10 combinations were narrowed to five primer

combinations that were used with all samples from all populations. A Sigma Genosys *Eco*RI -S1 primer was used with five different *Mse*I primers with base extensions CG, CC, GG, GA, and AG (S1, S4, S5, S8, and S10).

Selective amplification products of each sample (1.0 µl) were combined with 0.3 µl of the DNA size standard – 600 (Beckman-Coulter Inc., Fullerton, CA) and 39.7µl of sample loading solution (Beckman-Coulter Inc., Fullerton, CA). Capillary electrophoresis was performed using CEQ 8800 Genetic Analysis System (Beckman-Coulter Inc., Fullerton, CA). The Frag-4 analysis parameters of the CEQ software were applied to the samples and size standard (Beckman-Coulter Inc., Fullerton, CA). All AFLP fragments were scored as binary data (1, peak present; 0, peak absent) along with their sizes, which were verified with the electropherograms. The data set was trimmed to fragment lengths from 95-300bp, the most reliable range for the CEQ 8800 (Saunders et al., 2001). Data from the CEQ 8800 software was then exported into Excel for analysis.

Wood Density Analysis

Increment cores were dried to approximately 12% moisture content, glued into wooden holders and sawn lengthwise to 1.65mm thickness. The slices were scanned with a Quintek QTRS-01X Tree Ring Scanner (Knoxville, TN, USA) to obtain average wood density measurements for each tree sampled. Core density outputs were visually verified with scanned core images to ensure proper density measurements were acquired.

Statistical Analysis

NTSYSpc Numerical Taxonomy and Multivariate Analysis System v2.2 (Rohlf, 2005) was used to calculate genetic distance and similarity of the samples. The Dice similarity index of the samples and unweighted pair group method with arithmetic mean (UPGMA) cluster based on the Dice Similarity index (Dice, 1945, Nei and Li, 1979) were produced with NTSYSpc. Nei's original diversity index (Nei, 1973) and unbiased diversity index (Nei, 1978) were calculated using PopGene v.3.2 (Yeh and Boyle, 1997). UPGMA dendrogram based on Nei's unbiased genetic distance matrix

over all populations was created using PopGene v.3.2. Shannon's diversity index, Gst values: $G_{st}= D_{st}/ H_t$ (Nei, 1987), N_m: gene flow (McDermott and McDonald, 1993, Yeh and Boyle, 1997), and other population statistics were also calculated. An ANOVA was performed on the mean wood density values for each population using JMPv7 (SAS, 2007). A UPGMA cluster of the distance matrix of geographic distances between populations was also calculated using NTSYSpc v2.2 (Rohlf, 2005).

Results

Within Populations

The percent of polymorphic loci within populations ranged from 78.1% in Round Mountain, VA to 97.8% in Bolton, NC (Table 9). The Nei's gene diversity (H_e) was highest within the Bolton, NC population (0.38) and lowest in the Ichauway, GA population (0.24) (Table 9). Shannon's information index was highest for Oak Ridge, TN (0.52) and lowest for Ichauway, GA (0.37) (Table 9). Dice's Similarity Coefficient was used on each individual tree sampled and showed that in general, the degree of genetic similarity was higher within populations than been between them (Figure 10). This is also supported by the Nei's gene diversity and Shannon's information index for each population (Table 9).

The greatest mean annual precipitation was in Ichauway, GA (51.21 inches) and the lowest in Round Mountain, VA (39.05 inches) (Figure 11). Ichauway, GA had the highest mean annual temperature (65.42°F), and Round Mountain, VA had the lowest (51.29 °F) (Figure 11). Ichauway, GA also had the highest mean annual growing season (249 days) while Round Mountain, VA had the lowest (151 days) (Figure 11). The highest mean wood density was for the Bolton, NC population (647.56 kg/m^3) and the lowest mean was in the Round Mountain, VA population (523.47 kg/m^3) (Table 10). There were however no statistically significant differences in wood density.

Within Physiographic Provinces

The percentage of polymorphic loci in the Mountain populations were 86.94% and the Coastal Plain populations were 99.9% polymorphic. The number of alleles per locus was nearly the same of the Mountains (n_a: 1.990), Coastal Plain (n_a: 1.997), and overall (n_a: 2.000) (Table 9). Mountain

populations were more genetically similar (H_e: 0.32) than Coastal Plain populations (H_e: 0.37) (Table 9), which is also evident by the UPGMA cluster analysis showing that Mountain populations were more genetically similar than the Coastal Plain populations (Figure12). Nei's unbiased measures (Nei, 1978) were used to calculate a UPGMA dendrogram of genetic distance among populations with PopGene v3.2 (Yeh and Boyle, 1997) that showed populations were more similar within physiographic province than they were between (Figure 12). The UPGMA dendrogram shows that the Mountain populations and Coastal Plain populations clustered together, with the exception being Ichauway, GA, which was geographically further from any other population sampled. Nei's unbiased measures (Table 11) and Nei's original measures of genetic diversity (Nei and Feldman, 1972) (Table 12) produced similar results for between population genetic variations. The genetic distances calculated with the Nei's unbiased measures were generally lower than those calculated by Nei's original measures of genetic diversity. Dice's Similarity Coefficient was used on each individual tree sampled and showed that in general, the degree of genetic similarity was higher within physiographic provinces than between them (Figure 10). The expected number of alleles per locus was similar but more varied than the actual number: Mountains (n_e: 1.55), Coastal Plain (n_e: 1.63), and overall (n_e: 1.62) (Table 9). Shannon's Diversity Index was higher in the Coastal Plain (I: 0.54) than it was Mountains (I : 0.50) (Table 9).

The mean annual precipitation, temperature, and growing season were higher within the Coastal Plain (63.43 inches, 50.58°F, 229days) than the Mountain populations (53.69 inches, 43.32°F, 176 days) (Table 13). The mean annual climate data was also more similar within the Coastal Plain than it was within the Mountains (Figure 11). The average wood density values for the Mountain populations were 539.09 kg/m^3 and 575.43 kg/m^3 in the Coastal Plain (Table 10). The Coastal Plain populations were further apart geographically on average than the Mountain populations, largely due to the remoteness of the Ichauway, GA population (Figure 13).

Overall Variation

The overall percent polymorphic loci was 100% for the 993 loci identified in the 99 individual trees analyzed. Shannon's information index was overall 0.540, which was lower than the Coastal Plain 0.543, but higher than the Mountains 0.496 (Table 9). Nei's gene diversity was higher overall (*He*: 0.54) than it was for the Coastal Plain (*He*: 0.37) or the Mountains (*He*: 0.33). Wood density was on average lower for Mountain populations of *A. rubrum*, 539.09 kg/m^3, than Coastal Plain populations, 575.43 kg/m^3 (Table 14). The average range of wood density values was however higher in the Mountains, 159.03 kg/m^3, than in the Coastal Plain, 126.24 kg/m^3 (Table 14). The highest average density was in Bolton, NC, 647.56 kg/m^3, and the lowest average density was in Round Mountain, VA, 523.47 kg/m^3 (Table 14).

Discussion

Diversity within populations

Diversity was strong within populations with each site having at least 78% polymorphic loci. Levels of Shannon's Diversity and Nei's Diversity were both fairly consistent across all sites as well. It was expected that there would be higher levels of intra-site variation than there would be between sites, which was evident in past research and supported by the data in this study. A study of *Acer saccharum* found that genetic diversity was highest in the southern portions of its range, and that less than 2% of the species genetic diversity was attributed to regional differences (Gunter et al., 2000). *A saccharum* also has high inter-population genetic similarity in excess of 90%, which further contrasts the site level diversity patterns and agrees with high and widespread gene flow (Gunter et al., 2000). *A. rubrum* also exhibited within site phenotypic variation in the presence of increased levels of CO2 (Mohan et al., 2004). The strongest inter-site genetic differences were expected between the Mountain sites of highest latitude and those of Coastal Plain with lowest latitude, due in part to longer growing seasons in the Coastal Plain populations (Beck et al., 1990, Walters et al., 1990). This was supported by data collected from the Mountain populations, which were geographically closer to one another than

Coastal Plain populations, and more genetically similar than the Coastal Plain populations. *A. rubrum* seeds varied across the species range in germination and survivorship in the presence of elevated CO2, with Coastal Plain populations having more positive effects than Mountain or Piedmont populations (Mohan et al., 2004). This may also be due in part to effects of increased levels of precipitation, growing seasons, and temperature on seed development.

Genetic Structure

Spatial genetic structure studies in trees have generally shown high genetic diversity within populations and low diversity between populations, which could be due in part to poor sample sizes and sampling procedures (Jump and Penuelas, 2007). *A. rubrum* findings in the present study support the low diversity patterns between populations suggested by others (Jump and Penuelas, 2007). In the current study *A. rubrum* showed very little genetic structure and low inter-site and inter-physiographic province levels of diversity. A study on variation in patterns of spatial genetic structure (SGS) using SSR and AFLP on 210 samples of *Fagus sylvatica*, a Spanish wind-pollinated tree confirmed that genetic diversity is not homogenous across a landscape, with Shannon diversity index of 0.380 and expected heterozygosity of 0.244 for AFLP, and that AFLP has stronger results than SSR when working with smaller sample sizes (Jump and Penuelas, 2007). There have been many studies on intraspecific genetic and phenotypic variation in temperate forests, which are increasing in tropical forest species. AFLP studies on the widely distributed Caribbean tree *Pterocarpus officinalis* (Jacq.) found that approximately half of its genetic diversity lies within populations and geographic patterns of diversity are also evident (Rivera-Ocasio et al., 2002). *P. officinalis* is an insect pollinated tree (Little and Wadsworth, 1964) and this study involved continental South and Central American populations as well as Caribbean island populations (Rivera-Ocasio et al., 2002). Unlike *L. tulipifera*, *P. officinalis* can distribute via ocean water transport (Rivera-Ocasio et al., 2002). The overall percent polymorphism for the primer combinations used in the tropical study (68%) (Rivera-Ocasio et al., 2002) was much lower than that of the *L. tulipifera* in the present study. A study on intra and inter-

population AFLP variations of the dominant and economically important Indonesian trees *Shorea leprosula and S. parvifolia* showed the most genetic variation within populations, 70.2% and 66.2% respectively (Cao et al., 2006). Both *S. leprosula and S. parvifolia* have percentage of polymorphic loci (PPL) near 50% and expected heterozygosity (H_{ep}) of 0.16 and 0.14 respectively (Cao et al., 2006).

Wood Density Associations

There is potential for correlation between wood traits of interest and genetic markers. Many association genetics studies have been successfully performed including one involving single nucleotide polymorphisms and various wood property traits for the species *Pinus taeda,* which incorporated previous QTL and co-location studies was able to associate α-tubulin with a specific gene (Gonzalez-Martinez et al., 2006a). One approach to marker associations is with a bulk segregate analysis. Systematic ranges of favorable wood densities would have to be identified in order for pooling of samples to be successful, and in order to make associations between this type of anonymous marker and a quantitative trait like wood density it would help to have to have a highly saturated linkage map (Paterson et al., 1991). Some polymorphisms identified in this could be more variable when extrapolated to larger populations which has been experienced in mapping studies of other woody species including blackberry (Wagner, 1992). Tests on silvicultural and genetic treatment effects on production capacity in *P. taeda* (loblolly pine) and *Pinus elliottii* Engelm. *var. elliotttii* (slash pine) have shown economically important outcome for breeding programs (Roth et al., 2007). It has been determined that genotype and silvicultural treatment have the greatest positive effect on production and that genotype, silvicultural treatment, and location did not interact at a significant level (Roth et al., 2007). This would suggest that despite the natural variation of the species, selecting for desirable traits could yield valuable crop trees independent of site characteristics. While the reality is likely to be more a combination of the three in development of desirable crop trees, once desirable genotypes are identified it should be more likely to be able to use that genotype in more diverse sites.

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Table 8 Unmanaged *Acer rubrum* sites used in this study. Includes site name, physiographic province, sample size (number of trees of interest sampled) and the latitude and longitude of the *Acer rubrum* sites used.

Population Location	Physiographic Province	Sample Size	Latitude (N)	Longitude (W)
Blacksburg, VA	Mountains	11	37°18'04.02"	80°26'27.72"
Round Mountain, VA	Mountains	18	37°10'16.82"N	81° 9'35.78"W
Oak Ridge, TN	Mountains	16	35°56'11.59"N	84° 6'18.09"W
Bladen Lakes, NC	Coastal Plain	19	34°42'10.00"N	78°35'53.00"W
Bolton, NC	Coastal Plain	19	34°16'19.41"N	78°25'55.48"W
Ichauway, GA	Coastal Plain	16	31° 6'23.95"N	83°34'48.77"W
	Total	99		

Figure 9 Map of six *A. rubrum* study sites (blue balloons) generated with Google Earth (Google, 2008).

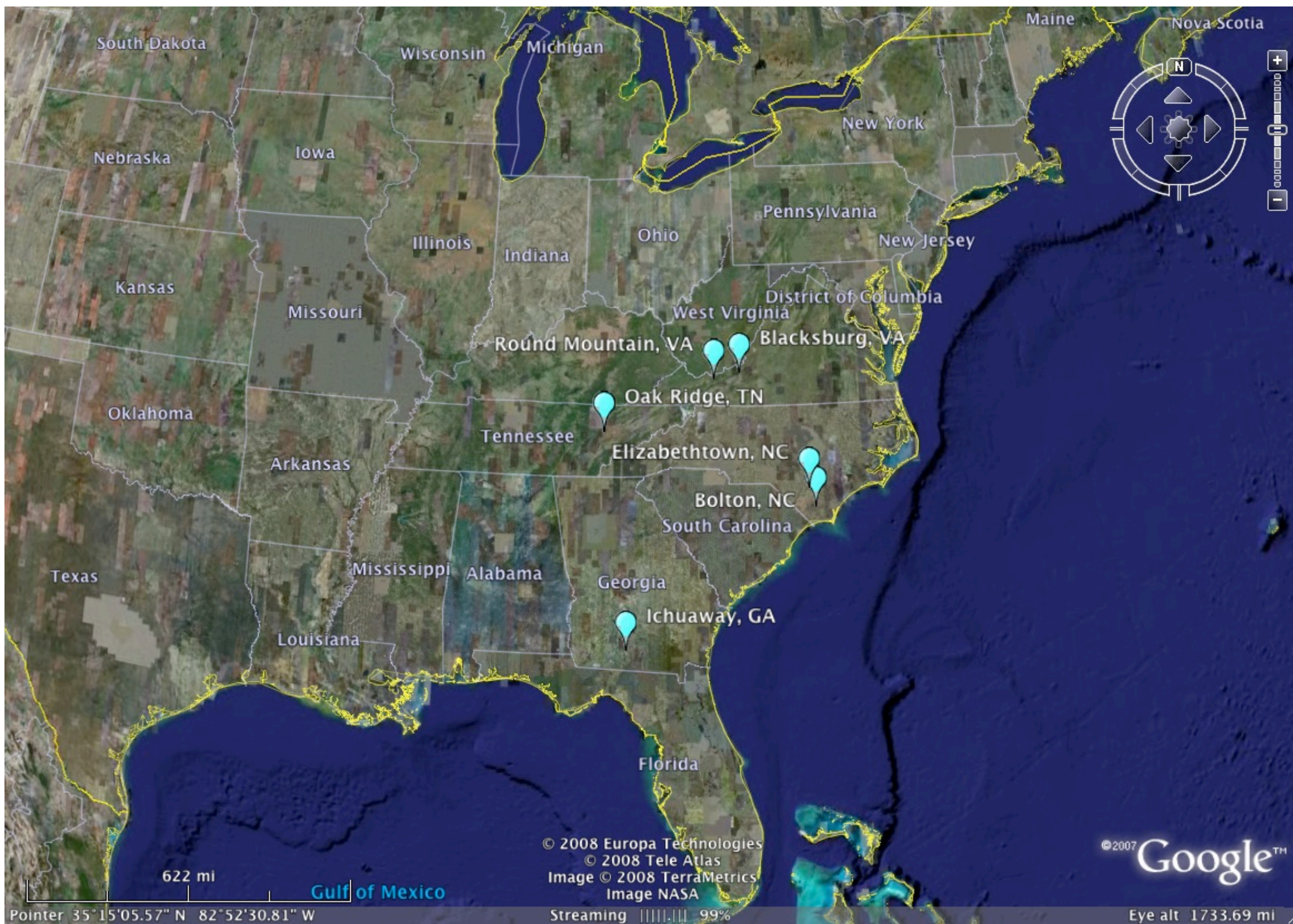


Table 9 Genetic diversity within individual, Mountain, Coastal Plain, and overall *A. rubrum* populations in this study. Statistics were calculated using PopGene v.3.2 (Yeh and Boyle, 1997).

