Summer 8-29-2013

Comparisons of Genetic Diversity Among Disjunct Populations of Magnolia tripetala

Victoria A. Gilkison
Western Kentucky University, victoria.gilkison856@topper.wku.edu

COMPARISONS OF GENETIC DIVERSITY

AMONG DISJUNCT POPULATIONS OF *MAGNOLIA TRIPETALA*

A Capstone Experience/Thesis Project

Presented in Partial Fulfillment of the Requirements for

The Degree Bachelor of Science with

Honors College Graduate Distinction at Western Kentucky University

By:

Victoria A. Gilkison

* * * * *

Western Kentucky University

2013

Approved By:

CE/T Committee:

Dr. Jarrett R. Johnson; Advisor

Advisor
Department of Biology

Dr. Albert J. Meier; Advisor

Dr. Leslie Baylis

Advisor
Department of Biology

ABSTRACT

Plant ranges are often made up of core areas where the distribution is continuous and the population density is high with small disjunct populations at the margins of the core. One well-studied type of disjunct population is formed by long distance dispersal as plants migrate away from disjunct Pleistocene refugial populations. The retreat of the Wisconsinan glaciation resulted in the outward dispersal of many plant species from their refugial locations to areas with suitable habitat. Many plants expanded their ranges through rare-long-distance dispersal.

This study used microsatellites to compare the genetic diversity, inbreeding levels, and gene flow frequency of disjunct *Magnolia tripetala* populations to main core *M. tripetala* populations. In addition, I determined that distance of dispersal is related to genetic diversity and identified source populations for the main core and northern disjunct populations.

There was no significant difference between the genetic diversity of disjunct populations and their counterparts. Inbreeding levels were high and gene flow was low among populations. Long-distance dispersal was determined to have a negative correlation with genetic diversity. Gene flow was traced from the refugial populations

through the main core and into the northern disjunct populations. *Magnolia tripetala* has a range made up of fragmented populations through the core with further disjunct populations at the border of the core. *M. tripetala* was determined to have abundant genetic diversity and disjunct populations are not in immediate danger of genetic deterioration.

When conducting a study on disjunct populations, species should be compared based on phylogenetic relationships. Furthermore, disjunct populations should not be generalized as having low genetic diversity. Instead, factors such as species traits and population history of a species should be taken into account in order for a more accurate hypothesis to be made.

Keywords: *Magnolia tripetala*, *g*enetic diversity, disjunct population, long-distance dispersal, Pleistocene refugia

ACKNOWLEDGEMENTS

I do not really know where to start.

Dr. Meier,

Thank you for being my mentor these past four years. I am truly blessed to have you as an advisor and a friend. Thank you for helping me to develop such an amazing project and for reading and editing my many thesis drafts.

Dr. Johnson,

Thank you for letting me—a crazy plant fanatic—conduct research in your clearly superior salamander lab and not giving me too much grief about it. Thanks for teaching me lab technique and helping me figure out how to grind up my plants the cheap—and fun—way. Thank you for helping me finish analyzing my data, getting the computer programs to work, and helping me edit my thesis.

Dr. Andersland,

According to Dr. Meier, I am supposed to blame you if anything with my thesis goes wrong. But I want to thank you for letting me use your liquid nitrogen! I still think your machine is half dragon.

Thank you to Ryan Vincent, Megan Laffoon, Kevin Tewell, and Elaine Flynn for helping me with lab work. And thank you Ryan for helping me to conquer the annoying computer programs that hate my guts.

I would also like to thank Paul Weigman, Rick Gardner, Scott Freidhof, Derrick Heckman, Virginia McDaniel and especially Donna Ware for helping me obtain samples. Without you guys, this project certainly would not have been possible. Donna, if I am ever in Virginia, I owe you some very delicious desserts.

Thank you to Dr. Meier's spring Ecology 2012 class for scouring a site in Alabama for remnants of a rare *M. tripetala* population.

Thank you to my family and friends for your love and support and for keeping me sane when I was about to go crazy with stress.

Thank you to the WKU Office of Research for awarding me a FUSE grant so that my project could be funded. Thanks to the Biology department and to the WKU Biotechnology Center for providing me the use of equipment and resources. I also want to thank the Kentucky Native Plant Society for funding me and helping me pay for travel to sample sights and for shipment of my samples. Thank you to Western Kentucky University's Upper Green River Biological Preserve for financial assistance.

And to anyone I failed to mention—thank you. This project could not have been possible without the help from so many wonderful people.

VITA

Victoria A. Gilkison victoria.gilkison@gmail.com

Education

Western Kentucky University

Current graduate student (first year Masters) at Western — August 2013—Present

Bachelor of Science Degree in Biology — August 2013

Carol Martin Gatton Academy of Mathematics and Science Graduate — August 2009—May 2011

Named Newsweek's #1 high-school in the nation 2012 and 2013

Anderson County High School Graduate — August 2007—May 2011

Research and Presentations

Undergraduate Honors Thesis Fall 2011—July 2013

Comparisons of Genetic Diversity Among
Populations of Magnolia tripetala

Gilkison, V. A., Johnson, J. R., and Meier, A. J. Comparisons of genetic diversity among disjunct populations of *Magnolia tripetala*. March 2013. Poster Presentation at WKU Student REACH Week Research Conference.

Malloy, E. M., **Gilkison, V. A.,** Meier, A. J., Grubbs, S., Yates, J. Food web analysis of an autumn riverine macro-invertebrate community. March 2012. Oral presentation at the Ecological Society of America's 97th Annual Anniversary Conference in Portland, Oregon.

Gilkison, V. A., Rauh, B., A., Clark, J. C., Hornback, J. A., Swiger, A. J., Sanford, J. C., Simpson, K. M., Kalantarzadeh, P. S., Wilson, R. M., Malloy, E. M., Jennings, A. B., Blackeman, E. A., Erwin, E. K., Meier, A. J., Grubbs, S., Yates, J. Stable isotopic analysis of the Upper Green River in Hart County, Kentucky. March 2012. Oral presentation at Western Kentucky University 42nd Annual Student Research Conference.

Gilkison, V. A., Effects of organic and inorganic fertilizer on the growth and survival of *Utterbackia Imbecillis* and *Alisma subcordatum.* November 2010. Oral presentation at the Kentucky Academy of Science.

Gilkison, V. A., Effects of organic and inorganic fertilizer on the growth and survival of *Utterbackia imbecillis* and *Alisma subcordatum.* October 2010. Oral presentations

at Western Kentucky University Honors Round Table. (Submitted paper to Siemens Competition)

Wigginton S. K., Racke, D., **Gilkison, V. A.,** American Ginseng conservation. February 2010. Poster presentation at the Western Kentucky University 40th Annual Student Research Conference.

Gilkison, V. A., Wigginton S. K., Racke, D., American Ginseng conservation. January 2010. Poster presentation at Posters of the Capitol in Frankfort, Kentucky.

Racke, D., **Gilkison, V. A.,** Wigginton S. K., American Ginseng conservation. November 2009. Oral presentation at the Kentucky Academy of Science.

Awards/Grants

2013 WKU Biology Department Outstanding Undergraduate Research March 2013

Larry Gleason Award

$400

WKU Fuse Grant October 2012

Comparisons of Genetic Diversity Among Populations of Magnolia tripetala

$5,000

Kentucky Native Plant Society September 2012

Comparisons of Genetic Diversity Among Populations of *Magnolia tripetala*

$250

Western Kentucky University's Center for Biodiversity Student of the Year March 2012

Ogden College Research Scholarship Awarded Presentations Fall Semester 2011—Spring Semester 2013

Stable isotopic analysis of the Upper Green River in Hart County, Kentucky — March 2012
Western Kentucky University 42nd Annual Student Research Conference
Presenting author; Oral presentation
First place

Effects of organic and inorganic fertilizer on the growth and survival of Utterbackia imbecillis and Alisma subcordatum — October 2010
Western Kentucky University Honors Round Table
Author; Oral presentation
Second place

American Ginseng Conservation — February 2010
Western Kentucky University Student 40th Annual Student Research Conference
Presenting author; Poster presentation
Second place

American Ginseng Conservation — November 2009
Kentucky Academy of Science
Co-author; Paper presentation
Second place

Skills and Technique

Lab Technique

Crop Callus Propagation

Carrot, Tobacco, Alfalfa

Crop Transformation with *Agrobacterium*

Carrot, Tobacco, Alfalfa

Gel Electrophoresis

IC and ICP analysis

Trained in June 2013

PCR

Plant DNA Extraction

Western Kentucky University Biotechnology Certification Program

Completed Fall Semester 2010

Field Technique

Measuring Water Quality Parameters

Temperature, Ph, TOC, Conductivity, TDS, TAN, and DO

Measuring Plant Growth

Measuring Environmental Conditions

Assembled and currently manage a small network of environmental data loggers and base stations at Western Kentucky University's Upper Green River Biological Preserve

Analysis Technique

Networking Analysis

Eigen vector and Eigen value

Biostatistics

Additional

Honors Biological Research Class Winter 2011

Cloud Bridge Nature Preserve, Costa Rica

Heliconias at Cloud Bridge Nature Preserve

Volunteer

Super Saturdays

Western Kentucky University

Introduction to Organic Chemistry November 2011

5th grade—6th grade

The Chemistry of Detective Work November 2011

5th grade—6th grad

Math is Everywhere November 2010

1st grade—3rd grade

Global Relief Club January 2010—May 2011

Carroll Martin Gatton Academy
of Mathematics and Science

Fundraising for Haiti

Fundraising for South Africa

Fundraising for Darfur

Middle School Teacher Student Aid August 2009

Anderson County Middle School

Extracurricular Activities

Global Relief Club August—May 2011

Co-vice-president

Green club August—May 2011

Western Kentucky Choral Society February 2011—December 2012

K-1st Sunday School teacher September—April;
2007— 2009

Academic Field of Study

Undergraduate Major

Biology

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Habitat heterogeneity ensures that very few plant species are continuously distributed throughout their entire range (Cain *et al.* 2000). Often, plant ranges include core areas where the distribution is continuous and the population density is high. Smaller disjunct populations border the core near the margins of the range.

