Genetic Variability in *Magnolia acuminata* (L.) Populations in the United States

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Introduction

Magnolia acuminata (**Figure 1**) has extremely variable morphological characteristics, particularly differing in pubescences, petals, base structure, and petiole anatomy (Hardin 1954). In addition, the morphology varies with geographical location. This study examines the genetic variability and diversity within *Magnolia acuminata* to determine whether these factors are reflected in genetic diversity of samples from the localities as demonstrated by **Figure 2** and listed, by sample, **in Table 1**.

Sample Number	Locality of Population
1-5	Patrick County, VA
6-10	Hocking County, OH
11-13	Florida
14-16	Colbert County, AL
17	Tuscaloosa County, AL
18-22	Pocahontas County, WV
23	Stoddard County, MO
24	Clarks Hill, SC
25-29	Pickens County, SC
30-32	Cultivar
33-35	Newberry, SC
36-37	Little Mountain, SC
38-42	Baldwinsville, NY
43-46	Rabun County, GA
47-48	Macon County, NC
49-53	Swain Country, NC
54-58	Rabun County, GA
59-63	Jackson County, AL
64-68	Saline, AR
69-73	Polk, AR
74-78	Newton, AR
79-83	Columbia, LA

Table 1. Sample number and locality with in the eastern United States

Figure 2. Localities of sampled individuals as demarcated with yellow markers http://www.hist-geo.co.uk/usa/50-states.php

Methods and Materials

Previously, the 84 individual samples were collected from locations as pictured in **Figure 2** by Magnolia Society members. The DNA was extracted with Qiagen DNeasy® kits. Primers were first optimized to obtain a functional annealing temperature. Then, polymerase chain reactions using NH_4 10x buffer, $MgCl_2$ buffer, dNTPs, forward and reverse primers, Biolase TAQ®, and BSA were performed as listed in **Table 2.** After the PCR had been performed, 3 µl of 3x loading dye was mixed with 2 µl of DNA template from the PCR product. To assess DNA sequence size and quality, these products were run on a 1.5% aragose gel stained with ethidium bromide. In the chloroplast spacer regions (psbk-F,R; atpf-F,R; and ndhf-F/rpl32-R), the products were Exo-Sapped in order to eliminate primers, the single stranded DNAs used to initiate replication. A cycle sequencing reaction was performed in order to replicate the DNA in only one direction through the use of a single primer with each DNA template strand by using .5 µl ABI Big Dye®, 1 µM forward or reverse primer, 1.75 µl 5x sequencing buffer, and 3.75 µl of Dnase free water. This product was run through hydrated sephadex plates to purify the sample and was sequenced on a 3730xl DNA Analyzer. The sequences were aligned with Geneious® software.

For microsatellite region stm200, the PCR product was diluted 1:10 with Dnase free water. Rox was diluted 1:10 with formamide and 10 µl of this mixture was added to 1 µl of the diluted PCR product. The plate was then submitted for fragment analysis. The chromatograms were analyzed with GeneMapper® software.

Table 2. The components added to each polymerase chain reaction in chloroplast regions and microsatellite regions.

Component	Chloroplast Spacer Regions (psbk (F,R); atpf (F,R); ndhf-F, rpl32-R) (µL)	Microsatellite stmo 200 (µL)
10x Bioline® ammonium buffer	2.0	1.0
10x dNTP's (10 µM)	0.8	0.5
MgCl2 (25 µM)	1.0	0.5
Forward Primer	1.0	0.04
Reverse Primer	1.0	0.4
BSA	0.0	0.5
Dnase free sterile water	14.8	6.84
TAQ® Enzyme	0.2	0.02
M13 Labeled Primer	0.0	0.4
Template DNA	2.0	1.0

Figure 1. *Magnolia acuminata* Arboretum Freiburg-Günterstal. Accessed July 23, 2010. <http://hubertus-nimsch.de/pages/arbor_magnolien-tal.htm>.