Physiographic Province	Population	Sample Size	Polymorphic loci	PPL (%)	n_a	n_e	H_e	I
Mountains	Blacksburg, VA	11	863	86.9 %	1.869	1.523	0.307	0.460
Mountains	Round Mountain, VA	18	775	78.1 %	1.781	1.423	0.253	0.385
Mountains	Oak Ridge, TN	16	952	95.9 %	1.959	1.597	0.347	0.518
Coastal Plain	Bladen Lakes, NC	19	956	96.3 %	1.963	1.590	0.343	0.512
Coastal Plain	Bolton, NC	19	971	97.8 %	1.978	1.655	0.375	0.553
Coastal Plain	Ichauway, GA	16	781	78.7 %	1.787	1.386	0.238	0.368
	Mountains	45	984	99.1 %	1.991	1.550	0.327	0.495
	Coastal Plain	54	990	90.7 %	1.997	1.631	0.365	0.543
	Overall	99	993	100.0%	2.000	1.624	0.364	0.542

Population: The city and state where the sampling took place
PPL: Percent polymorphic loci
n_a: the number of alleles per locus
n_e: the effective number of alleles per locus (Hartl and Clark, 1989)
H_e: gene diversity (Nei, 1973)
I: Shannon's information index (Lewontin, 1972)

Figure 10 UPGMA dendrogram based on Dice's Similarity Coefficient (Dice, 1945) of genetic distance among samples of *A. rubrum* from three Coastal Plain and three Mountain sites in Southeastern U.S. Generated with NTSYSpc Version 2.20k (Rohlf, 2005).

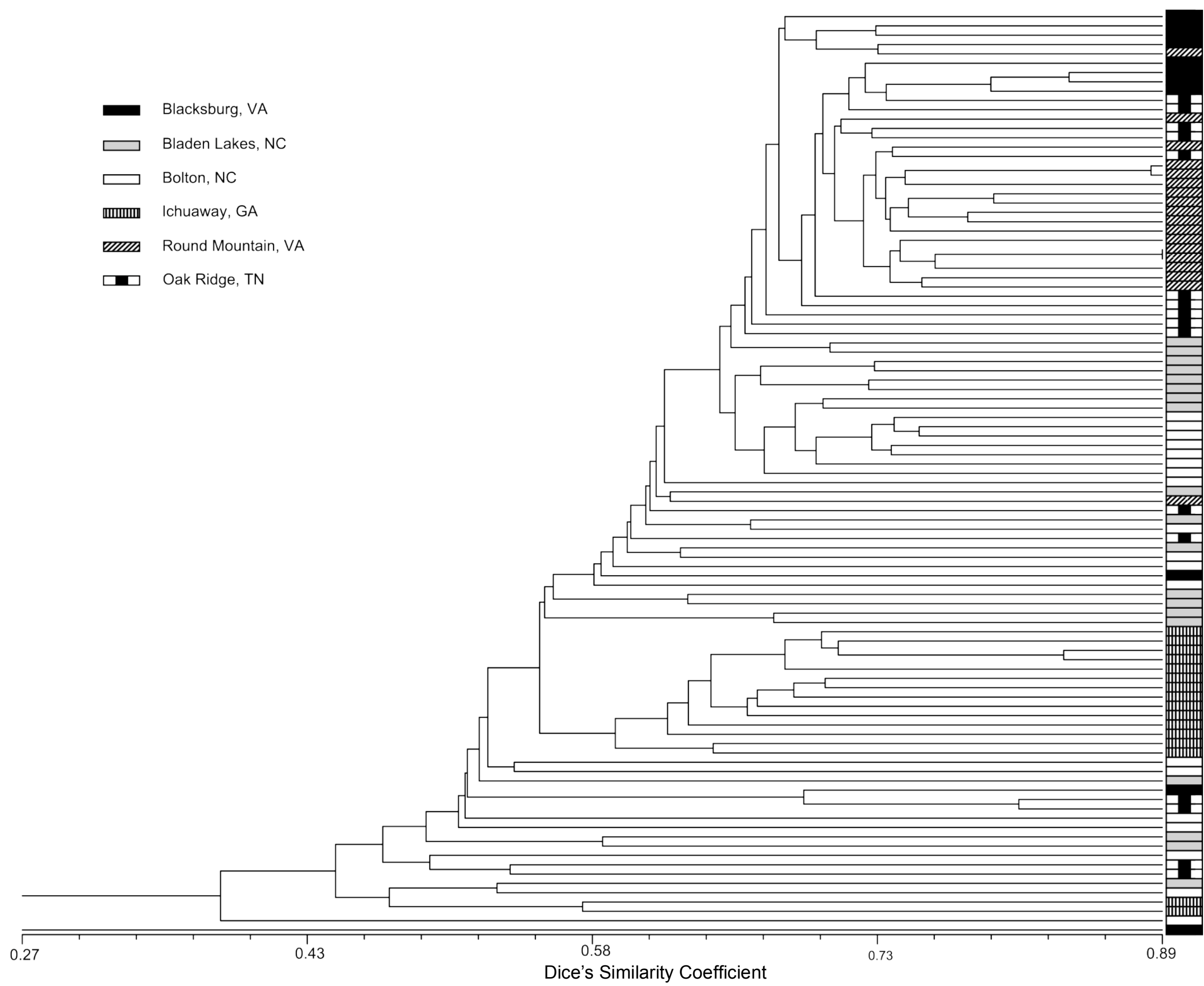
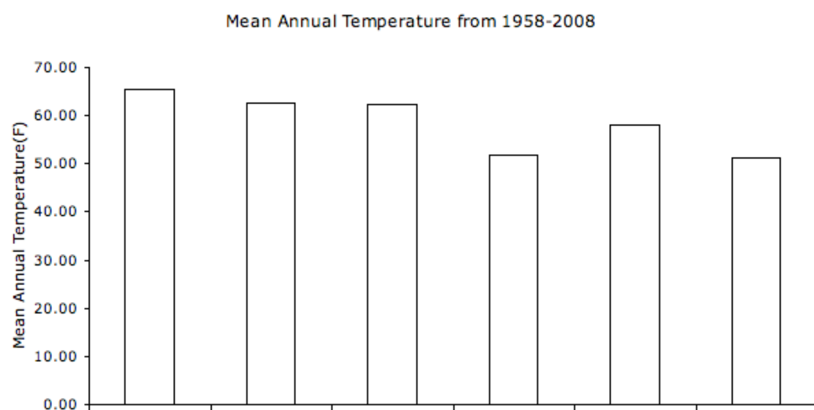
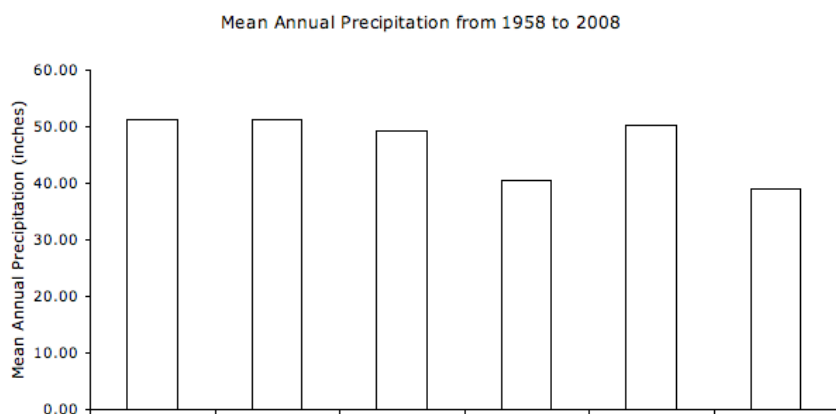


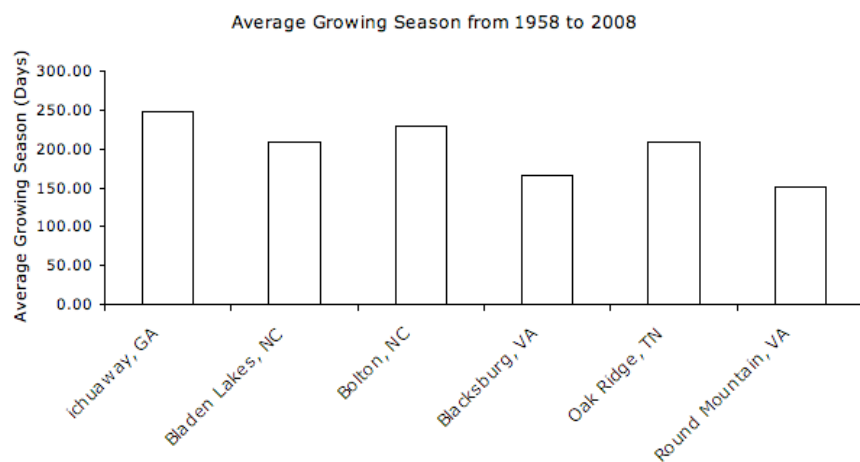
Figure 11 Mean annual temperature (**A**), mean annual precipitation (**B**), and mean annual growing season (**C**) for each population from 1958 to 2008.



A



B



C

Table 10 Wood density statistics for all *A. rubrum* populations.

	Population	Average (kg/m^3)	Range (kg/m^3)
Mountains	Blacksburg, VA	531.02	112.24
	Round Mountain, VA	523.47	182.70
	Oak Ridge, TN	562.76	182.16
	Average:	539.09	159.03
Coastal Plain	Bladen Lakes, NC	539.00	133.47
	Bolton, NC	647.56	129.72
	Ichauway, GA	539.72	115.54
	Average:	575.43	126.24

Figure 12 UPGMA dendrogram of genetic distance between populations of *A. rubrum* based on Nei's unbiased genetic distance (Nei, 1978). Mountain populations: Blacksburg, VA, Oak Ridge, TN, and Round Mountain, VA. Coastal Plain populations: Bladen Lakes, NC, Bolton, NC, and Ichauway, GA. Generated with PopGene v.3.2(Yeh and Boyle, 1997).

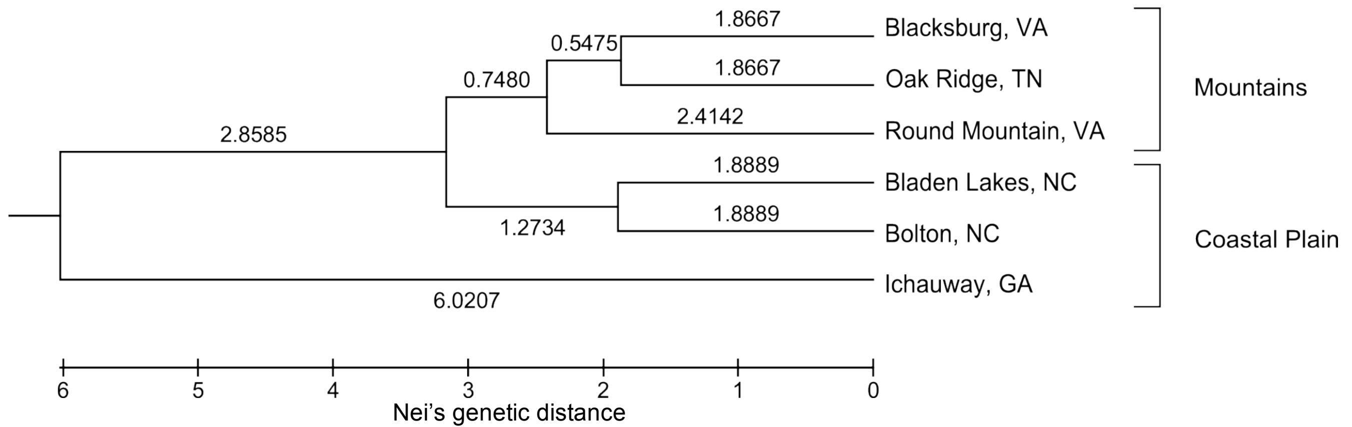


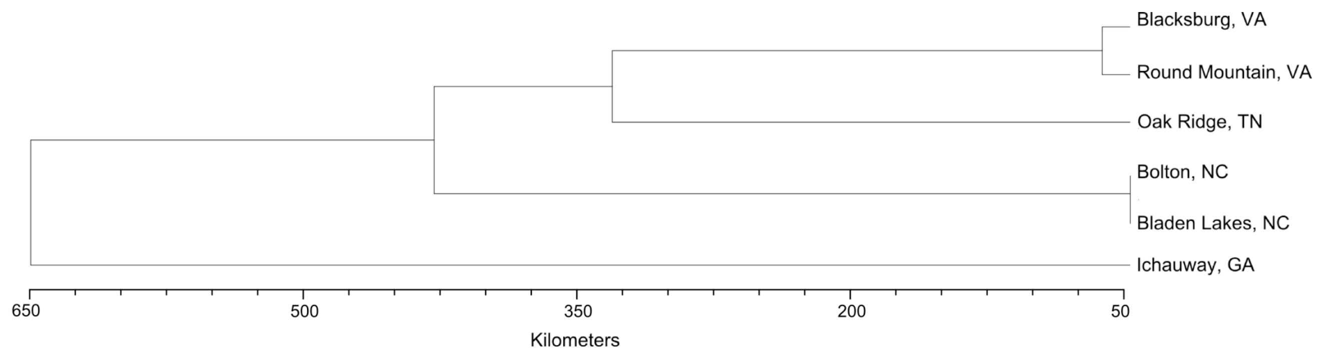
Table 11 Nei's unbiased measures of genetic identity (Nei, 1978) (above diagonal) and genetic distance (below diagonal) for each *A. rubrum* site. Calculated with PopGene v.3.2(Yeh and Boyle, 1997).