Plant populations can become disjunct from the core for a variety of reasons (Cain *et al* 2000, Bialozyt *et al*. 2006). Existing populations can become progressively more disjunct over time when distances caused by existing geographic barriers, such as rivers and deserts, become more extreme. Habitat fragmentation—including that of anthropogenic origin—causes formerly continuous distributions to become disjunct or more isolated (Gonzales and Hamrick 2005). Two well-studied types of disjunct population are those formed by dispersal events away from disjunct Pleistocene refugial populations and those formed by long-distance dispersal.

The last glacial time period in Earth's history occurred during the Pleistocene Epoch just over 2.5 million years ago (Hewitt 1999). The Pleistocene was characterized by many glacial and interglacial periods caused by oscillating temperatures (Web and Bartlein 1992) that led to the formation of many different ice sheets throughout the

epoch. During the warmer periods, altitudinal ranges would have shifted, allowing plants to either ascend or descend mountains (Larena *et al.* 2002). When temperatures dropped, plants persisted in refugial areas that provided protection from the otherwise harsh conditions (Paulo *et al.* 2001, Shepard and Burbrink 2008).

The Laurentide ice sheet in North America reached its maximum around 18 thousand years ago (Barrington and Paris 2007) and continued to retreat until approximately 13 thousand years ago (Dyke and Prest 1987). Warming temperatures resulted in the outward dispersal of many plant species from their refugial locations (thus forming disjunct refugial populations) to areas with less competition and environments that matched their climatic needs (Braun 1947, Webb and Bartlein 1992, Hewitt 1999, Cox and Moore 2000, Trapnell *et al.* 2007, Plues 2011). Some pockets remained in mountainous areas where cool, humid environments were maintained (McWilliam 1966, and Braun 1947), and these places protected cold-tolerant plants from the warming temperatures.

Given the position of the disjunct Pleistocene refugial populations relative to contemporary expanded ranges, it is evident that many plant species dispersed farther than their average seed dispersal distance per generation would suggest was possible (Clark *et al.* 1998). This phenomenon—known as Reid's Paradox—is thought to be caused by rare long-distance dispersal events (Clark *et al.* 1998).

Long distance dispersal occurs via the infrequent aid of biotic and abiotic factors. These biotic and abiotic dispersal agents—such as mammals, birds, flowing bodies of water, and heavy wind storms (Clark *et al.* 1998, Cain *et al.* 2000, Gonzales and Hamrick

2005, Nathan 2008)—result in the expansion of population ranges beyond the typical dispersion distance.

When long-distance dispersal occurs, one, or a few seeds are established at great distances from the main core population. These founder events as well as the separation and dispersal of many individuals away from refugial populations create new disjunct populations. However, high amounts of genetic diversity may exist between those few individuals to counteract the negative genetic effects associated with small population size (e.g., drift, inbreeding).

By definition, gene flow in the form of fertilization via pollen is limited between disjunct and core populations. If sufficient gene flow occurs, disjunct populations may be integrated into the main core. In this way, long-distance dispersal can cause both an expansion of a plant range and a shift of the main core.

The range of the *Magnolia tripetala* is an example of a shift in the main core. During the Pleistocene, the main core populations—now refugial populations—were located in Arkansas (suggested by McWilliam 1966), Florida and Virginia (Harvel Jr. 1975, Donna Ware), and certain regions of the Appalachian Mountains such as the Tunica Hills in Louisiana (Delcourt and Delcourt 1975). Today, the main core stretches from Alabama to Kentucky and east into North Carolina with numerous disjunct populations bordering the core (Fig.1). As more populations were established and the main core expanded, modern refugial populations were left behind in cooler pockets where they were able to escape the warming temperatures. Long-distance dispersal aided in the quick dispersal and lead to the formation of more disjunct populations further north in areas such as Ohio and Pennsylvania.

Disjunct populations are often of great interest to population biologists and of great concern to conservation biologists (Ellstrand 1992, Kikuchi and Isagi 2002). While plant populations near or at the Pleistocene refugia have been shown to have high levels of genetic diversity (Broyles 1998, Abbott 2000, Hewitt 2000, Persson 2003, Barrington and Paris 2007), population genetic theory predicts that disjunct populations formed by founder events will have lower genetic diversity with genetic diversity decreasing as distance from refugial population increases (Aide and Rivera 1998).

The maintenance of genetic diversity is very important for the long-term persistence of populations. Low genetic diversity can decrease a population's overall fitness and therefore make it more susceptible to extinction (Vellend and Geber 2005). The ability to adapt to future competitors, poor environmental conditions (Thomas *et al.*1999, Pluess and Stöcklin 2004), and pests or blights (Smithson and Lenné 1996, Sun *et al.*2001) are limited because individuals possessing the rare alleles that could aid in the adaptation or evolution of a population may have been lost.

Measuring genetic diversity is helpful for the conservation of rare species, usually occurring in small or disjunct populations (Ellstrand 1992), because they can alert conservationists to declining condition so that precautions to prevent extinction may be made (Kikuchi and Isagi 2002). However, Aide and Rivera (1998) concluded that conservation efforts that introduce new alleles into genetically distinct disjunct populations might result in the gradual homogenization of the species (Aide and Rivera 1998)—thus decreasing genetic diversity

Theory predicts that disjunct populations resulting from dispersal away from refugia (e.g., range expansion following glaciation) will have low genetic diversity for

reasons such as increased occurrences of inbreeding (Ellstrand and Elam 1993), clonal growth, genetic drift (Ellstrand and Elam 1993, Tomimatsu and Ohara 2003), and bottlenecks (Lammi *et al.* 1999). Inbreeding in plants mainly occurs in small populations through self-fertilization or bi-parental fertilization of close relatives (Ellstrand and Elam 1993). Inbreeding and clonal growth can leave populations very susceptible to disease as necessary adaptive alleles are lost (Jackson *et al.* 1985) and the population becomes more homogeneous.

Genetic drift is a powerful evolutionary force in small, newly established populations due to bottlenecks (i.e., reductions in genetic diversity) (Leberg 1992). Under normal conditions, gene flow balances the loss of diversity caused by drift but in disjunct populations where gene flow is expected to be low, genetic deterioration by drift is expected to have much more of an effect (Slatkin 1987).

After the initial establishment of disjunct populations, long-distance dispersal may to increase gene flow between populations (Nichols and Hewitt 1994), but chances are low. Gene flow becomes even more restricted for populations that are geographically isolated from each other (Gugger *et al.* 2008). In eastern North American, the Appalachian Mountains are considered the largest obstacle to the dispersal of plants (Gugger *et al.* 2008). Barriers such as these mountains isolate populations and can cause an increase in among population genetic diversity—especially between disjunct populations (Aide and Rivera 1998). When looking at the range of *M. tripetala*, the main core populations located in Kentucky are separated from the main core Virginia populations by the Appalachian Mountains. Therefore it can be speculated that these populations would have high levels among population diversity.

Many studies have been conducted on the relationship between disjunct populations and their counterparts in the core. Some studies reported low genetic diversity in disjunct populations (Karron 1987, Broyles 1998, Lammi *et al.* 1999, Hannon and Orick 2000, Landergott *et al.* 2001, Kikuchi and Isagi 2002, and Persson 2003, Gonzales and Hamrick 2005) as predicted by population genetic theory. Other studies found that disjunct populations and core populations had no significant difference in levels of genetic variability (Rossum *et al.* 2003, Baali-Cherif and Besnard 2005, Mandak *et al.* 2005).

To provide further insight on the dilemma of whether or not disjunct populations have low genetic diversity, I am conducting this study on disjunct *M. tripetala* populations with four main questions in mind: 1) Do the disjunct Pleistocene refugial populations and northern disjunct populations of *M. tripetala* have lower levels of genetic diversity than the main core populations? The disjunct Pleistocene refugial populations of *M. tripetala* are refugial as well as disjunct, so I expect them to have lower levels of genetic diversity than the core, but higher levels of genetic diversity than the northern disjunct. 2) Is inbreeding more prevalent in the disjunct populations of *M. tripetala* as compared to the more widespread main core populations? Inbreeding is common in small populations (Ellstrand and Elam 19933). 3) Do disjunct populations experience less gene flow as predicted by theory? Populations that are geographically isolated from each other experience less gene flow (Gugger *et al.* 2008). 4) Is the distance separating a population from a refugial population associated with its level of genetic diversity? Disjunct Pleistocene refugial populations are often areas of high genetic diversity (Abbott 2000, Hewitt 2000), and thus it is often thought that genetic diversity decreases as distance

from a refugial population increases (Aide and Rivera 1998). In addition to these four questions, I would like to determine source populations of the main core and disjunct populations of *M. tripetala*.

If disjunct populations of *M. tripetala* have lower genetic diversity, I will be able to determine if the low genetic diversity is associated with high amounts of inbreeding, little gene flow or long distance dispersal. If the genetic diversity levels of disjunct populations are not be significantly lower, this study will indicate that disjunct populations of *M. tripetala* should not be of high concern to conservation biologists.