Chloroplast Spacer Regions' Results and Discussion

• Chloroplast spacer regions are non-coding regions in the genome of the chloroplast

• Since spacer regions have no known function, they often exemplify evolution and can show how populations are related

• Three regions: psbk, atpf, and ndhf/rpL32(demarcated in **Figure 3**) were sequenced and assessed for variation

• There was no evident variability in these three regions

• Consensus sequences for ndhf/rpL32, atpf, and psbk are shown in **Table 3**

• Lack of geographical variability in chloroplast spacers may suggest a slow rate of evolution in these regions of the genome or that alleles are commonly shared between all the populations, making them non-divergent

• Chloroplast markers did not show variability between populations, therefore microsatellites were assessed

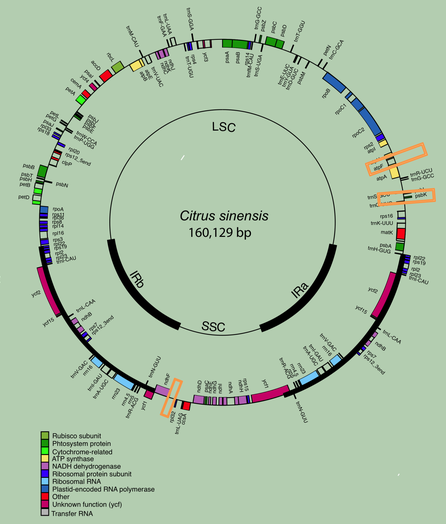

Figure 3. The Chloroplast genome of *Citrus sinensis* with markers at the three DNA regions sequenced in *Magnolia acuminata*: ndhf/rpl32, psbk, and atpF Bausher *et al.* 2006. BMC Plant Biology. 6:21.

Table 3. Consensus sequences of chloroplast spacers as shown in schematic above

Chloroplast Spacer Region	Consensus Sequence
ndhf/rpL32	AACTTTTTTTATTCTTATTAATTGTTTCCGATTCACCAGCTCTTCTCTCTTTCGGAAGTCAAATAAATAAATAAAAATCAAGATAGAAAAGAACTCAAATAAGATTTTTAATTCTTAATTATTCCGATTCTTTCCCAAATATCGTATTGAATAAAAAAGAAATTTAAATCAAGAAGTTACAATTGTTTAAATGACCAAGTCACTGGTTAAAACTGACCATTTAGTTATTAACTAAGATATTTATTAAGATAAAGAAAGAATATGAGATTTTCAATCATTCCACTATTACGGCGATTGATATCCATATAGTAAATAGTAAGGAAAAGGAATGACACCAAAAAAAGGTAAAAGTACTCTATTGGGATATGGATCATAGAATCCGCCAATAACTCGACCCCAATAAAATACTAGTTATTTTCGTTAGTTTATTCGGAGTCATTAATTAATTCATTGTAGAACTGATTGATTGGCTTCTATTCCAATAAAACAAACTTATGTTTATAGGAAATCCTATGATACTCGTCACTTCGCATAGAACTGAAATAAGAGATTACTAAAATTACCTTTATCAAGTAATGTATGGTTTAACAGATTAACTACCAATCTCATTTTCATTTAAATTATGAGTACTCTATCCTCGAGCTTGATCAATTAATAGAAAATTTTAAATTATTATTTTCTTAAATTAGGTAAGGATTCTTAAGCTTTTCGATTTATTCAATGTAAGACGACGAGATATAAATAGACTGAGATTGAGATATGAATTGGAACCCTTTTTGATTCTTATCAACAACAACCGGTTCGATTTCTATGGTAGCGGACCTCATAGACATAGATCGGAATGAAGATATGAAGGTACAAATTAGTACGAATTCTTCTTTCTTCGGGGCATTGTATGTAATAGAGATGTGGAACAAAAAAAAATTCCTTTGAAAATAACATCGTTTTTCTTTTTTCTATTTCTTTTTTTATCCCTAGTGTACTCTATGTGATGCGCACGTATCTATATTTTTGTATGTTATGAAGGAAGTATCCGCTATGGAATAAATATAGATAATGAATAGCAAGAACGATCTATTTTGAACAATACATGTCTTTCACATCCAACTATAAAAGTAACTTCTTTATTTTCAAATGGCGGTTCCAAAGAAAC
atpf	ATTTATTAGATTTGTTGCTAAAATATCGGTATTCAACCCGAAACTCCCGGCGGATGGCCAGTAACCCAAGGAAACGAAAGAATCGGTTACATTTTTCATATGCTCTCCTCTTATAGATAGGACTAACAAAATCGAACAGAGTTCTTTTTGTATCACTTCGTACCGTTTTTTTATTATTGATTTCTTTTTTTTTTATTAATGACTTATTTTAAAATATGAATATATTCATTTCATTTGAAATCATTTTCAAATTTCAAAAATGGATTCTTTATTATTATTTTATTTCAATTGAAGTTCCCAATAAGATACTTATTAGGTCCCCGGTTTCATGTCAATTGCGAAATACCTGCAACGCTTCCTAAAAGTCAAAAGGGGTTTCCATTAAATTAAGGACGGGAAGTGAGAAAGCGAGTGGATCTACTAATTCCTCATCCTCAAATCAGGCCTTCCCCGGAGTATTGTCTCAACGAAGAAGTGGAGTGAAGTTTTGATATAATTCGAAGAAGCAAGCGGTAAGTCGACGGCAATAAAATAAGAAAAGAAGTACGTATTTTCACGTTTATAGAATAGGATTAAACAAAAGGAT
psbk	AGTTTGAGAGTAAGCATTACACAATCTCCAAGATCATTTTTGGGGGAAATAAGGGAATAGATTCTCTATTTTTGTACCACATATCCCATTTTGACACCAAGAAATGGAGTGGTTTCTAGAAAAGAAAGGAATTTGCAGGAATTCATTTGTAATAAGATTCTGATTCCTTCGTTACCAAAATGATCTTTCATACCCACAATTAGGTATTGTGAGGGACCATACATAAGGTCTTTGACCTCCGGAAAGTCAGAATGAGAAAATGAGGTGATCCAGATTCATCGCGGCTATCCAAAAGAATTTCAATGTTTGAATCGAGAGTTCATAATGTAAGATTTATCTGATCTTATCAATTGTTAGAATAGAATTTTTCCTTTTTTAGCGAATCAATCATGAATGTTTCTAGGACAGTATT