Population	Blacksburg, VA	Bladen Lakes, NC	Bolton, NC	Ichauway, GA	Round Mountain, VA	Oak Ridge, TN
Blacksburg, VA	****	0.948	0.938	0.901	0.948	0.963
Bladen Lakes, NC	0.054	****	0.963	0.911	0.930	0.956
Bolton, NC	0.064	0.038	****	0.888	0.915	0.946
Ichauway, GA	0.105	0.093	0.118	****	0.853	0.882
Round Mountain, VA	0.054	0.072	0.089	0.159	****	0.958
Oak Ridge, TN	0.037	0.045	0.056	0.126	0.043	****

Table 12 Nei's (Nei and Feldman, 1972) original measures of genetic identity (above diagonal) and genetic distance (below diagonal) for each *A. rubrum* site. Calculated with PopGene v.3.2(Yeh and Boyle, 1997).

Population	Blacksburg, VA	Bladen Lakes, NC	Bolton, NC	Ichauway, GA	Round Mountain, VA	Oak Ridge, TN
Blacksburg, VA	****	0.931	0.920	0.886	0.933	0.945
Bladen Lakes, NC	0.072	****	0.948	0.900	0.919	0.941
Bolton, NC	0.084	0.053	****	0.876	0.903	0.930
Ichauway, GA	0.121	0.106	0.132	****	0.844	0.869
Round Mountain, VA	0.070	0.084	0.102	0.170	****	0.945
Oak Ridge, TN	0.057	0.060	0.072	0.140	0.056	****

Figure 13 Geographic distances between *A. rubrum* sites in kilometers.



Chapter 5: Conclusions

A. rubrum and *L. tulipifera* are common throughout the majority of their ranges and are increasing in dominance. In a time of increased rates of global climate change, human population growth, and utilization of natural resources, it is imperative that information is gathered about the ecosystems that many aspects of life rely heavily upon. Tree species that are not only common in their range, but increasingly so, should be of specific interest to those managing forests. Interest should focus not only on the volume and quality of wood but also on potential threats and complications that could arise with increased homogenization of forested lands. This study did not focus on management or global climate change, but a tool to study genetic diversity that could be useful in determining management and the capacity forests to adapt.

The results from the *L. tulipifera* study are summarized below:

- There were significant levels of molecular variation between populations
- The overall level of genetic differentiation was higher than either of the physiographic provinces
- Individuals within populations were more similar to each other than to individuals from other populations
- There were significant levels of molecular variation within populations
- Differences in wood density were more dependant on precipitation, temperature, and growing season than physiographic province
- Higher level of genetic differentiation among populations in the Mountains than the Coastal Plain
- Populations were more genetically similar within physiographic province than between
- Higher levels of precipitation and growing season lengths were present in the Coastal Plain
- Wood density did not correlate with physiographic province, but with individual site conditions
- The mean annual precipitation was higher for the Coastal Plain than for the Mountains
- Mean Annual growing season was lower for the Mountains than the Coastal Plains
- Mean Annual temperature was lower for the Mountains than the Coastal Plain

The results from the *A. rubrum* study are summarized below:

- Genetic diversity was higher in Coastal Plain populations than Mountain populations
- Populations were more genetically similar within physiographic province than between
- Level of genetic differentiation was lower overall than within populations
- Overall genetic diversity was low
- The degree of genetic similarity was higher within populations than between them
- There were no statistically significant differences in wood density
- The average wood density values were lower for the Mountain populations than the Coastal Plain
- The average range of wood density values was however higher in the Mountains, than in the Coastal Plain
- The mean annual precipitation, temperature, and growing season were higher within the Coastal Plain than the Mountain populations

Evolutionary Theory

Not all hardwoods of the Southeast U.S. have the same genetic structure or diversity. In this study the genetic diversity and structure of *A. rubrum* and *L. tulipifera* had contrasting results, both of which were in agreement with previous research. While *L.tulipifera* showed significant levels of genetic diversity between populations, *A. rubrum* did not. This leads to a view of migration for *A. rubrum* that contrasts that of *L. tulipifera*, and leads to the possibility that *A. rubrum* may have been present throughout it's distribution on a much smaller time scale than *L. tulipifera. A. rubrum* had high levels of genetic diversity within populations while *L. tulipifera* had strong levels of genetic structure with significant differences between the Mountain and the Coastal Plain populations. This suggests that *A. rubrum* is openly pollinated and homogenizing the distribution of its gene pool at a much higher rate than *L. tulipifera*.

Reproductive Strategies

Both species have high seed production and viability (Beck et al., 1990, Clark and Boyce, 1964), which could also contribute to the genetic patterns observed. Marker variation throughout *L. tulipifera*'s range could be attributed in part to historic evolution and migration of the species and also the environmental diversity that is present throughout the widespread range of this species. Environmental data was consistent for both species, with Mountains having shorter growing seasons and lower temperatures and precipitation levels than the Coastal Plain populations. There is evidence of historic migrations of *L. tulipifera* (Sewell et al., 1996b) including major shifts in the fitness and gene pool of the species. Both within population diversity and between population diversity have advantages. *A. rubrum* having low between population variation may cause it to rely more heavily on adaptation to endure introduced stresses such as invasive species or sudden and prolonged changes in climate. Evidence shows that *A. rubrum* can exhibit phenotypic, germination, and survivorship differences in the face of increased $CO2$ levels which suggests that *A. rubrum* will be able to adapt in the face of rapid ecosystem changes (Mohan et al., 2004). In terms of management options for these species, genetic tools could be useful in incorporating desired traits for both species into the gene pool. AFLP could be useful in determining genetic structure of a species and when populations and samples are large enough can lead to advantageous association genetics studies.

References

ABRAMS, M. (2003) Where has all the white oak gone? *BioScience,* 53**,** 927-939.

ABRAMS, M. D. (1998) The Red Maple Paradox What explains the widespread expansion of red maple in eastern forests? *BioScience,* 48**,** 355-364.

ABRAMS, M. D. & DOWNS, J. A. (1990) Successional replacement of old-growth white oak by mixed-mesophytic hardwoods in southwest Pennsylvania. *Canadian Journal of Forest Research,* 20**,** 1864-1870.

AUSUBEL, F. M., BRENT, R., KINGSTON, R. E., MOORE, D. D., SEIDMANN, J. G. & STRUHL, K. (1990) New York, NY, John Wiley & Sons, Inc. .

BAREFOOT, A. C. (1963) Selected wood characteristics in young yellow poplar (*Liriodendron tulipifera*). *Forest Products Journal,* 13**,** 443-448.

BECK, D. E., BURNS, R. M. & HONKALA, B. H. (1990) *Silvics of North America: 2. Hardwoods. Agriculture Handbook 654.* , U.S. Department of Agriculture, Forest Service, Washington, DC.

BECKAGE, B. & CLARK, J. S. (2003) Seedling survival and growth of three forest tree species: The role of spatial heterogeneity. *Ecology,* 84**,** 1849-1861.

BEEBEE, T. & ROWE, G. (2004) *An Introduction to Molecular Ecology,* New York, Oxford University Press.

BOYCE, S. G. & KAEISER, M. (1961) Why yellow-poplar seeds have low viability. IN USDA FOREST SERVICE, T. P. C. S. F. E. S. (Ed. Columbus, OH.

BROADFOOT, W. M. & WILLISTON, H. L. (1973) Flooding Effects on Southern Forests. 71**,** 587.

BROWN, G. R., BASSONI, DANIEL L., GILL, GEOFFREY P., FONTANA, JOSEPH R., WHEELER, NICHOLAS C., MEGRAW, ROBERT A., DAVIS, MARK F., SEWELL, MITCHELL M., TUSKAN, GERALD A., AND NEALE, DAVID B. (2003) Identificaiton of quantitative trait loci influencing wood property traits in loblolly pine (*Pinus taeda* L.). III. QTL verification and candidate gene mapping. *Genetics,* 164**,** 1537-1546.

BROWN, G. R., GILL, G. P., KUNTZ, R. J., LANGLEY, C. H. & NEALE, D. B. (2004) Nucleotide variation and linkage disequilibrium in loblolly pine. *Proceedings of the National Academy of Sciences of the United States of America,* 101**,** 15255-15260.

BURLEY, J. (2001) Genetics in sustainable forestry: the challenges for forest genetics and tree breeding in the new millennium. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere,* 31**,** 561-565.

BYRAM, T. D., MULLIN, T. J., WHITE, T. L. & VAN BUIJTENEN, J. P. (2005a) The future of tree improvement in the southeastern United States: Alternative visions for the next decade. *Southern Journal of Applied Forestry,* 29**,** 88-95.

BYRAM, T. D., MYSZEWSKI, J. H., GWAZE, D. P. & LOWE, W. J. (2005b) Improving wood quality in the western gulf forest tree improvement program: the problem of multiple breeding objectives. *Tree Genetics & Genomes,* 1**,** 85-92.

CAMPBELL, T. A., FORD, W. M., HALE, P. E., WENTWORTH, J. M., JOHNSON, A. S. & MILLER, K. V. (2002) Nutritional value of yellow-poplar flowers to deer in the southern Appalachians. *Southeastern Naturalist,* 1**,** 425-432.

CAO, C.-P., FINKELDEY, R., SIREGAR, I., SIREGAR, U. & GAILING, O. (2006) Genetic diversity within and among populations of Shorea leprosula Miq. and Shorea parvifolia Dyer (Dipterocarpaceae) in Indonesia detected by AFLPs. *Tree Genetics & Genomes,* 2**,** 225-239.

CERVERA, M. T., GUSMAO, J., STEENACKERS, M., VANGYSEL, A., VANMONTAGU, M. & BOERJAN, W. (1996) Application of AFLP(TM)-based molecular markers to breeding of Populus spp. *Plant Growth Regulation,* 20**,** 47-52.

CHANG, Y., VEILLEUX, R. E. & IQBAL, M. J. (2009) Analysis of Genetic Variability among *Phalaenopsis* Species and Hybrids Using Amplified Fragment Length Polymorphism. *Journal of the American Society for Horticultural Science,* 134**,** 58-66.

CHAVE, J., MULLER-LANDAU, H. C., BAKER, T. R., EASDALE, T. A., TER STEEGE, H. & WEBB, C. O. (2006) Regional and phylogenetic variation of wood density across 2456 neotropical tree species. *Ecological Applications,* 16**,** 2356-2367.

CLARK, F. B. & BOYCE, S. G. (1964) Yellow-poplar seed remains viable in the forest litter. *Journal of Forestry,* 62**,** 564-567.

CLARKE, C. R. E. (2000) Wood and pulp properties of four New South Wales provenances of Eucalyptus nitens grown on a warm and a cold site in South Africa. *Appita Journal,* 53**,** 231-236.

CLAYTON, L. R., SENECOFF, J. F., MEAGHER, R. B. & MERKLE, S. A. (1998) Development of transgenic yellow poplar for mercury phytoremediation. *Nature Biotechnology* 16**,** 925-928.

CONSTABLE, J. V. H. & RETZLAFF, W. A. (2000) Asymmetric day/night temperature elevation: Growth implications for yellow-poplar and loblolly pine using simulation modeling. *Forest Science,* 46**,** 248-257.

CORE, H. A., MOSCHLER, W. W. & CHANG, C. (1975) A Note on Prediction of Wood Properties in Yellow-Poplar. *Wood and Fiber Science,* 9**,** 258-261.

CORNELL (2007).

COTTRELL, J. E., MUNRO, R. C., TABBENER, H. E., MILNER, A. D., FORREST, G. I. & LOWE, A. J. (2003) Comparison of fine-scale genetic structure using nuclear microsatellites within two British oakwoods differing in population history. . *Forest Ecology and Management,* 176**,** 287-303.

DAI, J. L., VENDRAME, W. A. & MERKLE, S. A. (2004) Enhancing the productivity of hybrid yellow-poplar and hybrid sweetgum embryogenic cultures. *In Vitro Cellular & Developmental Biology-Plant,* 40**,** 376-383.

DANSEREAU, P. M. (1957) *Biogeography,* New York, Ronald Press.

DELLA-BIANCA, L. & JR., D. F. O. (1961) Soil-site studies in Piedmont hardwood and pine-hardwood upland forests. *Forest Science,* 7**,** 320-329.

DEMURA, T. & FUKUDA, H. (2007) Transcriptional regulation in wood formation. *Trends in Plant Science,* 12**,** 64-70.

DESTA, F., COLBERT, J. J., RENTCH, J. S. & GOTTSCHALK, K. W. (2004) Aspect induced differences in vegetation, soil, and microclimatic characteristics of an Appalachian watershed. *Castanea,* 69**,** 92-108.

DICE, L. R. (1945) Measures of the Amount of Ecologic Association Between Species. *Ecology,* 26**,** 297-302.

DYER, J. M. (2001) Using witness trees to assess forest change in southeastern Ohio. *Canadian Journal of Botany* 31**,** 1708-1718.

EXCOFFIER, LAVAL, L. G. & SCHNEIDER, S. (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online,* 1**,** 47-50.

EYRE, F. H. (1980) *Forest cover types of the United States and Canada. ,* Washington, DC, Society of American Foresters.

FARMER, J. D., RUSSELL, T. E. & KRINARD, R. M. (1967) Sixth-year results from a yellow-poplar provenance test. *Ninth Southern Conference on Forest Tree Improvement.* Knoxville, TN., Eastern Tree Seed Laboratory, Macon, GA. .

FEARNSIDE, P. M. (1997) Wood density for estimating forest biomass in Brazilian Amazonia. *Forest Ecology and Management,* 90**,** 59-87.

FEKEDULEGN, D., HICKS, R. R. & COLBERT, J. J. (2003) Influence of topographic aspect, precipitation and drought on radial growth of four major tree species in an Appalachian watershed. *Forest Ecology and Management,* 177**,** 409-425.

FOREST-SERVICE, U. S. D. A. (1974) Wood handbook: wood as an engineering material. Madison, WI, U.S. Department of Agriculture, Forest Service, Forest Products Laboratory.

FOREST-SERVICE, U. S. D. A. (August 2008) FIADB 3.0 ed.