METHODS

Choosing the Species:

Magnolia tripetala is a good candidate for this study because it has a range consisting of isolated, disjunct, putative Pleistocene refugial population, main core populations, and northern disjunct populations (Fig. 1). Molecular markers have been developed for two Asian lineages of *Magnolia—Magnolia obovata* and *Magnolia seiboldii* ssp. *Japonica* (Kikuchi an Isagi 2002). Belonging in the subsection known as Rhytidospermum, *Magnolia tripetala* is more related to several Asian Magnolia species than to the North American Magnolia species (Nie *et al.* 2008).

Collecting Samples:

Samples belonging to northern disjunct populations were collected from Fayette/Somerset, Pennsylvania; Jackson County, Ohio; and West Portsmouth, Ohio (Fig. 1). Samples belonging to main core populations were collected from Rowan County, Kentucky; Carter County, Kentucky; Albermarle County, Virginia; Madison County, Virginia; and Gallant County, Alabama (Fig. 1). Disjunct putative Pleistocene refugial populations were collected from Williamsburg/James City, Virginia; Virginia Beach, Virginia; and from the Caddo/Womble Ranger District of the Ouachita National Forest Service, Arkansas (Fig. 1).

One leaf was collected from each of at least twenty trees in a population. Trees were sampled biasedly. Samples were taken from trees not directly beside each other to avoid double sampling with clonal growth. The Pennsylvania population was the only case where sample size deviated from the standard twenty. *M. tripetala* is so sparse in Pennsylvania that only five trees were located and sampled from in the visited population.

DNA Extraction:

Between 60-100mg of tissue were excised from each leaf. Tissue was placed in individual 1.5mL tubes, immediately frozen with liquid nitrogen and then ground. DNA extractions proceeded with the use of a QIAGEN DNeasy Plant Mini Kit ®. DNA was suspended in buffer, and concentrations were determined using NanoDrop. DNA was re-extracted from any individual having a DNA concentration lower than 10μg/nL.

Molecular Markers & PCR:

Microsatellites are short sequences of nucleotide repeats (Morgante and Olivieri 1993) used in genetic studies for reasons such as higher mutation rate (Li *et al.* 2004). Microsatellites are appropriate for studies involving phylogenetic relationships and the conservation of rare or disjunct species (Kikuchi and Isagi 2002).

Microsatellites markers isolated by Isagi *et al.* (1999) were used for this experiment. I was only able to use ten out of the eleven primer pairs as the eleventh primer did not work for my samples. CAG primers (Nunziata *et al.* 2011) were attained to attach to the forward primers of the working Isagi *et al.* (1999). Fluorescently labeled

dyes were attached to the CAG primers. PCR protocol was as described in Isagi *et al.* (1999) with the denaturation temperature at 94^{o}C, the annealing temperature at 55^{o}C, and the extension temperature at 72^{o}C. The successful amplification of primer pairs was assessed using agarose gel electrophoresis. Based on band strength, DNA was diluted then allocated to a submission plate. Submission plates were shipped to the University of Georgia Genome Facility for genotyping.

Data Analysis:

Raw data received from the University of Georgia Genome Facility were imported into GeneMapper (Applied Biosystems, Foster City, California) where allele calls could be made, reviewed and edited. Loci were reduced to six for this study: M6D1, M6D3, M6D4, M10D6, M10D8, and M15D5 due to primer bonding complications or lack of variation within a locus. Individuals missing data for more than three loci were deleted from the final data set.

MicroChecker (Oosterhout *et al.* 2004) software was used to check for the presence of null alleles in my data set. Null alleles are areas on the DNA sequence that were unable to be replicated (Callen *et al.* 1993). Null alleles can cause individuals to be scored as homozygotes instead of heterozygotes—thus skewing results (Callen *et al.* 1993). Possible null alleles were identified for the M6D1, and M6D8 loci however, these loci were not consistently deemed null across all populations. In a study done by Carlsson (2008), high frequencies of microsatellites with null alleles had non-significant effects on the correct assignment of populations in programs such as STRUCTURE. Therefore, the four loci remained included for further analysis.

The program STRUCTURE (Pritchard *et al.* 2000) was used as an unbiased approach to randomize the data and assign individuals into populations. STRUCTURE assigns individuals into populations assumed to be under Hardy-Weinberg equilibrium based on genotypes and allele frequencies (Pritchard *et al.* 2000). The K (or number of populations) was set from 1 to 11 meaning that ultimately the individuals would be grouped into eleven different populations. Log-likelihood values (delta K) were determined for each K. The program HARVESTER (Dent and vonHoldt 2012) uses the Evanno method (Evanno *et al.* 2005) to determine the optimal number of populations for my data set.

GenAlEx (Peakall and Smouse 2012) was used to assess the genetic diversity of each population based on four parameters: the number of alleles per population (Na), the number of effective alleles per population (Ne), the number of private alleles per population (Np), and the expected number of heterozygotes (He) for each population. An ANOVA was conducted to determine if there were significant differences in the number of alleles per population (Na),the number of effective alleles (Ne), the number of private alleles (Np), and for the expected number of heterozygotes (He) for the three population types (disjunct putative Pleistocene refugial, main core and northern disjunct).

An AMOVA was used to determine the proportions of genetic variance within individuals, among individuals, and among populations over the entire range of *Magnolia tripetala.* Individuals were separated into main groups (main core and disjunct) so that an AMOVA could detect how genetic variation relates to disjunct populations versus their widespread congeners. Individuals were further into their three population types (northern disjunct, main core, and disjunct putative Pleistocene refugial) so that an

AMOVA could detect the genetic variance differences between the two types of disjunct populations (northern and Pleistocene refugial).

GenePop (Raymond and Rousset 1995) was used to calculate F_{IS} values and associated P-values per locus for each population. F_{IS} values showed the amount of inbreeding occurring. Any value close to zero represented allele frequencies that might be expected during random mating. Values closer to one represented allele frequencies that might occur in an inbred population. Pairwise F_{ST} values were calculated in GenAlEx between every population to determine genetic differentiation among populations. Numbers closer to one meant that populations were highly differentiated from each other.

I used BayesAss v 3.0.3 (Wilson and Rannala 2003) to determine the amount of gene flow between every population through immigration and emigration.

Google Earth (Google Inc, Santa Clara, California) was used to determine the straight distance between every population sampled and the nearest accessible refugial population. These distances were plotted against the He (number of expected heterozygotes for every population based on the individuals sampled) to determine if there was a linear correlation. A linear regression was performed in R (R Core Team 2012) to determine if the correlation was significant or not.

Google Earth was used to determine the straight distance between all the populations. These distances were plotted against the associated pairwise F_{ST} values calculated in GenAlEx to determine if there was any correlation.

A neighbor joining tree with a bootstrap value of 1000 was constructed in Poptree2 (Takezaki *et al.* 2010) to estimate the degree of relation between populations. The tree was made using allele frequencies determined with GenAlEx.

RESULTS

In this study I was able to successfully genotype 187 individual *Magnolia tripetala* trees across 6 loci and from 11 different populations. Missing data accounted for 3.65% of the data set.

The core Alabama population (ALG) and the putative Pleistocene refugial ARJ had the highest genetic diversity among all the populations as they had the greatest number of different alleles, number of effective alleles, number of private alleles and the highest expected frequency of heterozygotes (Fig. 3) In contrast, the refugial VAA and VAVB populations had the lowest genetic diversity. VAVB had no private alleles present in population, and both populations had very few heterozygotes present.

When comparing the remaining eight populations, there appear to be no significant difference between the values. Even after including the high ALG, and ARJ populations and the low VAA and VAVB populations, an ANOVA showed no significant difference in the genetic diversity values (Na, Ne, Np, and He) for any of the populations. Thus the level of genetic diversity maintained in a population does not correlate with the population type (disjunct Pleistocene refugial, main core, or northern disjunct).

When looking at the entire range, the AMOVA showed that 40% of the variation occurred within individuals, 22% of the variation of the data occurred among individuals, and 38% of the variation occurred among populations. When comparing the AMOVA of the main core individuals versus the individuals belonging to disjunct populations, it is clear that disjunct populations contain much more variation among populations. Disjunct populations contained 22% of the variation within individuals, 28% among individuals, and 50% among populations. Main core populations in contrast contained 46% of the variation within individuals, 21% among individuals, and 33% of the variation among populations. When the disjunct populations were further divided into northern disjuncts and disjunct Pleistocene refugial populations, the putative Pleistocene refugial disjunct populations had the highest among population diversity. The northern disjunct populations contained 46% of their genetic variation within individuals, 31% among individuals and 23% among populations. The refugial disjunct populations contained 19% of their variation within individuals, 17% among individuals, and 64% among populations.

F_{IS} values, which provide a measure of deviation from expected levels of heterozygosity, and Fisher exact tests of deviation from Hardy-Weinberg equilibrium (HWE) indicate that there are significant deviations for every locus and in every population (Table 1). M6D8 had the highest amount of inbreeding out of all 6 loci as the F_{IS} values all indicated extreme heterozygote deficiency. Some loci also demonstrate heterozygote excess in some populations (negative F_{IS} values; Table 1). Heterozygote

excess can result from negative assortative mating, or other evolutionary forces such as hybrid vigor or selection against homozygotes.

F_{ST} values indicated high amounts of genetic differentiation between the majority of the populations sampled (Table 2). There was low genetic differentiation occurring between the main core KYR population and the main core KYGC population as well as between KYR and the northern disjunct OHJ population.

The Bayesian assignment values from BayesAss suggested low proportions of gene flow between population pairs (Table 3). Dispersing 6.847 individuals to the main core KYR population, the main core KYGC population sent out the most individuals. The northern disjunct OHJ and OHS populations also sent out dispersers to KYR. OHJ sent 6.580 individuals per one generation of KYR, and OHS dispersed 6.025 individuals per one generation of KYR. The main core VAA population also dispersed individuals. VAA sent 5.019 individuals to the main core VAM population per one generation of VAM. The remaining populations experienced the majority of their gene flow with themselves.

There was a negative correlation ($R^2 = 0.7222$) found between a population's genetic diversity (represented in this case by He) and its distance from the nearest refugial population (Fig. 4). A linear regression performed in R had a T-statistic of -3.94, six degrees of freedom and a P-value = 0.0076 (< alpha=0.05). Therefore, there is a significant correlation between the distance a population was located from the nearest refugial population and the number of heterozygotes in the population. There was no linear correlation (R^2=0.0012) found between the distance between populations and the associated pairwise F_{ST} values.

There was only one clade strongly supported by the Neighbor Joining (NJ) tree created by PopTree2 (Fig. 5). The clade is comprised of the two main core Kentucky populations (KYGC and KYR), the two northern disjunct Ohio populations (OHJ and OHS), and the northern disjunct PAFH population. ALG was an out group for this data.

HARVESTER results showed that the optimal log-likelihood score was at K=2 meaning that instead of having eleven populations, my study appeared to have two heavily supported genetically discernible entities (i.e., populations) (Fig. 2). The second optimal log-likelihood score was at K=5.

DISCUSSION

This study was designed to compare the genetic diversity of disjunct *Magnolia tripetala* with the genetic diversity of *M. tripetala* belonging to core of the range. To accomplish this goal I asked three main questions. 1) Do disjunct populations have lower genetic diversity than their counterparts in the core? 2) Are disjunct populations of *M. tripetala* more inbred than main core populations? 3) Do disjunct populations experience less gene flow than main core populations? In addition, I tested the effects of long-distance dispersal on the genetic diversity of populations by asking a fourth question: 4) is geographic distance from the nearest sampled disjunct putative Pleistocene refugial population of *M. tripetala* correlated with the number of heterozygotes in any given population? Lastly, I identified potential source populations to provide a better picture of the gene flow occurring within the *M. tripetala* populations.

Results led me to conclude that *Magnolia tripetala* has a population structure shaped by limited gene flow. Disjunct populations appear to experience the same levels genetic diversity, gene flow and inbreeding as main core populations. Ultimately, at least as measured by microsatellite markers, being disjunct appeared to have little genetic effect on populations of *M. tripetala*.

GENETIC DIVERSITY, INBREEDING, AND GENE FLOW

The first main goal of this study was to determine if disjunct populations of *M. tripetala* had lower genetic diversity than main core populations. There was no significant difference between the amount of genetic diversity possessed by disjunct populations (both the northern disjunct and the disjunct Pleistocene refugial) and the main core populations. This result coincides with other studies where the genetic diversity of disjunct populations was found to not be significantly different from main core populations (Rossum *et al.* 2003, Baali-Cherif and Besnard 2005, Mandak *et al.* 2005).

The putative Pleistocene refugial disjunct VAJC and VAVB populations had the lowest genetic diversity of all the populations, though the overall genetic diversity was not significantly different. These populations likely underwent severe bottlenecks that greatly decreased their genetic diversity. Overall population sizes are unknown for these populations, but if they are small they could be experiencing high levels of drift. Small populations are at higher risk for genetic drift (Ellstrand and Elam 1993).

In contrast to the VAA and the VAVB populations, the main core ALG population and the refugial ARJ populations had almost double the number of alleles and the number of private alleles in the population (Fig. 3). In the early stages of this study, I designated the ALG population as a disjunct Pleistocene refugial population. Upon further observation of the locality and the presence of more *M. tripetala* populations nearby, I renamed the ALG population as a main core population. Results indicate that ALG could be a refugial population that is not disjunct from the main core. Refugial populations have been identified as areas of high genetic diversity (Abbott 2000, Hewitt

2000, Barrington and Paris 2007, Broyles 1998, Persson 2003). Another possibility is that ALG is receiving high amounts of gene flow from surrounding populations that were not sampled for this study. If populations in the vicinity of ALG were sampled, a second clade might be established on the NJ tree. I cannot accurately predict these surrounding populations to be either refugial or main core.

As for the putative refugial ARJ population, there is no clear answer that would explain why ARJ has high genetic diversity when the other refugial populations have very low diversity. VAJC and VAVB do have higher levels of genetic differentiation from the other populations than ARJ. Thus, it is possible that ARJ is experiencing more gene flow and thus able to counteract negative genetic effects such as drift however, this claim is not supported by my results.

Although population type did not seem to have an affect or be associated with a certain level of genetic diversity, the genetic variation percentages per population type varied. An AMOVA conducted on the entire range of *M. tripetala* showed that the molecular variance was spread out somewhat unequally within individuals (38%), among individuals (22%) and among populations (40%). The high percentage among populations supports the high genetic differentiation found between populations (Table 2). When AMOVAs were conducted on certain population range types, the percentages shifted for the disjunct populations. The main core population shifted the variation to within individuals (46%), making among populations 33% and among individuals 21%. These numbers suggest that the main core populations are undergoing gene flow that allow for mutations to accrue and individuals to differ. These varying individuals are equally spread among the five different main core populations.

The disjunct populations had 28% within individuals, 22% among individuals, and 50% among populations. This would suggest that while individuals within a population do not differ greatly from each other, populations vary considerably.

When the disjunct populations were further subdivided into northern disjuncts and refugial populations, it appears that the putative Pleistocene refugial core populations are even more genetically differentiated. Northern disjunct populations had the majority of their diversity (46%) of their within individuals, whereas the disjunct Pleistocene refugial populations had the majority among populations (64%). This percentages account for over half of the genetic variance in the disjunct populations. This result supports the lower genetic diversity in refugial VAA and VAJC populations and how they differ from the refugial ARJ population. Considering that there were signs of gene flow between the Kentucky, Ohio, and Pennsylvania populations (Table 3, Fig. 5), it makes sense that they would be showing similar patterns of variance The disjunct populations are genetically differentiated from each other, which is often the case for disjunct populations. Often at the margin of the species range where conditions are not optimal, disjunct populations face strong evolutionary pressures which can cause genetic divergence to occur as populations strive to adapt (Rossum *et al.* 2003).

The second goal of this study was to determine if disjunct populations of *M. tripetala* have higher levels of inbreeding than main core populations. Population-level F_{IS} values (Table 1) indicate a significant deviation in each population. This deviation is likely caused by non-random mating because each marker also shows significant deviations from HWE (Table 1). Across all markers, the general trend is for each population to demonstrate a reduction in heterozygotes (positive F_{IS} values) indicating an

elevated degree of inbreeding in every population including populations that were expected to have lower levels (e.g. main core). However, some markers appear to demonstrate heterozygote excess, indicating possible selection at linked loci. Evolutionary pressures are certainly acting on *Magnolia tripetala* that lead to the high levels of deviation from HWE expectations. In addition, high inbreeding could be due to the breeding system of *Magnolia* trees. For example, Ishida (2006) found that *Magnolia obovata*, a sister taxa to *Magnolia tripetala,* has high self-fertilization rates often resulting from self-pollination.

In a similar study conducted by Matsuki *et al.* (2008), three main pollinators were observed to pollinate *Magnolia obovata*. These pollinators were denoted by the authors as bumblebees, flower beetles and small beetles. The flower beetles, which are by nature less likely to travel long distances (Somanathan *et al.* 2004), had the higher amounts of genetic diversity in the pollen. Insects more likely to be involved in long-distance dispersal of pollen—the bumblebees—were observed to have higher proportions of self-pollen, meaning that they were often involved in inbreeding. Perhaps they are the main reason for the inbreeding depression noted in both the Ishida (2006) study and the Matsuki *et al.* (2008) study. *Magnolia tripetala* must also have high self-pollination rates in order to explain the high amounts of inbreeding shown by the results of this study (Table 1).

The third goal of this study was to determine whether disjunct populations of *M. tripetala* experience lower gene flow than the main core populations. Results indicate low levels of gene flow and high genetic differentiation among all populations, even populations that are geographically near (Table 2, Table 3). The majority of individuals

dispersing into any given population were individuals already located within that population (Table 3) meaning that the majority of gene flow occurred within populations not between.

As the distance increases, the likelihood of successful pollination decreases due to the rare occurrence of long-distance dispersal. The Kentucky main core populations and the Ohio northern disjunct populations are in close proximity and were thus expected to experience high levels of gene flow. The Bayesian Analysis showed that there are migrations occurring between main core KYR and main core KYGC and KYR and northern disjunct populations OHJ and OHS (Table 3). The Virginia populations are also in close proximity and were also experiencing gene flow. Dispersal events occurred between the main core VAA population and the main core VAM population (Table 3). Evidence of these migrations and past migrations can be seen on the NJ tree (Fig. 5). As more gene flow occurs, populations become more related. Therefore, it makes sense to have a main clade forming between the Kentucky and Ohio populations.

The NJ tree confidently showed the presence of only a single clade comprised of the Kentucky, Ohio and Pennsylvania populations. The NJ tree is supported by the STRUCTURE plot (Fig. 2). The optimal log-likelihood score was at K=2 (Fig. 2). K=2 placed the main core ALG, the refugial ARJ, the main core KYGC and KYR, and the northern disjunct OHJ, OHS, and PAFH populations together. The split outlines the split of the Appalachian Mountains where the Virginia populations are separated from the rest of the populations. When the K is increased to 3, we see the formation of a Kentucky-Ohio-Pennsylvania clade similar to what is seen on the NJ tree (Fig. 5). However, in the K=3 plot ALG and ARJ are paired with one of the Virginia populations (VAA) leaving

the other three Virginia populations (VAJC, VAM, and VAVB) in a different group. I expected the Virginia population's to be more related since they are separated from the rest of the populations by the Appalachian Mountains. In addition, as shown by my results, very little gene flow is occurring between the Virginia populations and the populations west of the mountains (Table 3). A better picture is painted when K =5 (the second highest optimal log-likelihood score). The Kentucky-Ohio-Pennsylvania clade is still in place—still supporting the NJ tree—and the Virginia populations are forming their own group.