GAUDEUL, M., TILL-BOTTRAUD, I., BARJON, F. & MANEL, S. (2004) Genetic diversity and differentiation in Eryngium alpinum L. (Apiaceae): comparison of AFLP and microsatellite markers. *Heredity,* 92**,** 508-518.

GERHOLD, H. D., ZEASER, D. B., STEINER, K. C. & TOWNSEND, A. M. (1976) Genetic Variation in Red Maple Rootstocks. *23rd Northeastern Forest Tree Improvement Conference.* New Brunswick, NJ.

GIVEN, D. R. (1986) *Principles and Practice of Plant Conservation,* Portland, Oregon, Timber Press.

GOLDSTEIN, D. B. & WEALE, M. E. (2001) Population genomics: Linkage disequilibrium holds the key. *Current Biology,* 11**,** R576–R579.

GONZALEZ-MARTINEZ, S., ERSOZ, E., BROWN, G., WHEELER, N. & NEALE, D. B. (2006a) DNA sequence variation and selection of tag SNPs at candidate genes for drought-stress response in Pinus taeda L. . *Genetics,* 172**,** 1915-1926.

GONZALEZ-MARTINEZ, S. C., KRUTOVSKY, KONSTANTIN V., AND NEALE, DAVID B. (2006) Forest-tree population genomics and adaptive evolution. *New Phytologist.,* 170**,** 227-238.

GONZALEZ-MARTINEZ, S. C., WHEELER, N. C., REZOS, E., NELSON, C. D. & NEALE, D. B. (2006b) Association Genetics in Pinus taeda L. l. Wood Property Traits. *Genetics,* 175**,** 399–409.

GOOGLE (2008) Google Earth. 4.3.7284.3916 (beta) ed. Mountain View, CA.

GUNTER, L. E., TUSKAN, G. A., GUNDERSON, C. A. & NORBY, R. J. (2000) Genetic variation and spatial structure in sugar maple (Acer saccharum Marsh.) and implications for predicted global-scale environmental change. . *Global Change Biology,* 6**,** 335–344.

HANSEN, M., KRAFT, T., GANESTAM, S., SALL, T. & NILSSON, N. O. (2001) Linkage disequilibrium mapping of the bolting gene in sea beet using AFLP markers. *Genetical Research,* 77**,** 61-66.

HARTL, D. L. & CLARK, A. G. (1989) *Principles of population genetics,* Sunderland, MA, Sinauer.

HELMS, J. A. (1998) *The Dictionary of Forestry,* Bethesda, MD, Society of American Foresters.

HIBBS, D. E. (1983) Forty Years of Forest Succession in Central New England. *Ecology,* 64**,** 1394-1401.

HICKS, R. R. (1998) *Ecology and management of central hardwood forests,* New York, John Wiley.

IKE, A. F. J. & HUPPUCH, C. D. (1968) Predicting tree height growth from soil and topographic site factors in the Georgia Blue Ridge Mountains. Macon, GA, Georgia Forest Resources Council.

JOHNSON, J. E., BOLLIG, J. J. & RATHFON, R. A. (1997) - Growth Response of Young Yellow-Poplar to Release and Fertilization. - 21**,** - 179.

JONES, C. J., EDWARDS, K. J., CASTAGLIONE, S., WINFIELD, M. O., SALA, F., WIEL, C. V. D., BREDEMEIJER, G., VOSMAN, B., MATTHES, M., DALY, A. & AL., E. (1997) Reproducibility testing of RAPD, AFLP, and SSR markers in plants by a network of Eurpean laboratories. *Molecular Breeding,* 3**,** 381-390.

JONES, D. P., ZEDAKER, S. M., ZINK-SHARP, A. & FULLER, J. J. (2006) Summary Report for 2006 SEMI Summer Field Sampling and Final Summary of Research Project.

JUMP, A. S. & PENUELAS, J. (2007) Extensive spatial genetic structure revealed by AFLP but not SSR molecular markers in the wind-pollinated tree, Fagus sylvatica. *Molecular Ecology,* 16**,** 925-936.

KELLISON, R. (1968) Natural variation of yellow-poplar in North Carolina. *Proc. Northeast. For. Tree Improv. Conf.*, 15, 7-14.

KOPISCH-OBUCH, F. J. & DIERS, B. W. (2006) Segregation at the SCN resistance locus rhg1 in soybean is distorted by an association between the resistance allele and reduced field emergence. . *Theoretical and Applied Genetics,* 112.

KOSSUTH, S. V., YOUNG, J. J., VOELLER, J. E. & HOLT, H. A. (1980) Year-round ardwood control using the hypo-hatchet injector. *Southern Journal of Applied Forestry,* 4, 73-76.

KRUTOVSKY, K. V. (2006) From Population Genetics to Population Genomics of Forest Trees: Integrated Population Genomics Approach. *Russian Journal of Genetics.,* 42, 1088-1100.

KUKLEWSKI, K. M., BLANKENHORN, P. R. & RISHEL, L. E. (1985) Comparison of selected physical and mechanical properties of red maple (Acer rubrum L.) and aspen (Populus grandidentata michx.) flakeboard. *Wood and Fiber Science,* 17, 11-21.

LARSEN, J. A. (1953) A Study of an Invasion by Red Maple of an Oak Woods in Southern Wisconsin. *American Midland Naturaliast.,* 49, 908-914.

LEDIG, F. T. (1996) Ch. 11: FOCUS Pinus torreyana at the Torrey Pines State Reserve, California. IN DONALD A. FALK, C. I. M., AND MARAGARET OLWELL. (Ed.) *Restoring Diversity. Strategis for Reintroduction of Endangered Plants.* Washington D.C. , Island Press.

LEWONTIN, R. C. (1972) The apportionment of human diversity. *Evolutionary Biology,* 6, 381-398.

LITTLE, E. L. & WADSWORTH, F. H. (1964) *Agriculture Handbook No. 249: Common trees of Puerto Rico and the Virgin Islands,* Washington, D.C.

LITTLE, E. L. J. (1979) Checklist of United States trees (native and naturalized). Washington, DC, U.S. Department of Agriculture.

LOFTIS, D. (1990) A Shelterwood Method for Regenerating Red Oak in the Southern Appalachians. . *Forest Science,* 36, 917-929.

LUPPOLD, W. R., PRESTEMON, J. P. & SCHULER, A. (2002) Changing markets for hardwood roundwood. IN ZHANG, D. (Ed. *Proceddings of the 2001 Southern Forest Economics Workshop.* School of Forestry and Wildlife Sciences, Auburn University, Atlanta, GA.

MARQUIS, D. A. (1975) Seed storage and germination under northern hardwood forests. *Canadian Journal of Forest Research,* 5, 478-484.

MCCARTHY, E. F. (1933) Yellow-poplar characteristics, growth, and management. Washington DC, US Department of Agriculture.

MCDERMOTT, J. M. & MCDONALD, B. A. (1993) Gene flow in plant pathosystems. *Annuual Review Phytopathology,* 31, 353-373.

MCGEE, C. E. (1975) Regeneration alternatives in mixed oak stands. Southeastern Forest Experiment Station, Asheville, NC, USDA Forest Service.

MCKEAND, S. E., ABT, R. C., ALLEN, H. L., LI, B. L. & CATTS, G. P. (2006a) What are the best loblolly pine genotypes worth to landowners? *Journal of Forestry,* 104, 352-358.

MCKEAND, S. E., JOKELA, E. J., HUBER, D. A., BYRAM, T. D., ALLEN, H. L., LI, B. L. & MULLIN, T. J. (2006b) Performance of improved genotypes of loblolly pine across different soils, climates, and silvicultural inputs. *Forest Ecology and Management,* 227, 178-184.

MOHAN, J. E., CLARK, J. S. & SCHLESINGER, W. H. (2004) Genetic variation in germination, growth, and survivorship of red maple in response to subambient through elevated atmospheric CO2. *Global Change Biology,* 10, 233-247.

N.O.A.A. (2008) http://www.noaa.gov/.

NCBI (2008) The Single Nucleotide Polymorphism database.

NEALE, D. B. & SAVOLAINEN., O. (2004) Association genetics of complex traits in conifers. *TRENDS in Plant Science.,* 9, 325-330.

NEHRA, N. S., BECWAR, M. R., ROTTMANN, W. H., PEARSON, L., CHOWDHURY, K., CHANG, S. J., WILDE, H. D., KODRZYCKI, R. J., ZHANG, C. S., GAUSE, K. C., PARKS, D. W. & HINCHEE, M. A. (2005) Forest biotechnology: Innovative methods, emerging opportunities. *In Vitro Cellular & Developmental Biology-Plant,* 41**,** 701-717.

NEI, M. (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America,* 70**,** 3321-3323.

NEI, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics,* 89**,** 583-590.

NEI, M. (1987) *Molecular evolutionary genetics.,* New York, Columbia University Press.

NEI, M. & FELDMAN, M. (1972) Identity of Genes by Descent Within and Between Populations Under Mutuation and Migration Pressures. *Theoretical and Applied Genetics,* 3**,** 460-465.

NEI, M. & LI, W. H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America,* 76**,** 5269-5273.

OLSON, D. F. (1969) Silvical characteristics of yellow-poplar (Liriodendron tulipifera L.). Southeastern Forest Experiment Station, Asheville, NC, USDA Forest Service.

ORWIG, D. A. & ABRAMS, M. D. (1994) Land-use history (1720-1992), composition, and dynamics of oak-pine forests within the Piedmont and Coastal Plain of Northern Virginia. *Canadian Journal of Forest Research,* 24**,** 1216-1225.

PANG, X. M., WEN, X. P., HU, C. G. & DENG, X. X. (2006) Genetic diversity of Poncirus accessions as revealed by amplified fragment length polymorphism (AFLP). *Journal of Horticultural Science & Biotechnology,* 81**,** 269-275.

PARKS, C. R. & WENDEL, J. F. (1990) Molecular divergence between Asian and North American species of *Liriodendron* (Magnolicaceae) with implications for interpretation of fossil floras. *American Journal of Botany,* 77**,** 1243-1256.

PARKS, C. R., WENDEL, J. F., SEWELL, M. M. & QIU, Y. L. (1994) The significance of allozyme variation and introgression in the *Liriodendron tulipifera* complex (Magnoliaceae). *American Journal of Botany,* 81**,** 878-889.

PATERSON, A. H., DAMON, S., HEWITT, J. D., ZAMIR, D., RABINOWITCH, H. D., LINCOLN, S. E., LANDER, E. S. & TANKSLEY, S. D. (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. *Genetics,* 127**,** 181-197.

PERRY, T. O. & WU, W. C. (1960) Genetic Variation in the Winter Chilling Requirement for Date of Dormancy Break for Acer Rubrum. *Ecology,* 41**,** 790-794.

PHILLIPS, J. J. (1966) Site index of yellow-poplar related to soil and topography in southern New Jersey. Northeastern Forest Experiment Station, Broomall, PA, USDA Forest Service.

PRITCHARD, J. K. & PRZEWORSKI, M. (2001) Linkage Disequilibrium in Humans: Models and Data. *American Journal of Human Genetics,* 69**,** 1-14.

QIU, Y. L. & PARKS, C. R. (1994) Disparity of allozyme variation levels in three *Magnolia* (Magnoliaceae) species from the southeastern United States. *American Journal of Botany,* 81**,** 1300-1308.

QUELLERA, D. C., STRASSMANNA, J. E. & HUGHESB, C. R. (1993) Microsatellites and kinship *Trends in Ecology & Evolution,* 8**,** 285-288.

RENSHAW, J. F. & DOOLITTLE, W. T. (1958) Silvical characteristics of yellow-poplar. USDA Forest Service, Southeastern Forest Experiment Station, Asheville, NC.

RIDLEY, M. (2003) *Evolution*, Blackwell Publishing.

RIVERA-OCASIO, E., AIDE, T. M. & MCMILLAN, W. O. (2002) Patterns of genetic diversity and biogeographical history of the tropical wetland tree, *Pterocarpus officinalis* (Jacq.), in the Caribbean basin. *Molecular Ecology,* 11**,** 675-683.

ROHLF, F. J. (2005) Numerical Taxonomy and Multivariate Analysis System. 2.2 ed. New York, Exeter Software.
ROSS, D., R., BUCKNER, E., CORE, H. & WOODS, F. (1979) Nitrogen Fertilization Decreases Yellow-Poplar Wood Density. *Southern Journal of Applied Forestry,* 3**,** 119-122.
ROTH, B. E., JOKELA, E. J., MARTIN, T. A., HUBER, D. A. & WHITE, T. L. (2007) Genotype x environment interactions in selected loblolly and slash pine plantations in the Southeastern United States. *Forest Ecology and Management,* 238**,** 175-188.
SALICK, J. (1995) TOWARD AN INTEGRATION OF EVOLUTIONARY ECOLOGY AND ECONOMIC BOTANY - PERSONAL PERSPECTIVES ON PLANT/PEOPLE INTERACTIONS. *Annals of the Missouri Botanical Garden,* 82**,** 25-33.
SAS (2007) JMP. 7 ed. Cary, NC.
SAUNDERS, J. A., MISCHKE, S. & HEMEIDA, A. A. (2001) The Use of AFLP Techniques for DNA Fingerprinting in Plants., Beckman Coulter.
SCHNEIDER, S., ROESSLI, D. & EXCOFFIER, L. (2000) Arlequin: A software for population genetics data analysis. Ver 2.000 ed., Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.
SEWELL, M., PARKS, C. & CHASE, M. (1996a) Intraspecific chloroplast DNA variation and biogeography of North American *Liriodendron* L. (Magnoliaceae). *Evolution,* 50**,** 1147-1154.
SEWELL, M., PARKS, C. & CHASE, M. (1996b) Intraspecific chloroplast DNA variation and biogeography of North American Liriodendron L. (Magnoliaceae). . *Evolution,* 50**,** 1147-1154.
SIERRA-LUCERO, V., MCKEAND, S. E., HUBER, D. A., ROCKWOOD, D. L. & WHITE, T. L. (2002) Performance Differences and Genetic Parameters for Four Coastal Provenances of Loblolly Pine in the
Southeastern United States *Forest Science,* 48.
SIMPSON, W. T. (1993) Specific gravity, moisture content, and density relationship for wood. Madison, WI, USDA Forest Service, Forest Products Laboratory.
SLUDER, E. R. (1972) Variation in specific gravity of yellow-poplar in the southern Appalachians. . *Wood Science,* 5**,** 132-138.
SMALLEY, G. W. (1964) Topography, soil, and the height of planted yellow-popla. *Journal of Alabama Academy of Science,* 35**,** 39-44.
SMALLWOOD, M. (2006) The impact of genomics on crops for industry. *Journal of the Science of Food and Agriculture,* 86**,** 1747-1754.
SPINNEY, M. (September 9, 2003) Summer 2003 Field Season: Summary Report.
STEPHENSON, S. L. (1974) Ecological composition of some former oak-chestnut communities in western Virginia. *Castanea,* 39**,** 278-286.
TAYLOR, F. W. (1968) Specific gravity differences within and among yellow-poplar trees. *Forest Products Journal,* 18**,** 75-81.
TAYLOR, F. W. (1977) VAriation in specif gravity and fiber length of selected hardwoods throught the Mid-South. *Forest Science,* 23**,** 190-194.
THOR, E. & CORE, H. (1977) Fertilization, irrigation, and site factor relationships with growth and wood properties of yellow-poplar. *Wood Science,* 9**,** 130-135.
THORBJORNSEN (1961) Variation in density and fiber length in wood of yellow poplar. *Tappi Journal,* 44**,** 192-195.
TOWNSEND, A. M. (1972) Geographical variation in fruit characteristics of Acer
rubrum. *Bulletin of the Torrey Botanical Club,* 99**,** 122-126.
TOWNSEND, A. M., WRIGHT, J. W., KWOLEK, W. F., BEINEKE, W. F., LESTER, D. T., MOHN, C. A. & DODGE, A. F. (1979) GEOGRAPHIC-VARIATION IN YOUNG RED MAPLE GROWN IN NORTH CENTRAL UNITED-STATES. *Silvae Genetica,* 28**,** 33-36.