The low gene flow, high genetic differentiation, and high inbreeding indicate that *M. tripetala* has a range that consists of an area with a high density of fragmented populations (the main core) with further dispersed disjunct populations at the margin of the core, rather than a continuous main core range with bordering disjunct populations. Fragmented population structure is supported by the low dispersing beetles (Somanathan *et al.* 2004) that pollinate *M. tripetala* and most other magnolias (Thien 1974).

THE EFFECTS OF LONG-DISTANCE DISPERSAL

The effects of long-distance dispersal on the genetic diversity of plant species has been a topic in several previous studies (Broyles 1998, Griffin and Barrett 2004, Bohrer *et al.* 2005, Bialozyt *et al.* 2006). In most cases, long-distance dispersal was noted to decrease genetic diversity especially in recently established small populations when the likelihood of founder effects was greatest.

In this study, a linear regression (with a p-value of 0.0076 and an associated R^2 value of 0.7222) showed that there was a strong negative correlation between the distance

a *M. tripetala* population was located from the nearest refugia and the number of heterozygotes present in a population. Long-distance dispersal is a possible cause of small decreases in genetic diversity of dispersed *M. tripetala* populations. Although there is a negative trend of genetic diversity as related by distance, genetic diversity values do not appear to decline significantly with distance from refugia.

CONSERVATION OF *M. TRIPETALA* AND DISJUNCT POPULATIONS

The genetic variation of *M. tripetala* is spread somewhat unequally among populations (40%), among individuals (38%), and within individuals (22%). The level of inbreeding I detected may be affecting some populations such as the refugial VAJC and VAVB populations—which may explain the slight imbalance with high variation found among populations. As for the rest of the populations, the gene flow occurring appears sufficient enough to limit extreme differentiation among populations.

The relatively stable genetic diversity in *M. tripetala* can be interpreted one of two ways. The first—and more probable—is that the populations have been disjunct since the Pleistocene, but *M. tripetala* has retained genetic diversity. The alternative explanation is that that the populations only recently became disjunct from each other and the negative genetic effects have not yet begun to take place. In either case, habitat fragmentation will likely cause existing populations to remain disjunct or become further isolated, which may be detrimental in the future. The refugial VAJC and VAVB populations will likely continue to undergo genetic deterioration, and begin experiencing negative genetic effects much sooner than the other populations. For now, *M. tripetala*

populations do not suffer from a lack of genetic diversity—whether disjunct or main core—and thus should not be of highest concern to conservation biologists.

Karron (1987) felt that generalizing all rare species as possessing the same genetic patterns (i.e. low genetic diversity, low gene flow, high inbreeding) was futile without attempting to incorporate the life history aspect of individual species. Gitzendanner and Soltis(2000) took the Karron(1987) study into account and concluded that rare species should be compared according to phylogenetic relationships. Furthermore, species sharing a recent common ancestor should not be treated as independent samples when they are in fact related (Gitzendanner and Soltis 20000).

Much like rare species, disjunct populations are often generalized as having low levels of genetic diversity, despite studies that have shown otherwise (e.g. Rossum *et al.* 2003, Baali-Cherif and Besnard 2005, Mandak *et al.* 2005). The concept devised by Karron (1987) and others (e.g. Kruckeberg and Rabinowitz 1987), can be applied to species with disjunct populations. Disjunct populations sharing a recent common ancestor are more likely to have similar life history characteristics and thus more likely to have similar evolutionary forces acting on them and similar population histories. In addition, predictions about genetic diversity should be based after taking into consideration past events such as bottlenecks and migrations from refugia. Hannan and Orick (2000) concluded that factors such as these were important because of their potential to affect the genetic diversity of that population. Populations will experience different evolutionary forces as supported by my study. Despite being putative Pleistocene refugial disjunct populations, ARJ, VAJC and VAVB differed in their amount of genetic diversity (although these values were once again not significantly different).

I propose that instead of asking if disjunct populations have more or less genetic diversity than possessed by their counterparts, we should instead be asking if disjunct populations should possess low genetic diversity due to their population history such as historic bottlenecks and species specific traits such as limited dispersal mechanisms. This would help to prevent the placing of species with genetically stable disjunct populations under conservation management and redirect efforts to species in more immediate danger of endangerment or extinction.

I returned to the original ten conflicting papers and tried to discern patterns in the methods of the studies as a way to justify why some found that disjunct populations have lower genetic diversity (Karron 1987, Broyles 1998, Lammi *et al.* 1999, Hannon and Orick 2000, Landergot *et al.* 2001, Kikuchi and Isagi 2002, Persson 2003, Gonzales and Hamrick 2005) and why others found that disjunct populations have no significantly different or in some cases higher genetic diversity(Rossum *et al.* 2003, Baali-Cherif and Besnard 2005, Mandak *et al.* 2005). Studies varied drastically with no conceivable pattern. Authors used many different genetic analysis techniques including microsatellites, enzyme analyses (e.g. isozymes, and allozymes), and RAPD. Some studies adopted the Karron (1987) point of view and only studied populations of the same species or genus (Karon 1987, Lammi *et al.* 1999, Person 2003, Rossum *et al.* 2003, Mandak *et al.* 2005). Other studies compared their findings to findings in other papers with a different study species (Broyles 1998).

There was only one common thread I could find between these differing papers and my own study. Despite the result, whether lower genetic diversity, higher, or not significantly different, results were explained once a species' traits (such as pollinators,

fertilization mechanisms, dispersal mechanisms, geographic barriers) and population history (such as previous bottlenecks, glacial events, founder effects, and long-distance dispersal events) were taken into account. This further reiterates that these factors should be taken into account when trying to determine the amount of genetic diversity pertaining to disjunct populations.

LITERATURE CITED

Abbott, R. J. 2000. Molecular analysis of plant migration and refugia in the Artic. *Science.* 1343-1345.

Aide, T. M. and Rivera, E. 1998. Geographic patterns of genetic diversity in *Poulsenia armata* (Moraceae): Implications for the theory of Pleistocene refugia and the importance of riparian forest. *Journal of Biogeography*. 25: 695-705.

Baali-Cherif, D. and Besnard, G. 2005. High genetic diversity and clonal growth in relict populations of *Olea europaea* subsp. *Laperrinei* (Oleaceae) from Hoggar, Algeria. *Annals of Botany*. 96: 823-830.

Barrington, D. S. and Paris, C. A., 2007. Refugia and migration in the quaternary history of the New England flora. *RHODORA.* 109: 369-386.

Becker, R. A. Wilks, A R. R version by Brownrigg, R.. Enhancements by Minka, T. P. 2013. Maps: Draw Geographical Maps. R package version 2.3-2. http://CRAN.R-project.org/package=maps

Bialozyt, R., Ziegenhagen, B., and Petit, R. J. 2006. Contrasting effects of long-distance seed dispersal on genetic diversity during range expansion. *Department of Conservation Biology*. 19: 12-20.

Bohrer, G., Nathan, R., and Volis, S. 2005. Effects of long-distance dispersal for metapopulation survival and genetic structure at ecological time and spatial scales. *Journal of Ecology*. 93: 1029-1040.

Braun, L. 1947. Development of the deciduous forests of Eastern North America. *Ecological Monographs.* 17: 211-219.

Broyles, S. B. 1998. Postglacial migration and the loss of allozyme variation in northern populations of *Asclepias exaltata* (Asclepiadaceae). *American Journal of Botany.* 9: 1091-1097.

Cain, M. L., Milligan, B., G., and Strand, A. E. 2000. Long-distance seed dispersal in plant populations. *American Journal of Botany*. 87: 1217-1227.

Carlsson, J. 2008. Effects of microsatellite null alleles on assignment testing. *Journal of Heredity.* 6:616-623.

Cox, C. B. and Moore, P. D. 2000. Biogeography: An ecological and evolutionary approach. Blackwell Science, London, UK.

Clark, J. S., Fastie, C., Hurtt, G., Jackson, S. T., Johnson, C., King, G. A., Lewis, M., Lynch, J., Pacala, S., Prentice, C., Schupp, E. W., Webb III, T., and Wycoff, P. 1998. Reid's paradox of rapid plant migration. *Bioscience*. 48:13-24.

Davies, S., White, A., and Lowe, A. 2004. An investigation into effects of long-distance seed dispersal on organelle population genetic structure and colonization rate: a model analysis. *Heredity*. 93: 566-576.

Delcourt, H. R. and Delcout, P. A. 1975. The Blufflands: Pleistocene pathway into the Tunica Hills. *American Midland Naturalist.* 94: 385-400.

Dent, E. A. and vonHoldt, B. M. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 2:359-361.

Dyke, A. S. and Prest, V. K., 1987. Late Wisconsinan and Holocene history of the Laurentide Ice Sheet. *Géographie physique et Quaternmaire.* 41: 237-263.

Ellstrand, N. C. 1992. Gene flow by pollen: Implications for plant conservation genetics. *Oikos*. 63: 77-86.

Ellstrand, N. C. and Elam, D. R. 1993. Population genetic consequences of small population size: Implications for plant conservation. *Annual Review of Ecology and Systematics.* 24: 217-242.

Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology.* 14: 2611-2620.

Gitzendanner, M. A. and Soltis, P. A. 2000. Patterns of genetic variation in rare and widespread plant counterparts. *American Journal of Botany.* 87: 783-792.

Gonzales, E. and Hamrick, J. L. 2005. Heredity. Distribution of genetic diversity among disjunct populations of the rare forest understory herb *Trillium reliquum. Heredity*. 1-9.

Griffin, S. R. and Barrett, S. C. H. 2004. Post-glacial history of *Trillium grandiflorum* (Melanthiaceae) in Eastern North America: Inferences from phylogeography. American *Journal of Botany.* 91: 465-473.

Gugger, P. F., McLachlan J. S., Manos, P. S., and Clark, J. S. 2008. Inferring long-distance dispersal and topographic barriers during post-glacial colonization from the genetic structure of red maple (*Acer ruburm* L.) in New England. *Journal of Biogeography*. 35: 1665-1673

Hannan, G. L. and Orick, M. W. 2000. Isozyme diversity in *Iris cristata* and the threatened glacial endemic *I. lacustrus* (Iridaceae). *American Journal of Botany*. 3: 293-301.

Harvill Jr., A. M. 1975. Disjunct populations and the antiquity of species. *Castanca.* 40: 1-3.

Hewitt, G. M. 1999. Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*. 68: 87-112.

Hewitt, G. M. 2000. The genetic legacy of the Quaternary ice ages. *Nature.* 405: 907–913.

Hirayama, K. Ishida, K., and Tomaru, N. 2005. Effects of pollen shortage and self-pollination on seed production of an endangered tree, *Magnolia stellate. Annals of Botany.* 95: 1009-1015.

Ishida, K. 2006. Maintenance of inbreeding depression in a highly self-fertilizing tree, *Magnolia obovata* Thunb. *Evolutionary Ecology.* 20:173-191.

Jackson, J. B., Winston, J. E., and A. G. Coates. 1985. Niche breadth, geographic range, and extinction of Caribbean reef-associated cheilostome Bryozoa and Scleractinia. Proc. 5th Int. Coral Reef Symp. 4: 151-158.

Karron, J. D. 1987. A comparison of levels of genetic polymorphism and self-compatibility in geographically restricted and widespread plant counterparts. *Evolutionary Ecology.* 1: 47-58.

Kikuchi, S. and Isagi, Y. 2002. Microsatellite genetic variation in small and isolated populations of *Magnolia sieboldii* spp. *japonica. Heredity*. 88: 313-321.

Kruckebeg, A. R. and Rabinowitz, D. 1985. Biological aspects of endemism in higher plants. *Annual Review of Ecology and Systematics.* 16: 447-479.

Lammi, A., Siikarnäki, P., and Mustarjärvi, K. 1999. Genetic diversity, population size, and fitness in central and peripheral populations of a rare plant *Lychnis viscaria. Society for Conservation Biology.* 13: 1069-1078.

Landergott, U., Holderegger, R., Kozlowsi, G., and Schneller, J. J. 2001. Historical bottlenecks decrease genetic diversity in natural populations of *Dryopteris cristata. Heredity.* 87: 344-355.

Larena, B. G, Aguilar, J. F., and Feliner, G. N. 2002. Glacial-induced altitudinal migrations in *Armeria* (Plumbaginaceae) inferred from patterns of chloroplast DNA haplotype sharing. *Molecular Ecology.* 11: 1965-1974.

Lebeg, P. L. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. *Evolution.* 46: 477-494.

Li, Y., Korol, A. B., Fahima, T., and Nevo, E. 2004. Microsatellites within genes: Strucutre, function and evolution. *Molecular Biology and Evolution.* 21: 991-1107

Mandák B., Bímová, K., Plačkova, I., Mahelka, V., and Chrtek, J. 2005. Loss of genetic variation in geographically marginal populations of *Atriplex tatarica* (Chenopodiaceae). *Annals of Botany.* 96: 901-912.

Matsuki, Y., Tateno, R., Shibata, M., and Isagi, Y. 2008. Pollination efficiencies of flower-visiting insects as determined by direct genetic analysis of pollen origin, *American Journal of Botany*. 95: 925-930.

McWilliam, A. 1966. Observations on occurrence and range of three species of *Dentaria* (Cruciferae) in the Ouachita Mountains. *Arkansas Academy of Science Proceedings.* 20: 22-24.

Morgante, M., and Olivieri, A. M. 1993. PCR-amplified microsatellites as markers in plant genetics. *The Plant Journal.* 3:175-182.

Nathan, R. 2008. Long-distance dispersal of plants. Science. 313: 786-790.

Nichols, R. A. and Hewitt, G. M. 1994. The genetic consequences of long-distance dispersal during colonization. *Heredity.* 72: 312-317.

Nie, Z., Wen, J., Azuma, H., Qiu, Y., Sun, H., Meng, Y., Sun, W., and Zimmer, E. A. Phylogenetic and biographic complexity of Magnoliaceae in the Northern Hemisphere inferred from three nuclear data sets. 2008. *Molecular Phylogenetics and Evolution*. 48: 1027-1040.

Nunziata, S. O., Scott, D. E., Jones, K. L., Hagen, C., and Lance, S. L. 2011. Twelve novel microsatellite markers for the marbled salamander, *Ambystoma opacum*. *Conservation Genetics Resources.*

Oosterhout, C. V., Hutchinson, W. F., Wills, D. P. M., and Shipley, P. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes.* 4: 535-538.

Paulo, O. S., Dias, C., Bruford, M. W., Jordan, W. C., and Nichols, R. A. 2001. The persistence of Pliocene populations through the Pleistocene climatic cycles: Evidence from the phylogeography of an Iberian Lizard. *Proceedings: Biological Sciences.* 268: 1625-1630.

Peakall, R. and Smouse P.E. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537-2539.

Persson, H., Widén, B., Andersson, S., and Svensson, L. 2003. Allozyme diversity and genetic structure of marginal and central populations of *Corylus avellana* L. (Betulaceae) in Europe. *Plant Systematics and Evolution.* 244: 157-179.

Pluess, A. R. 2011. Pursuing glacier retreat: genetic structure of a rapidly expanding *Larix deciua* population. *Molecular Ecology*. 20: 473-485.

Pluess, A. R. and Stöcklin, J. 2004. Population genetic diversity of the clonal plant *Geum reptans* (Rosaceae) in the Swiss Alps. *American Journal of Botany*. 91: 2013-2021.

Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. STRUCTURE. Inference of population structure using multilocus genotype data. *Genetics*. 2: 945-959.

R Core Team (2012). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Raymond, M and Rousset, F. 1995. GENEPOP (version 1.2): population genetic software for exact tests and ecumenicism. J. *Heridty.* 86:248-249 .

Rossum, F. V., Vekemans, X., Gatia, E., and Meerts, P. 2003. A comparative study of allozyme variation of peripheral and central populations of *Silene nutans* L. (Caryophyllaceae) from Western Europe: Implications for conservation. *Plant Systematics and Evolution.* 242: 49-61.

Shepard, D.B. and Burbink, F.T. 2008. Lineage diversification and historical demography of a sky island salamander, *Plethodon ouachitae*, from the Interior Highlands. *Molecular Ecology.* 17: 5325-5335.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science.* 236: 787-792.

Somanathan, H., Borges, R. M., and Chakravarthy, S. 2004. Does neighborhood floral display matter? Fruit set in Carpenter Bee-pollinated *Heterophragma quadriloculare* and beetle-pollinated *Lasiosiphon ericephalus.* 36: 129-147.

Soons, M. B. and Bullock, J. M. 2008. Non-random seed abscission, long-distance wind dispersal and plant migration rates. *Journal of Ecology*. 96: 581-590.

Takezaki, N., Nei, M., Tamura, K. 2010. POPTREE2: Software for constructing population trees from allele frequency data and computing other population statistics with Windows-interface. *Molecular Biology and Evolution*. 27: 747-752.

Thien, L. B. 1974. Floral biology of *Magnolia*. *American Journal of Botany.*61:1037-1045.

Thomas, B. R., Macdonald, S. E., Hicks, M., Adams, D. L., and Hodgetts, R. B. 1999. Effects of reforestation methods on genetic diversity of lodgepole pine: an assessment using microsatellite and randomly amplified polymorphic DNA markers. *Theoretical Applications of Genetics.* 1999. 793-801.

Tomimatsu, H. and Ohara, M. 2003. Genetic diversity and local population structure of fragmented populations of *Trillium camschatcense* (Trilliaceae). *Biological Conservation*. 109: 249-258.

Trapnell, D. W., Schmidt, J. P., Quintana-Áscencio, P. F., and Hamrick, J. L. 2007. Genetic insights into the biogeography of the southeastern North American endemic, *Ceratiola ericoides* (Empetraceae). *Journal of Heredity.* 98:587-593.

Vellend, M., and Geber, M. A. 2005. Connections between species diversity and genetic diversity. Ecology Letters 8: 767-781.

Wickham, H. ggplot2: elegant graphics for data analysis. Springer New York, 2009.

Wilson, G.A., and Rannala B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics.* 163: 1177-1191.

Webb III, T. and Bartlein, P.J. 1992. Global changes during the last 3 million years: Climatic controls and biotic responses. *Annual Review of Ecology and Systematics.*23: 141-173.