TUSKAN G.A., D., S., JANSSON, S., BOHLMANN, J., GRIGORIEV, I., HELLSTEN, U., PUTNAM, N., RALPH, S., ROMBAUTS, S., SALAMOV, A., SCHEIN, J., STERCK, L., AERTS, A., BHALERAO, R. R., BHALERAO, R. P., BLAUDEZ, D., BOERJAN, W., BRUN, A., BRUNNER, A., BUSOV, V., CAMPBELL, M., CARLSON, J., CHALOT, M., CHAPMAN, J., CHEN, G.-L., COOPER, D., COUTINHO, P. M., COUTURIER, J., COVERT, S., CRONK, Q., CUNNINGHAM, R. DAVIS, J., DEGROEVE, S., DÉJARDIN, A. , DEPAMPHILIS, C., DETTER, J., DIRKS, B., DUBCHAK, I., DUPLESSIS, S., EHLTING, J., ELLIS, B., GENDLER, K., GOODSTEIN, D., M. GRIBSKOV, GRIMWOOD, J., GROOVER, A., GUNTER, L., HAMBERGER, B., HEINZE, B., HELARIUTTA, Y., HENRISSAT, B., HOLLIGAN, D., HOLT, R., HUANG, W., ISLAM-FARIDI, N., JONES, S., JONES-RHOADES, M., JORGENSEN, R., JOSHI, C., KANGASJÄRVI, J., KARLSSON, J., KELLEHER, C., KIRKPATRICK, R., KIRST, M., KOHLER, A., KALLURI, U., LARIMER, F., LEEBENS-MACK, J., LEPLÉ, J.-C., LOCASCIO, P., LOU, Y., LUCAS, S., MARTIN, F., MONTANINI, B., NAPOLI, C., NELSON, D. R., NELSON, C., NIEMINEN, K., NILSSON, O., PEREDA, V., PETER, G., PHILIPPE, R., PILATE, G., POLIAKOV, A., RAZUMOVSKAYA, J., RICHARDSON, P., RINALDI, C., RITLAND, K., ROUZÉ, P., RYABOY, D., SCHMUTZ, J., SCHRADER, J., SEGERMAN, B., SHIN, H., SIDDIQUI, A., STERKY, F., TERRY, A., TSAI, C.-J., UBERBACHER, E., UNNEBERG, P., VAHALA, J., WALL, K., WESSLER, S., YANG, G., YIN, T., DOUGLAS, C., MARRA, M., SANDBERG, G., VAN DE PEER, Y., ROKHSAR, D. (2006) The genome of black cottonwood, Populus trichocarpa(Torr. & Gray). *Science,* 313**,** 1596-1604.

VAARTAJA, O. (1961) Demonstration of photoperiodic ecotypes in Liriodendron and Quercus. *Canadian Journal of Botany,* 39**,** 649-654.

VICK, C. B. (1985) Yellow poplar, an American wood. FS-272. *U.S. Department of Agriculture, Forest Service.* Washington D.C.

VOS, P., HOGERS, R., BLEEKER, M., REIJANS, M., VAN DE LEE, T., HORNES, M., FRITERS, A., POT, J., PALEMAN, J., KUIPER, M. & ZABEAU, M. (1995) AFLP: new technique for DNA fingerprinting. *Nucleic Acids Research,* 23**,** 4407-4414.

WAGNER, D. (1992) Nuclear, chloroplast, and mitochondrial DNA polymorphisms as biochemical markers in population genetic analyses of forest trees. *New Forests,* 6**,** 373-390.

WALTERS, R. S., YAWNEY, H. W., BURNS, R. M. & HONKALA, B. H. (1990) *Silvics of North America: 2. Hardwoods. Agriculture Handbook 654.* , U.S. Department of Agriculture, Forest Service, Washington, DC.

WHITNEY, G. G. (1984) Fifty Years of Change in the Arboreal Vegetation of Heart's Content, An Old-Growth Hemlock-White Pine-Northern Hardwood Stand. *Ecology,* 65**,** 403-408.

WILCOX, J. R. & TAFT, K. A. (1969) Genetics of Yellow-Poplar. *US Forest Service Res. Pap. US Department of Agriculture No. WO-6.*

WILCOX, P. L., CARSON, S. D., RICHARDSON, T. E., BALL, R. D., HORGAN, G. P. & CARTER, P. (2001) Benefit-cost analysis of DNA marker based-selection in progenies of Pinus radiata seed orchard parents. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere,* 31**,** 2213-2224.

WILDE, H. D., MEAGHER, R. B. & MERKLE, S. A. (1992) Expression of Foreign Genes in Transgenic Yellow-poplar Plants. . *Plant Physiology,* 98**,** 114-120.

WILFINGER, W. W., MACKEY, K. & CHOMCZYNSKI, P. (1997) *BioTechniques,* 22**,** 474-478.

WINSTEAD, J. E., SMITH, B. J. & WARDELL, G. I. (1977) Fruit weight dines in populations of ash, ironwood, cherry, dogwood, and maple. . *Castanea,* 42**,** 56-60.

YEH, F. & BOYLE, T. (1997) Popluation genetic analysis of co-dominant and dominant markers and quanitative traits. *Belgian Journal of Botany,* 129.

YOSHIDA, M., OHTA, H., YAMAMOTO, H. & OKUYAMA, T. (2002) Tensile growth stress and lignin distribution in the cell walls of yellow poplar, Liriodendron tulipifera Linn. *Trees-Structure and Function,* 16**,** 457-464.

ZELEZNIK, J. D. & SKOUSEN, J. G. (1996) Survival of three tree species on old reclaimed surface mines in Ohio. *Journal of Environmental Quality,* 25**,** 1429-1435.

ZHANG, L. J., OSWALD, B. P. & GREEN, T. H. (1999) Relationships between overstory species and community classification of the Sipsey Wilderness, Alabama. *Forest Ecology and Management,* 114**,** 377-383.

ZINK, A. G., HANNA, R. B. & STELMOKAS, J. W. (1997) Measurement of Poisson's ratios for yellow-poplar. *Forest Products Journal,* 47**,** 78-80.

ZOBEL, B. J. & BUIJTENEN, B. (1989) *Wood variation: Its causes and control,* Berlin, Springer-Verlag.

ZOBEL, B. J. & SPRAGUE, J. R. (1998) *Juvenille wood in forest trees,* Berlin, Springer-Verlag.

ZOBEL, B. J. & TALBERT, J. (1984) *Applied forest tree improvement,* New York, John Wiley.

Appendix A: AFLP Protocol for Daylily, Dr. M. Javed Iqbal

Outline of Steps

1. Extraction of DNA from frozen leaf samples
2. Quantifying the samples
3. Checking the quality of samples (Running on Agarose Gel)
4. Dilution of samples (if needed)
5. Restriction Ligation
6. Comparison of RL products with Uncut DNA samples
7. Pre-selective Amplification
8. Selective Amplification
9. Preparing the samples for running on CEQ 8800
10. Running the CEQ 8800

1. DNA ISOLATION

1 Grind approximately 0.4 g of leaf tissue in liquid nitrogen in a separate mortar and pestle.

2 Transfer 0.2 g of powder in a 1.5 ml eppendorf tube.

3 Add 600 µl of plant DNAzol (Invitrogen)

4 Vortex briefly

5 Mix for 5 min by inverting the tubes @ room temperature (RT)

6 Add 600 µl of chloroform

7 Mix by inverting the tubes

8 Mix by inverting for 5 min at RT

9 Spin in microfuge @ 12000 g for 10 min

10 Transfer the supernatant (aqueous phase, bright colored in this case) to a new tube

11 Add 500 µl of 95% ethanol, mix gently by inverting 6-8 times & incubate @RT for 5 min

12 Spin @ 10000 g for 5 min

13 Remove the supernatant

14 Add 600 µl of DNAzol-Ethanol wash solution (1 ml of DNAzol + 750 µl of 100% ethanol), mix

15 Mix by inverting and incubate @ RT for 5 min.

16 Spin @ 5000 g for 4 min

17 Discard the supernatant, keep the pellet

18 Add 600 µl of 75% ethanol. Vortex and spin @ 5000 g for 4 min. We can repeat this step if necessary.

(You can stop here and leave the sample at 4°C)

19 Discard liquid and keep the pellet

20 Add 100 µl of TE and RNase A (40 ug/ml concentration). Mix/ break the pellet by pipette.

21 Incubate @37°C for 1 hour

22 Add 100 µl of dH_20, mix, add 200 µl of Phenol:Choloroform:Isoamyl alcohol. Mix well by inverting. Spin for 5 min @12000 g. transfer the upper phase (aqueous) to a new tube. Perform this step in hood.

23 Add 20 µl of 5 M NH_4OAC, mix

24 Add 400 µl of 100% ethanol, mix gently and incubate @ -20°C overnight.

It is better to stop here

25 Centrifuge @ 12,000 g for 10 min. Discard the liquid carefully. Keep the pellet.

26 Be careful. The pellet will be clear/transparent and hard to see.

27 Add 75% ethanol, spin for 5 min @ 12000 g

28 Air dry pellet for 20-30 min

29 Add 50 µl of TE

30 Measure DNA concentration

31 Run 1 ug on 0.8% Agarose Low EEO Gel

2. QUANTIFYING THE SAMPLES

We can measure DNA concentration by Spectophotometer or by nanometer drop.

While using spectrophotometer, dilution of DNA is required

Make samples as 2 µl DNA+ 98 µl water. Use dsDNA mode of spectrophotometer.

While using nanometer drop there is no need of dilution.

3. QUALITY CHECKING OF DNA (RUNNING ON GEL)

1 Use 0.8 % (0.8 g in 100 ml of 1X TBE buffer) Agarose Low EEO gel. Bring the contents to boiling and then stir to cool down.

2 For preparing samples to run on gel, combine the following

DNA (1 µg)	3 µl or as much needed
5X DNA loading dye	2 µl
DNA stain, SYBR Green	1 µl
Water (sigma)	X µl
Total volume	10 µl

Mix all these (except DNA), pulse centrifuge and then mix DNA

Run the gel on 96-97 V for 1- 1.30 hours

After running take the photograph by using Alpha Imager 34

4. DILUTION OF SAMPLES

Since all the samples have different DNA concentration, so it a better idea to make their concentration uniform before further steps.

In this experiment, we tried to set the DNA concentration around 125 ng/µl.

Dilution is done with water.

5. RESTRICTION LIGATION

Make the master mix as follows. DO NOT ADD DNA in the MASTER MIX.

Sr No	Reagents	Vol. for One reaction
1	10 X Ligase Buffer	1.10 µl
2	NaCl (0.5 M)	0.11 µl
3	BSA (1 mg.ml)	0.05 µl
4	*Eco* RI adapter (5 pmol/µl)	2.2
5	*Mse* I adapter (50 pmol/µl)	1.1
6	*Eco* RI Restriction enzyme	0.25
7	*Mse* I Restriction enzyme	0.50
8	T4 DNA Ligase (10 U/µl)	0.1 µl
9	Water (Sigma)	1.59
10	DNA Sample(125 ng/µl)	4.0 µl
11	Total Rx Volume	11.0 µl

Restriction and Ligation

1. Heat *Eco* RI and *Mse* I adapters (mix of adapter 1 and 2) at 95°C for 5 min and then cool down to room temperature.
2. Make RL master mix as above.
3. Aliquot 7 µl in labeled 0.5 ml tubes and then add corresponding DNA.
4. Spin briefly.
5. Incubate the mix at 37°C c for 3 hours in a water bath
6. Check success of RL products on gel as below

6. RUNNING THE RL PRODUCTS ON GEL AND THEIR COMPARISON WITH UNCUT DNA

1. RL products- 4 µl of RL product + 2 µl of loading dye (5X) + 1 µl of SYBR Green
2. Uncut DNA- 2 µl of diluted DNA (125 ng/µl) + 2 µl dye + 1 µl of SYBR Green
3. On both ends Kb+ ladder was used- 4 µl of Kb+ ladder + 1 µl of SYBR Green
4. Running Condition- Gel 1.2%, 97-98 V, 1.30 hours
5. Photograph gel using Alpha Imager
6. Dilute RL product 10X with 0.1 X TE buffer and store it at -20 C. This is called t DNA.

7. PRESELECTIVE AMPLIFICATION

Make the master mix as following except tDNA.

Sr No	Reagents	Vol. for One reaction
1	10 X PCR Buffer (Sigma)	1.30 µl
2	dNTPs Mix (2.5 mM)	1.04 µl
3	$MgCl_2$ (50 mM) Sigma	0.52 µl
4	*Eco* RI PS Primer(10 pM) Sigma	0.39 µl
5	*Mse* I PS Primer(10 pM) Sigma	0.39 µl
6	*Taq* Polymerase (Sigma Jumpstart)	0.10 µl
7	Water(Sigma)	6.26 µl
8	tDNA Sample(diluted RL product)	3.0 µl
9	Total Rx Volume(one reaction mix)	13.0 µl

Aliquot 10 µl of Master mix and then add 3.0 µl of tDNA

Pre-selective Amplification program

1	72^oC – 2 min	1 cycle
2	94^oC – 20 sec	20 cycles
	56^oC – 30 sec	
	72^oC – 2 min	
3	72^oC – 2 min	1 cycle
5	60^oC – 30 min	1 cycle

Following Pre-selective amplification, dilute reaction products 10 fold with 0.1 X TE buffer. This is called will be called as PSA.

8. SELECTIVE AMPLIFICATION

Make the master mix as following except PSA

Sr No	Reagents	Vol. for One reaction
1	10 X PCR Buffer (Sigma)	0.8 µl
2	dNTPs Mix(2.5 mM)	0.64µl
3	$MgCl_2$ (50 mM) Sigma	0.64µl
4	**Eco*RI labeled Sel Primer (EcoRI+3) (10 pM) Sigma	0.5 µl
5	**Mse*I Sel Primer (MSe I+ 3) (10 pM) Sigma	0.5 µl
6	Taq Polymerase (Sigma Jumpstart)	0.08 µl
7	Water(Sigma)	2.84 µl
8	Diluted Preselective Amplification product) PSA	2.0 µl
9	Total Rx Volume(one reaction mix)	8.0 µl

* For different primer combinations use respective *Eco* Sel and *Mse* Sel primers.

Selective Amplification program

1	94°C – 2 min	1 cycle
2	94°C – 20 sec	10 cycles
	66°C – 30 sec decrease 1.0 C /cycle	
	72°C – 2 min	
3	94°C – 30 sec	25 cycle
	56°C – 30 sec	
	72°C- 3 min	
4	60°C – 30 min	1 cycle

9. PREPARING THE SAMPLES FOR RUNNING ON CEQ 8800

1 Prepare a Sample Loading Solution (SLS) and Size standard master mix by combining 39.6 µl SLS and 0.4 µl of DNA size standard 600.

2 Add 0.5 µl of Selective amplified reactions to sample plate containing 40 µl of sample loading solution and DNA size standard.

3 Put one drop of mineral oil in each well. Centrifuge it and sample is ready for running on CEQ

4 Fill the buffer plate well (2/3) corresponding to the DNA samples with separation buffer

10. RUNNING THE CEQ 8800

1 Before loading the samples and running the CEQ samples should be ready.

2 Setup and save your sample plates.

2 Check the gel life and water in the wetting trays. It is good idea to clean the water tray and refill it with water.

3 Load the sample plates and buffer plates and start the CEQ.

4 Select the Frag-4 method and IALR size standard 600 analysis.

Run the sample plates.

Appendix B: AFLP Primer Combinations

Species	Primer Name	Primer Sequence: (5'-3')	Size
L. tulipifera	*Eco*RI-S1*	5'(dyeD4)GACTGCGTACCAATTCAG	18
	*Mse*I S1*	GATGAGTCCTGAGTAACCG	19
	*Mse*I S2	GATGAGTCCTGAGTAACCA	19
	*Mse*I S3	GATGAGTCCTGAGTAACCT	19
	*Mse*I S4*	GATGAGTCCTGAGTAACCC	19
	*Mse*I S5*	GATGAGTCCTGAGTAACGG	19
	*Mse*I S6	GATGAGTCCTGAGTAACGC	19
	*Mse*I S7	GATGAGTCCTGAGTAACGT	19
	*Mse*I S8*	GATGAGTCCTGAGTAACGA	19
	*Mse*I S9	GATGAGTCCTGAGTAACAC	19
	*Mse*I S10*	GATGAGTCCTGAGTAACAG	19
A. rubrum	*Eco*RI-S4*	5'(dyeD4)GACTGCGTACCAATTCAA	18
	*Mse*I S1	GATGAGTCCTGAGTAACCG	19
	*Mse*I S2	GATGAGTCCTGAGTAACCA	19
	*Mse*I S3*	GATGAGTCCTGAGTAACCT	19
	*Mse*I S4	GATGAGTCCTGAGTAACCC	19
	*Mse*I S5	GATGAGTCCTGAGTAACGG	19
	*Mse*I S6*	GATGAGTCCTGAGTAACGC	19
	*Mse*I S7	GATGAGTCCTGAGTAACGT	19
	*Mse*I S8*	GATGAGTCCTGAGTAACGA	19
	*Mse*I S9*	GATGAGTCCTGAGTAACAC	19
	*Mse*I S10*	GATGAGTCCTGAGTAACAG	19

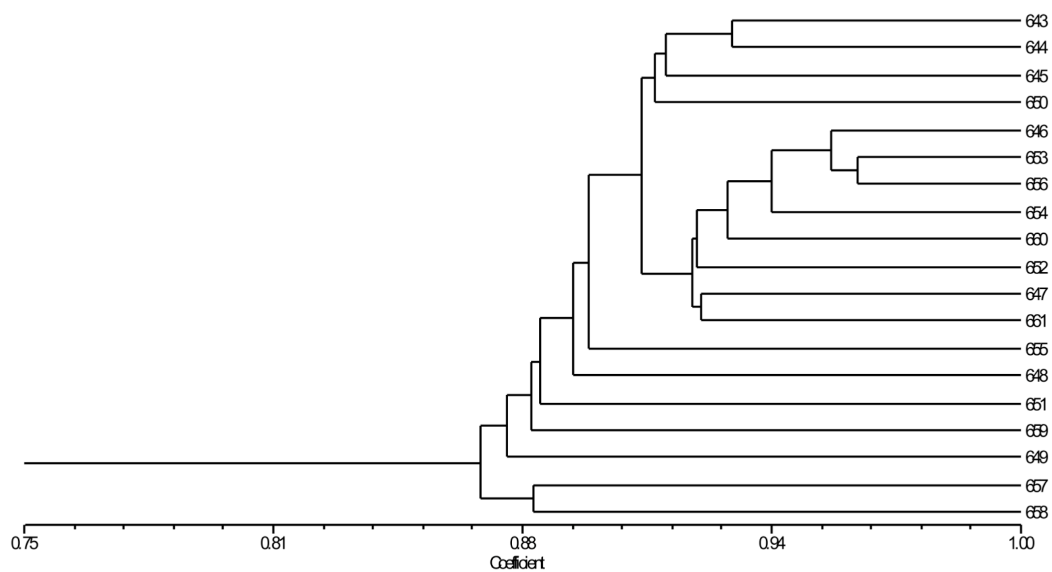
* = Primer combinations used in AFLP analysis. Each *Mse*I primer was used in combination with the *Eco*RI primer.

Appendix C: Contact Information for Field Sites

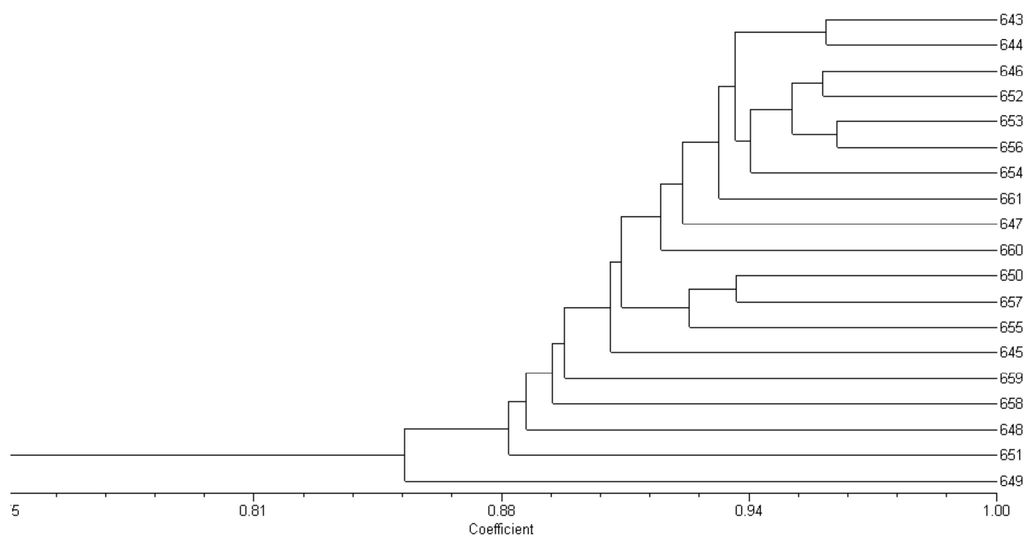
Population Location	Latitude (N)	Longitude (W)	Landowner	Contact Person
Blacksburg, VA	37°18'04.02"	80°26'27.72"	Virginia Polytechnic Institute and State University	Shep Zedaker/Mike Aust
Chillicothe, OH	39°02'45.66"	83°11'40.98"	F&W Forestry Services, Inc.	Peter Gayer
Oak Ridge, TN	36°00'09.00"	84°13'01.00"	University of Tennessee	Richard Evans
Summerville, SC	32°56'46.68"	80°22'41.40"	MeadWestvaco	David Bourgeois
Tappahannock, VA	37°40'43.26"	76°56'16.44"	Virginia Department of Forestry	Tommy Walker
Vicksburg, MS	32°25'40.52"	90°43'08.80"	Anderson-Tully Company	Glynn Brown
Blacksburg, VA	37°18'04.02"	80°26'27.72"	Virginia Polytechnic Institute and State University	Shep Zedaker/Mike Aust
Round Mountain, VA	37°10'16.82" N	81° 9'35.78"W	Jefferson National Forest	Ed Leonard
Oak Ridge, TN	35°56'11.59" N	84° 6'18.09"W	University of Tennessee	Richard Evans
Bolton, NC	34°16'19.41" N	78°25'55.48" W	Resource Management Service	Clay Jenkins
Bladen Lakes, NC	34°42'10.00" N	78°35'53.00" W	Bladen Lakes State Forest	Michael Chesnutt
Ichauway, GA	31° 6'23.95"N	83°34'48.77" W	Jones Ecological Research Center	Steve Jack

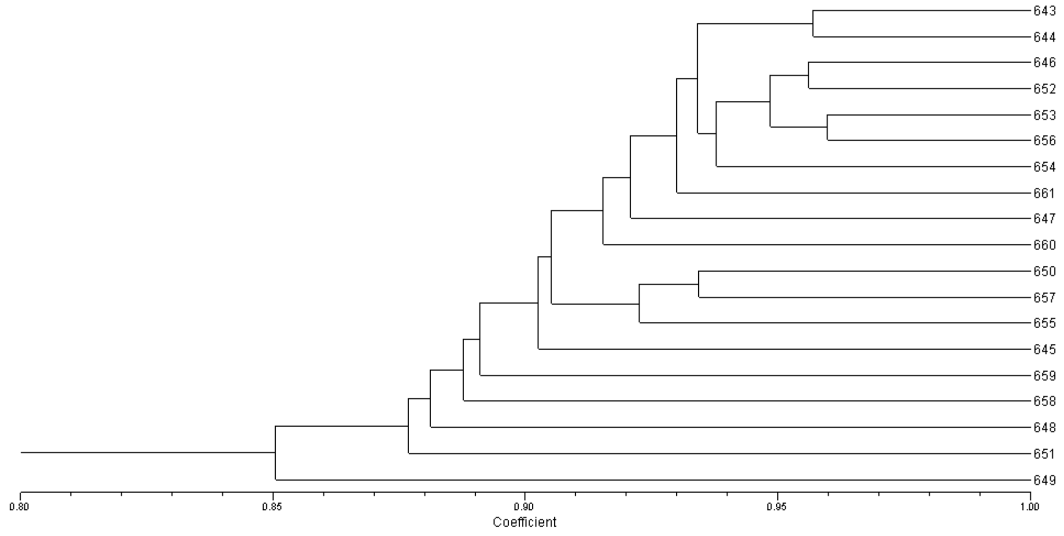
Appendix D: Dendrograms of 10 Initial Primer Combinations for *L. tulipifera* Samples from Tappahannock, VA Population

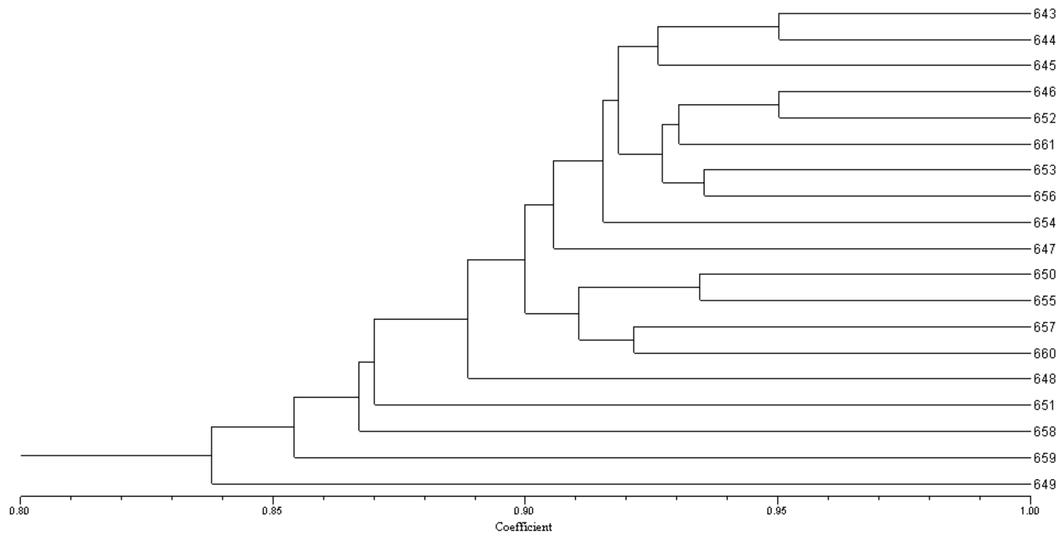
Ten initial primer combinations.