APPENDIX OF FIGURES

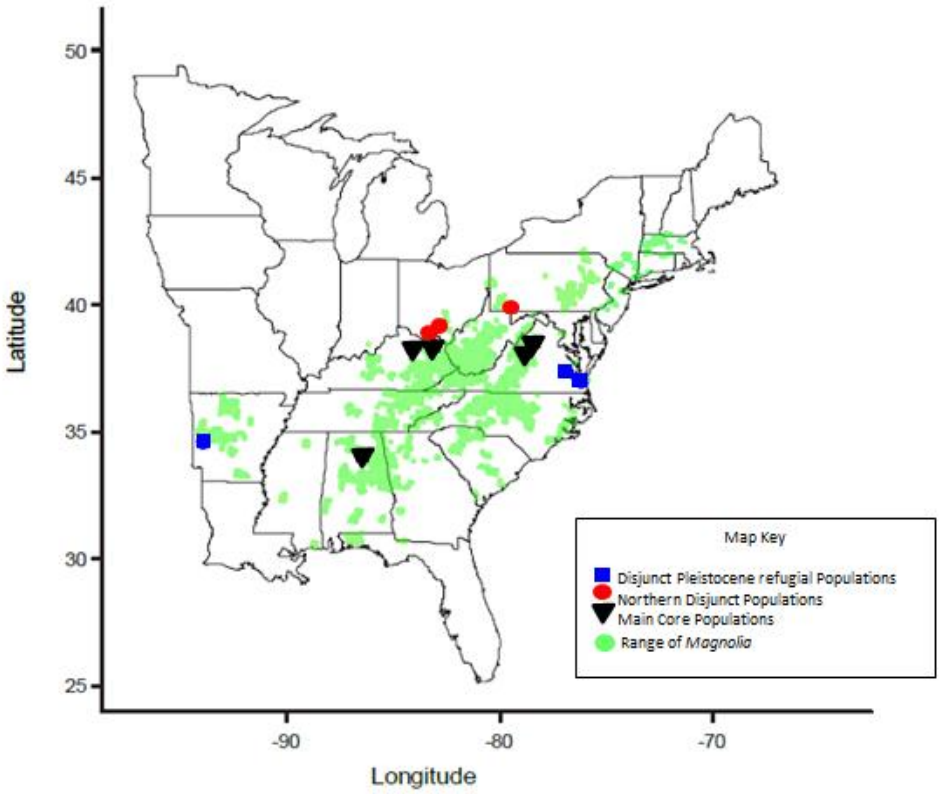

Figure 1: Range map of *Magnolia tripetala*. The colored shapes denote sampling locations.

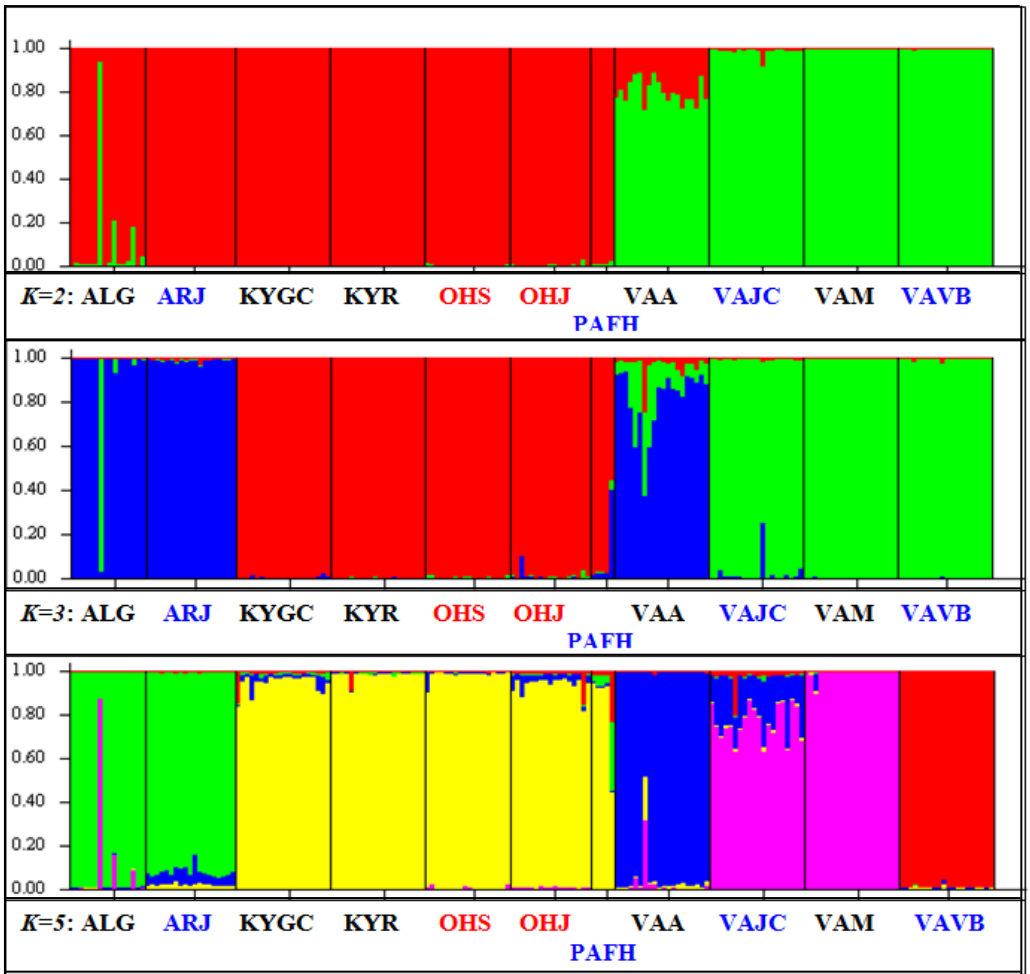

Figure 2: K=2, K=3, and K=5 barplots constructed in STRUCTURE. Populations of *Magnolia tripetala* that have been grouped will have the same color. Pleistocene refugial populations (ARJ, VAJC, and VAVB) have been labeled in blue. Main core population (ALG, KYGC, KYR, VAA, and, VAM) have been labeled in black. Northern disjunct populations (OHJ, OHS, and PAFH) have been labeled in red.

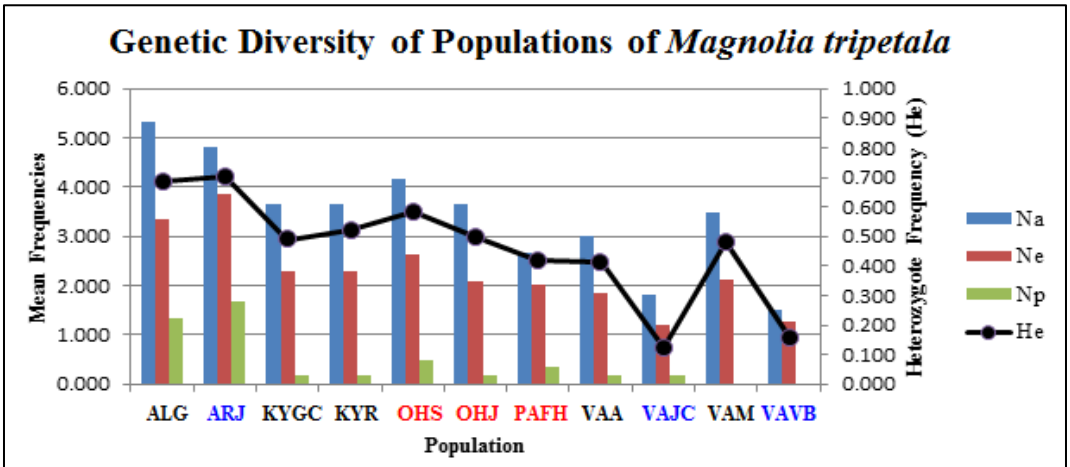

Figure 3: Genetic diversity of each population of *M. tripetala* as defined by four major parameters: the number of different alleles belonging to a population (Na), the number of effective alleles belonging to a population (Ne), the number of private alleles belonging to a population (Np), and the number of expected heterozygotes for each population. Pleistocene refugial populations (ARJ, VAJC, and VAVB) have been labeled in blue. Main core population (ALG, KYGC, KYR, VAA, and, VAM) have been labeled in black. Northern disjunct populations (OHJ, OHS, and PAFH) have been labeled in red.

Table 1: F_{IS} values per locus calculated in GenePop for populations of *Magnolia tripetala*.

	M10D8	M15D5	M6D1	M6D3	M6D4	M6D8	Chi^2	D.f.	P-value
ALG	0.0596	0.4101	0.0863	*-0.1798*	0.0769	1.0000	Infinity	12.000	**<0.01**
ARJ	0.6667	0.0769	0.2755	0.2174	0.0204	1.0000	Infinity	12.000	**<0.01**
KYGC	0.4783	*-0.4927*	1.0000	0.1237	0.3298	-	Infinity	10.000	**<0.01**
KYR	0.8348	*-0.9000*	0.6320	0.0142	*-0.1132*	1.0000	Infinity	12.000	**<0.01**
OHJ	0.3486	*-0.1712*	0.5579	0.3379	0.3905	1.0000	Infinity	12.000	**<0.01**
OHS	*-0.2632*	0.0769	0.6364	0.1860	*-0.0112*	1.0000	Infinity	12.000	**<0.01**
PAFH	-	*-0.3333*	1.0000	-0.0001	*-0.1429*	1.0000	18.475	10.000	**0.0475**
VAA	0.3091	*-0.0588*	0.6381	0.5210	*-0.0556*	1.0000	Infinity	12.000	**<0.01**
VAJC	-	-	1.0000	0.4648	0.6647	-	25.485	6.0000	**0.0003**
VAM	0.3955	*-0.1565*	0.5598	0.349	0.0236	1.0000	52.443	12.000	**0.0000**
VAVB	-	-	0.1047	-	1.0556	1.0000	Infinity	102.00	**<0.01**
Chi^2	56.472	97.172	Infinity	61.335	76.882	Infinity			
D.f.	16.000	18.000	22.000	20.000	22.000	18.000			
P-value	**0.0000**	**0.0000**	**<0.001**	**0.0000**	**0.0000**	**<0.001**			

Notes: F_{IS} values represent the amount of inbreeding occurring in each population over the six loci used for this study. Values closer to 0 indicate random mating, whereas values closer to 1 indicate high amounts of inbreeding. The bolded numbers represent significant P-values signifying that a population or locus is out of Hardy-Weinberg-Equilibrium. The italicized numbers represent outbreeding occurring. Pleistocene refugial populations (ARJ, VAJC, and VAVB) have been labeled in blue. Main core population (ALG, KYGC, KYR, VAA, and, VAM) have been labeled in black. Northern disjunct populations (OHJ, OHS, and PAFH) have been labeled in red.