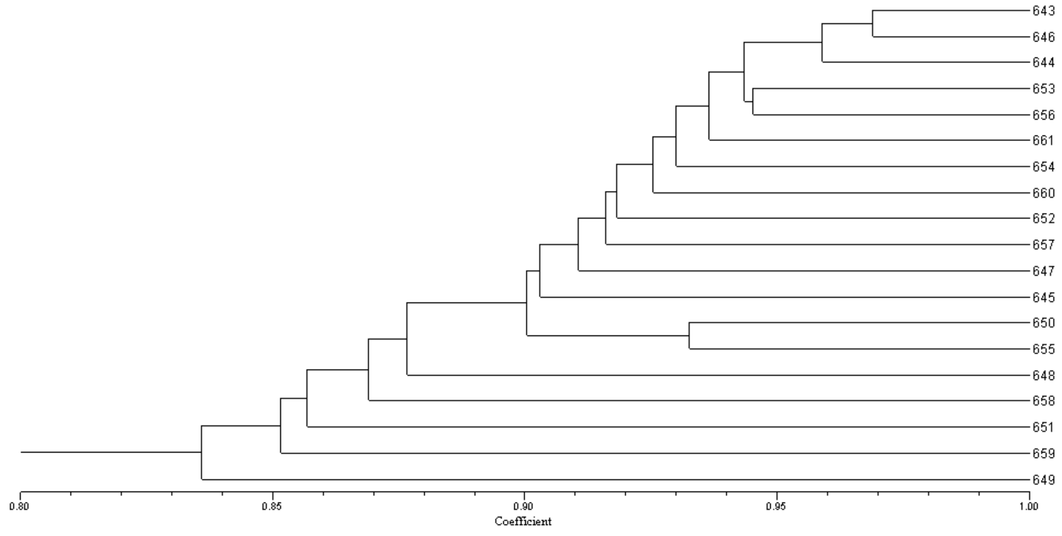


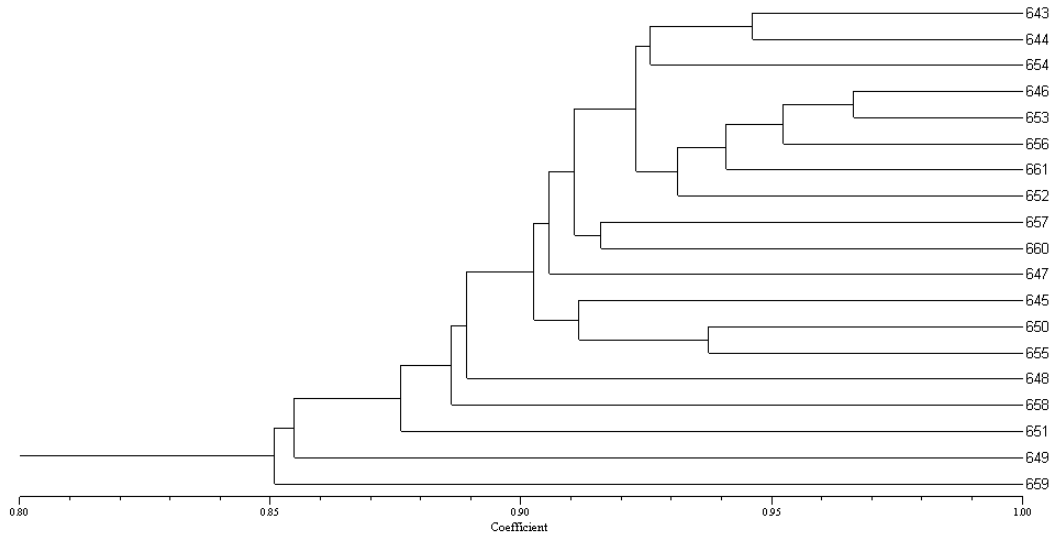
Final Five Chosen Primer Combinations

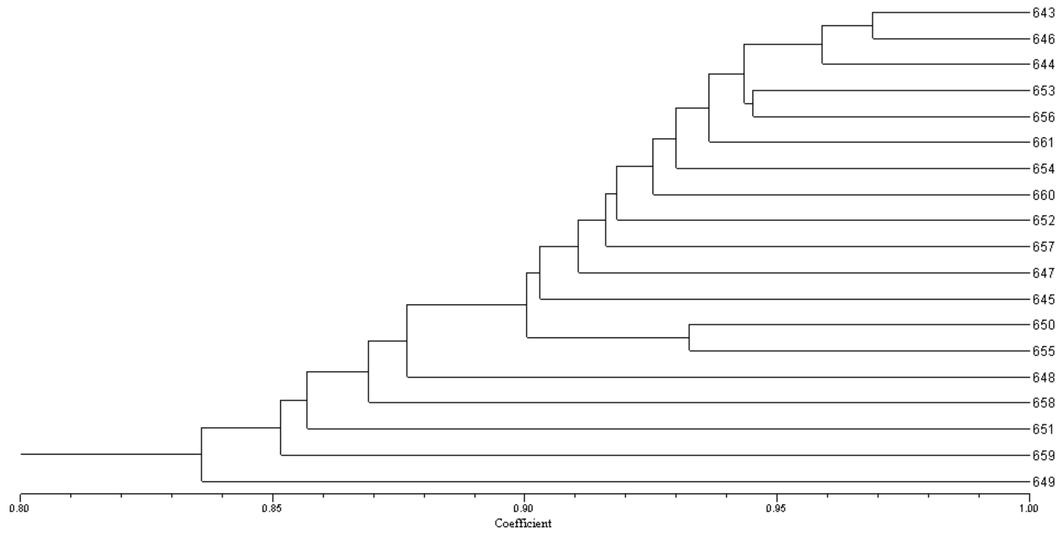



SPZ with 5 Primer Combinations
S1S1, S1S2, S1S3, S1S5, S1S6
643
644
646
652
653
656
654
661
647
660
650
657
655
645
659
658
648
651
649
0.80
0.85
0.90
0.95
1.00
Coefficient

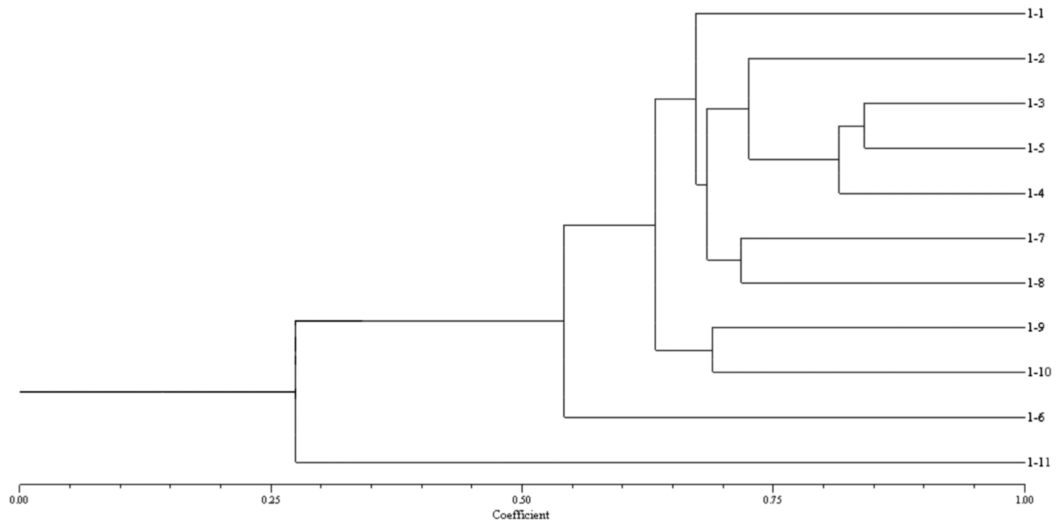

SPZ with 5 Primer Combinations
S1S1, S1S2, S1S4, S1S5, S1S6
643
644
645
646
652
661
653
656
654
647
650
655
657
660
648
651
658
659
649
0.80
0.85
0.90
0.95
1.00
Coefficient


SPZ with 5 Primer Combinations
S1S1, S1S2, S1S3, S1S4, S1S5
643
646
644
653
656
661
654
660
652
657
647
645
650
655
648
658
651
659
649
0.80
0.85
0.90
0.95
1.00
Coefficient

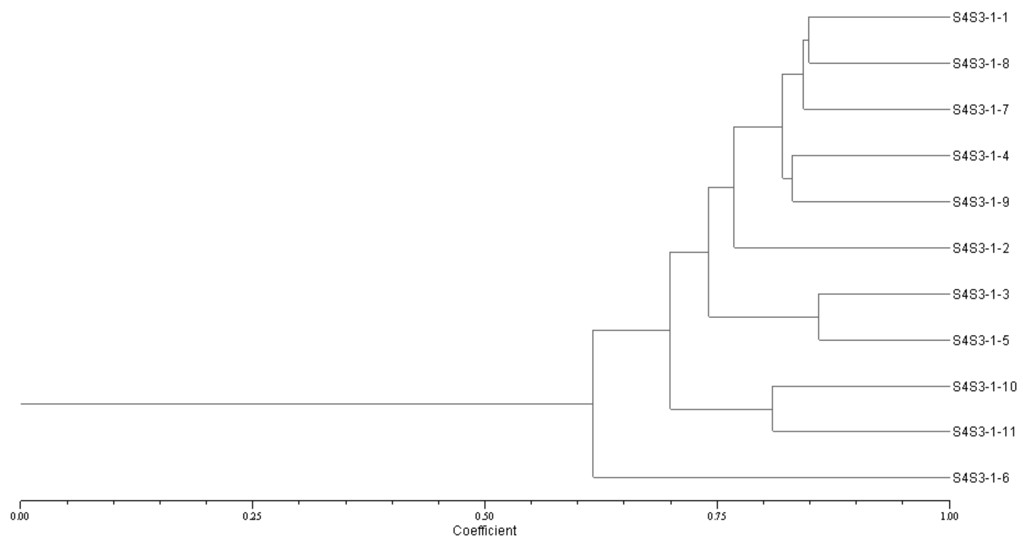

SPZ With 5 Primer Combinations
S1S2, S1S3, S1S4, S1S5, S1S6
643
644
654
646
653
656
661
652
657
660
647
645
650
655
648
658
651
649
659
0.80
0.85
0.90
0.95
1.00
Coefficient


SPZ with 5 Primer Combinations
S1S1, S1S2, S1S3, S1S4, S1S5
643
646
644
653
656
661
654
660
652
657
647
645
650
655
648
658
651
659
649
0.80
0.85
0.90
0.95
1.00
Coefficient

Appendix E: Dendrograms of Initial 10 Primer Combinations for *A. rubrum*

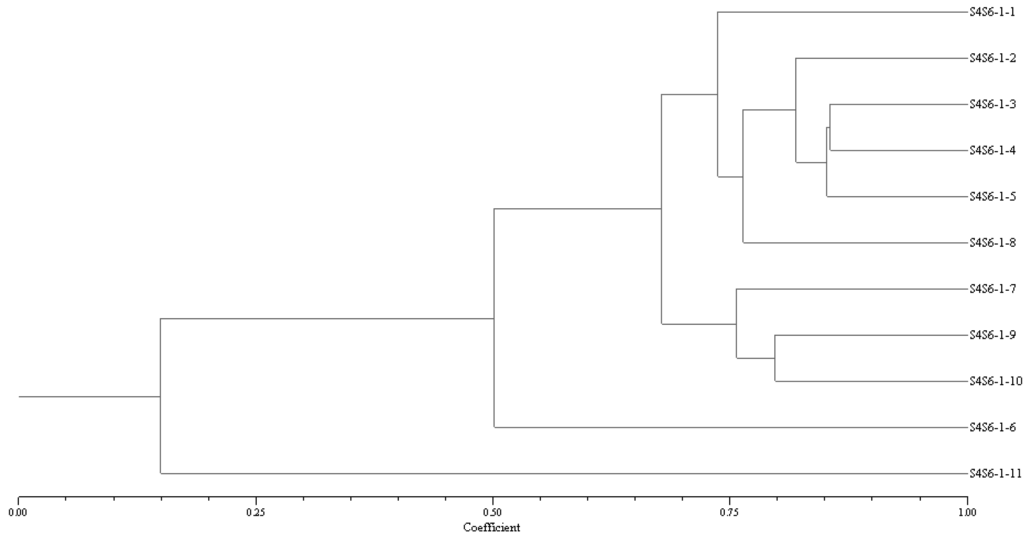


Chosen Primer Combination



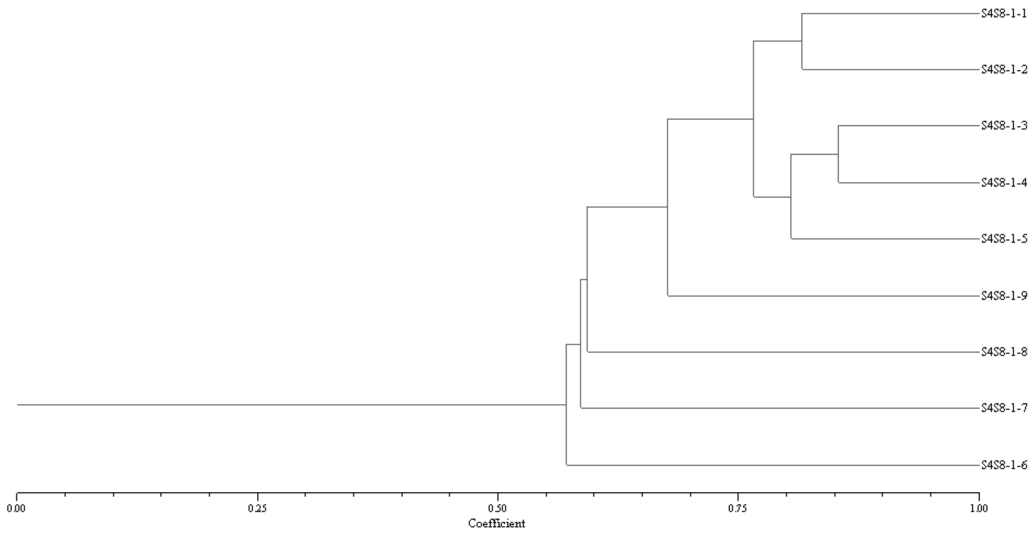
Chosen Primer Combination

S4S6 BJ RM



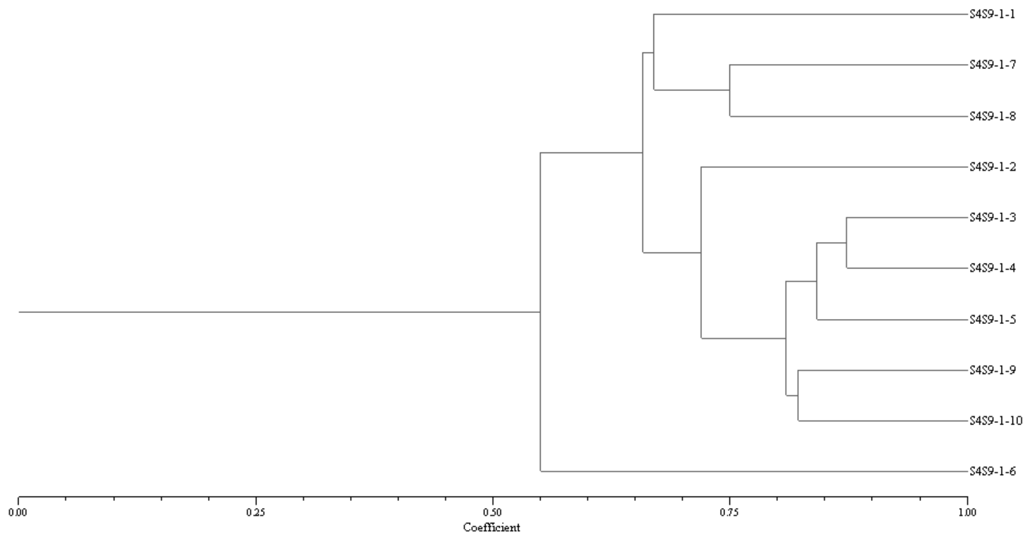
Chosen Primer Combination

S4S8 BJ RM



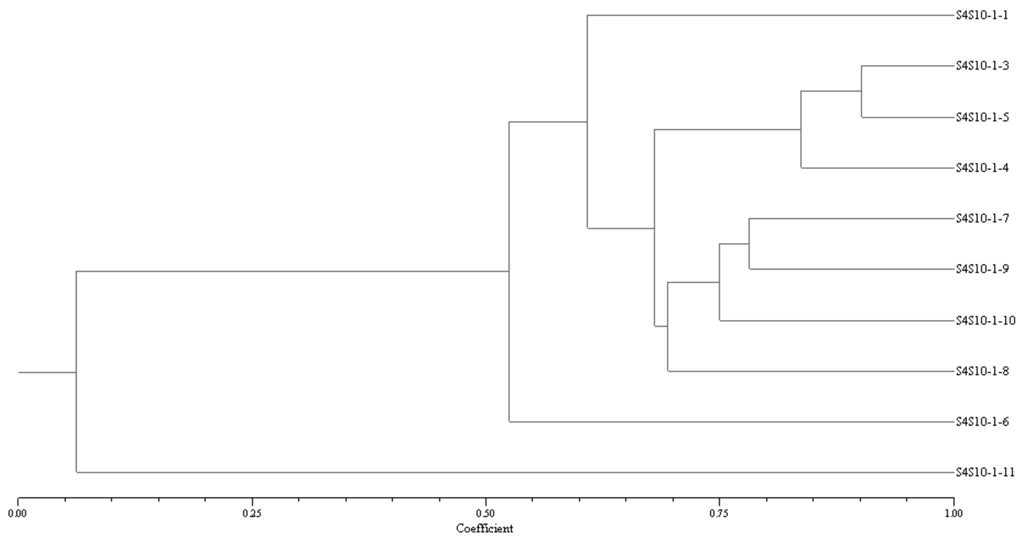
Chosen Primer Combination

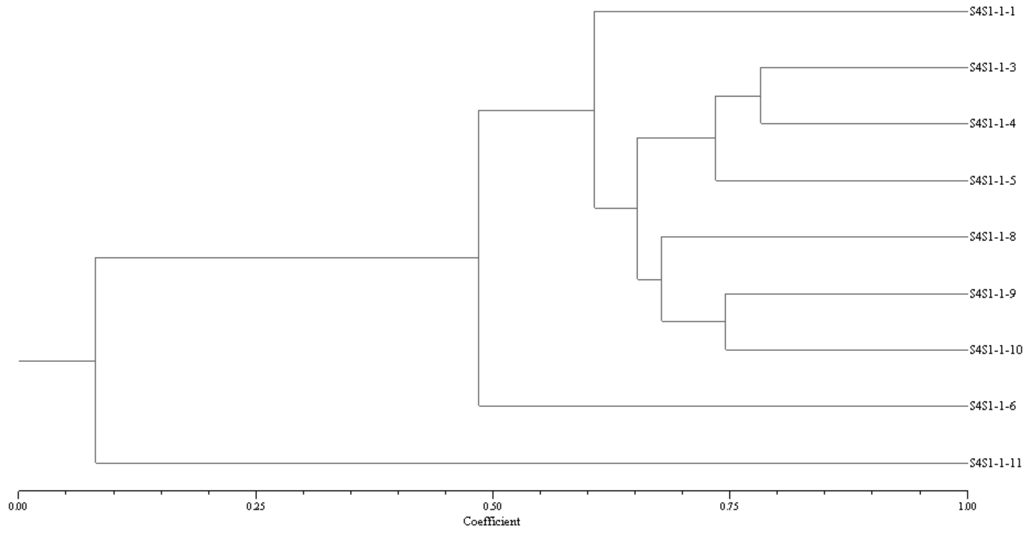
S4S9 BJ RM

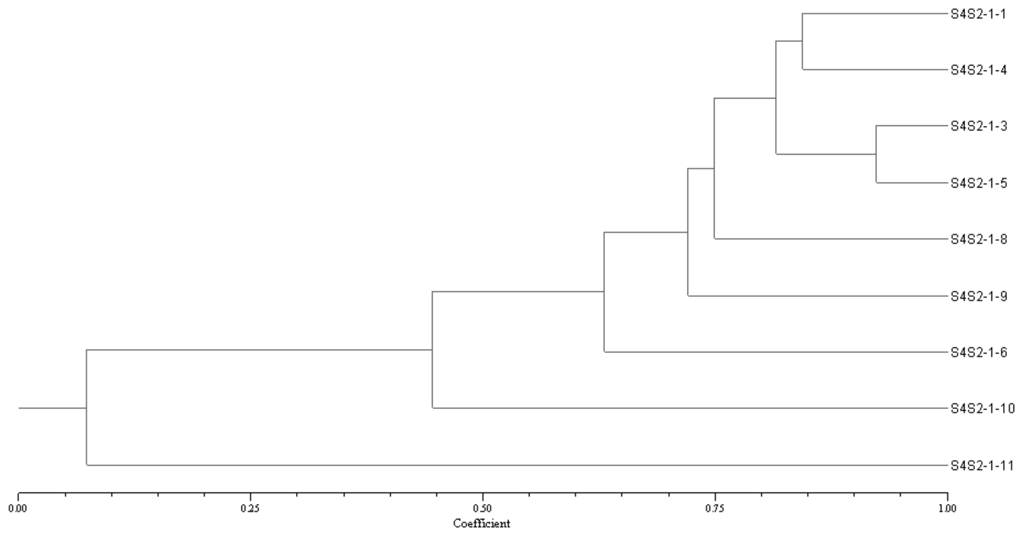


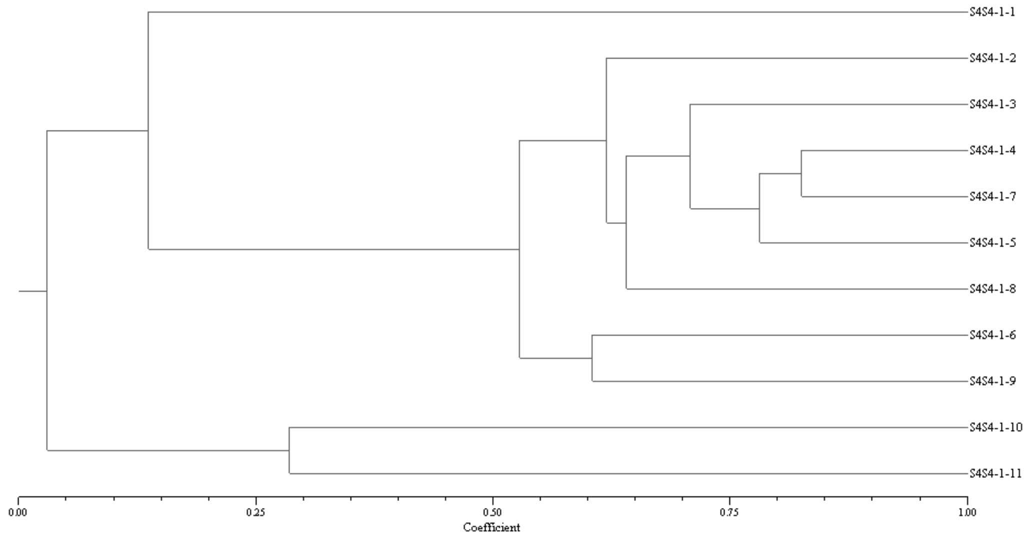
Chosen Primer Combination

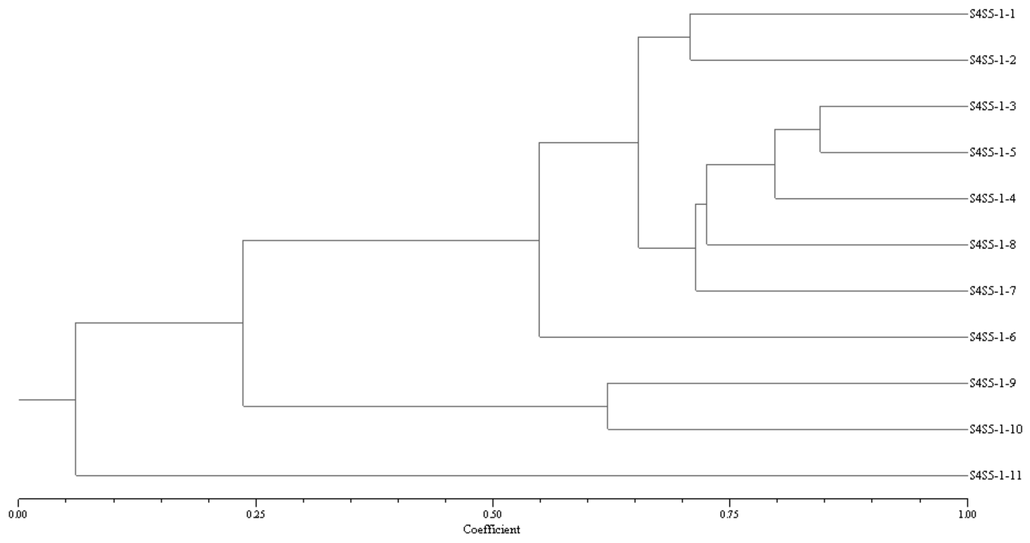
S4S10 RM BJ

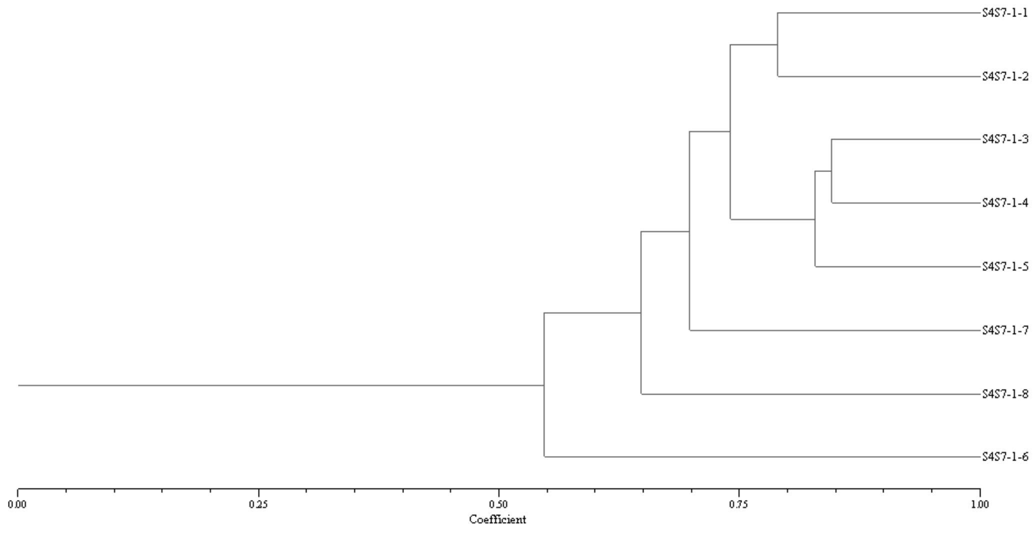



S4S1 BJ RM
S4S1-1-1
S4S1-1-3
S4S1-1-4
S4S1-1-5
S4S1-1-8
S4S1-1-9
S4S1-1-10
S4S1-1-6
S4S1-1-11
0.00
0.25
0.50
0.75
1.00
Coefficient


S4S2 BJ RM
S4S2-1-1
S4S2-1-4
S4S2-1-3
S4S2-1-5
S4S2-1-8
S4S2-1-9
S4S2-1-6
S4S2-1-10
S4S2-1-11
0.00
0.25
0.50
0.75
1.00
Coefficient


S4S4 BJ RM
S4S4-1-1
S4S4-1-2
S4S4-1-3
S4S4-1-4
S4S4-1-7
S4S4-1-5
S4S4-1-8
S4S4-1-6
S4S4-1-9
S4S4-1-10
S4S4-1-11
0.00
0.25
0.50
0.75
1.00
Coefficient


S4S5 BJ RM
S4S5-1-1
S4S5-1-2
S4S5-1-3
S4S5-1-5
S4S5-1-4
S4S5-1-8
S4S5-1-7
S4S5-1-6
S4S5-1-9
S4S5-1-10
S4S5-1-11
0.00
0.25
0.50
0.75
1.00
Coefficient


S4S7 BJ RM
S4S7-1-1
S4S7-1-2
S4S7-1-3
S4S7-1-4
S4S7-1-5
S4S7-1-7
S4S7-1-8
S4S7-1-6
0.00
0.25
0.50
0.75
1.00
Coefficient

Appendix F: Climate Data

Species	Physiographic Province	Population	Mean Annual Temperature (F)	Mean Annual Precipitation (inches)	Mean Growing Season
A. rubrum	Coastal Plain	Ichauway, GA	65.42	51.21	248.65
A. rubrum	Coastal Plain	Bladen Lakes, NC	62.51	51.21	208.48
A. rubrum	Coastal Plain	Bolton, NC	62.34	49.32	228.36
A. rubrum	Mountains	Blacksburg, VA	51.80	40.57	165.18
A. rubrum	Mountains	Oak Ridge, TN	57.99	50.33	209.42
A. rubrum	Mountains	Round Mountain, VA	51.29	39.05	151.00
L. tulipifera	Coastal Plain	Summerville, SC	64.08	53.18	231.44
L. tulipifera	Coastal Plain	Tappahannock, VA	58.16	43.54	220.28
L. tulipifera	Coastal Plain	Vicksburg, MS	65.25	55.80	256.95
L. tulipifera	Mountains	Oak Ridge, TN	57.99	50.33	209.42
L. tulipifera	Mountains	Blacksburg, VA	51.80	40.57	165.18
L. tulipifera	Mountains	Chillicothe, OH	53.00	38.14	180.33

Appendix G: Geographic Distance Matrices

A. rubrum Populations

	Blacksburg, VA	Round Mountain, VA	Oak Ridge, TN	Bolton, NC	Bladen Lakes, NC	Ichauway, GA
Blacksburg, VA	0.00	65.33	360.86	382.87	333.46	747.55
Round Mountain, VA	65.33	0.00	297.05	406.09	358.90	710.91
Oak Ridge, TN	360.86	297.05	0.00	548.76	518.63	539.86
Bolton, NC	382.87	406.09	548.76	0.00	50.31	597.17
Bladen Lakes, NC	333.46	358.90	518.63	50.31	0.00	613.90
Ichauway, GA	747.55	710.91	539.86	597.17	613.90	0.00

L. tulipifera Populations

	Blacksburg, VA	Chillicothe, OH	Oak Ridge, TN	Summerville, SC	Tappahannock, VA	Vicksburg, MS
Blacksburg, VA	0.00	309492.17	364665.01	442432.73	312245.78	1086883.86
Chillicothe, OH	309492.17	0.00	349318.37	689345.35	566808.89	1005621.12
Oak Ridge, TN	364665.01	349318.37	0.00	469019.32	672388.70	724295.52
Summerville, SC	442432.73	689345.35	469019.32	0.00	568996.12	987049.88
Tappahannock, VA	312245.78	566808.89	672388.70	568996.12	0.00	1386794.93
Vicksburg, MS	1086883.86	1005621.12	724295.52	987049.88	1386794.93	0.00