Table 2: Pair-wise F_{ST} values as calculated by GenAlEx per population of *Magnolia tripetala*

	ALG	ARJ	KYGC	KYR	OHJ	OHS	PAFH	VAA	VAJC	VAM	VAVB
ALG	0.000										
ARJ	0.148	0.000									
KYGC	0.243	0.135	0.000								
KYR	0.227	0.137	**0.072**	0.000							
OHJ	0.179	0.152	0.162	**0.092**	0.000						
OHS	0.241	0.183	0.132	0.124	0.166	0.000					
PAFH	0.283	0.216	0.151	0.202	0.237	0.214	0.000				
VAA	0.245	0.187	0.243	0.250	0.228	0.270	0.329	0.000			
VAJC	0.385	0.393	0.499	0.415	0.436	0.520	0.603	0.523	0.000		
VAM	0.237	0.206	0.207	0.229	0.242	0.240	0.282	0.205	0.323	0.000	
VAVB	0.384	0.311	0.293	0.282	0.352	0.380	0.441	0.391	0.665	0.361	0.000

Notes: Values indicate amount of genetic differentiation between populations. Values closer to one indicate high levels of genetic differentiation. Bolded values indicate low levels of genetic differentiation. Pleistocene refugial populations (ARJ, VAJC, and VAVB) have been labeled in blue. Main core population (ALG, KYGC, KYR, VAA, and, VAM) have been labeled in black. Northern disjunct populations (OHJ, OHS, and PAFH) have been labeled in red.

Table 3: Bayesian assignment values calculated by BayesAss v3.0.3 to show gene flow occurring between populations of *Magnolia tripetala*

`	ALG	ARJ	KYGC	KYR	OHJ	OHS	PAFH	VAA	VAJC	VAM	VAVB
ALG	**25.54**	1.070	1.058	1.088	1.127	1.035	1.039	1.050	1.082	1.045	1.029
ARJ	0.979	**12.69**	1.014	1.077	0.850	1.020	1.040	1.290	0.940	1.025	1.061
KYGC	1.019	1.028	**55.70**	**6.847**	0.950	1.018	1.058	1.135	0.991	1.075	0.991
KYR	1.034	1.033	1.065	**27.46**	1.056	1.051	1.052	0.980	1.016	1.058	1.036
OHJ	1.072	1.052	0.986	**6.580**	**52.76**	1.107	1.085	1.060	1.079	1.007	1.071
OHS	1.087	1.050	1.090	**6.025**	1.067	**48.37**	1.014	1.045	1.037	1.000	1.103
PAFH	1.106	0.970	0.917	1.639	1.014	1.043	**28.06**	1.065	1.040	1.018	0.989
VAA	1.035	1.012	1.037	0.930	0.963	1.074	1.117	**48.36**	1.053	**5.019**	0.985
VAJC	1.096	1.035	1.076	1.090	1.077	0.858	0.922	1.090	**35.97**	1.465	1.029
VAM	1.055	1.068	1.028	1.083	1.013	1.034	1.092	1.063	1.000	**22.99**	1.093
VAVB	1.143	1.006	1.051	1.007	0.841	1.046	1.056	1.070	1.077	1.627	**23.13**

Notes: Gene flow is represented by migrations between populations. Migrations occur via the dispersal of individuals from populations listed at the left of the table into populations listed above the table. High levels of gene flow are bolded. Pleistocene refugial populations (ARJ, VAJC, and VAVB) have been labeled in blue. Main core population (ALG, KYGC, KYR, VAA, and, VAM) have been labeled in black. Northern disjunct populations (OHJ, OHS, and PAFH) have been labeled in red.

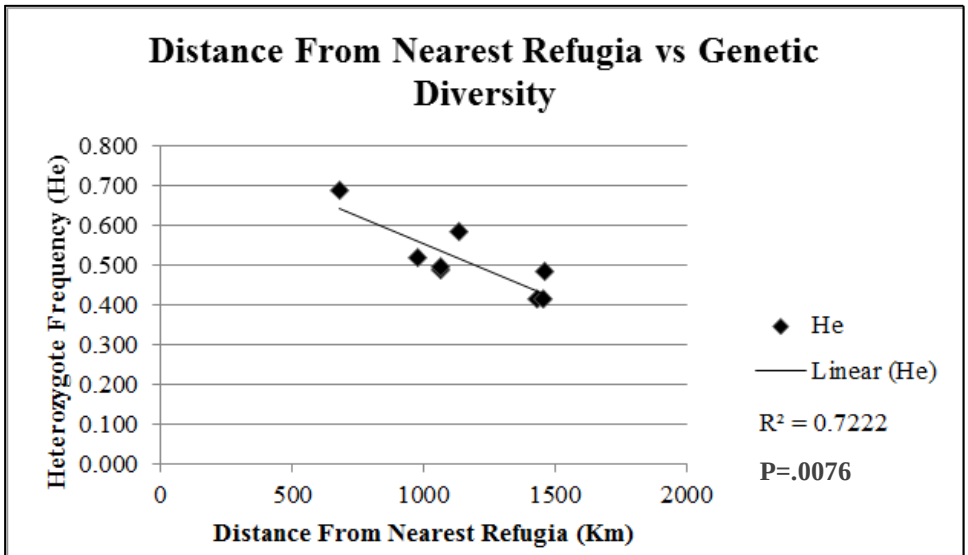

Figure 4: The expected number of heterozygotes in *Magnolia tripetala* populations plotted against the distance from the nearest Pleistocene population of *M. tripetala.*

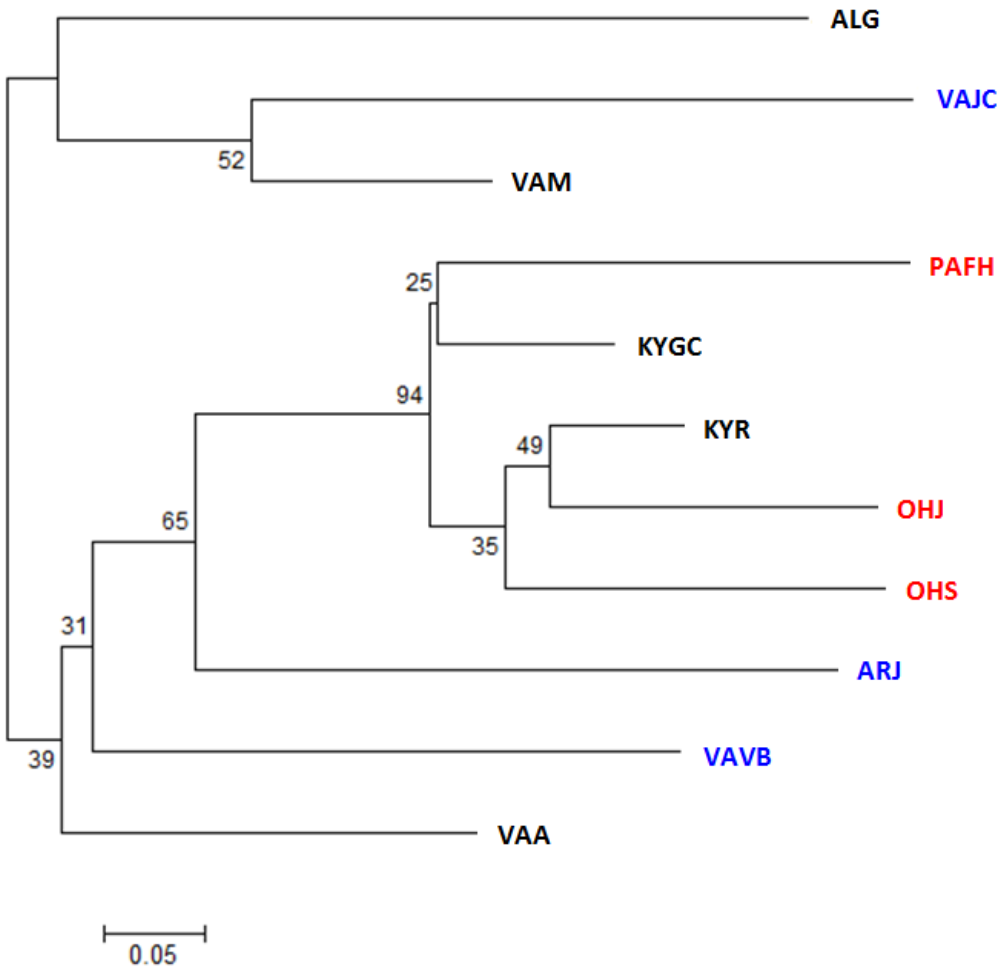

Figure 5: An un-rooted Neighbor Joining tree created in PopTree. Populations on the same branch are more likely related to other populations. Pleistocene refugial populations (ARJ, VAJC, and VAVB) have been labeled in blue. Main core populations (ALG, KYGC, KYR, VAA, and VAM) have been labeled in black. Northern disjunct populations (OHJ, OHS, and PAFH) have been labeled in red.