Microsatellite Results and Discussion

• Microsatellites, also known as single sequence repeats, are small regions with repeating nucleotide bases (ATATATAT…) and often show high variability because they are easily mutated during DNA replication

• Primer pair stm200 amplifies a region that repeats:$(CT)_{13}(TC)_{11}$ with sizes ranging from 167 to 211 base pairs (Setsuko et al. 2005)

• In sister species, *Magnolia stellata,* stm200 contained 11 alleles while this study found 14 in *Magnolia acuminata* mapssite.blogspot.com

• **Figure 4** shows the allele frequency in each of the sampled populations

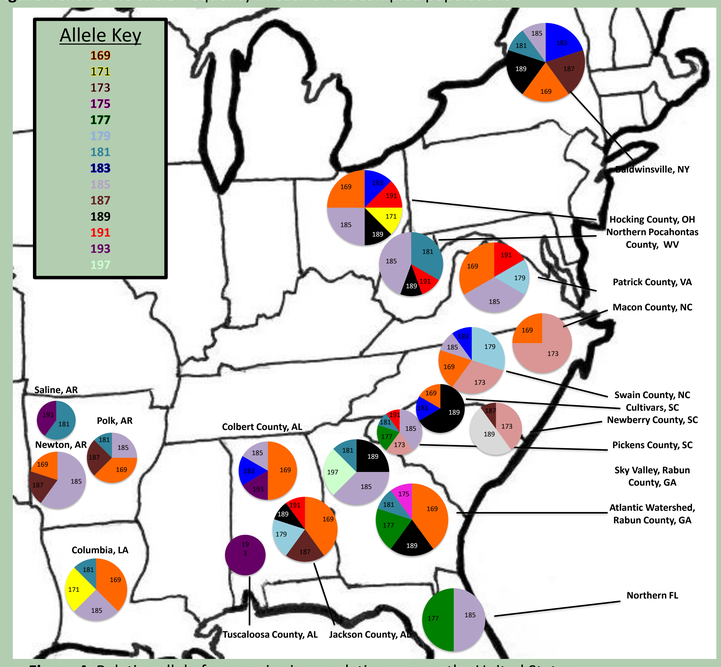

Figure 4. Relative allele frequencies in populations across the United States map from: mapssite.blogspot.com

• Some alleles showed widespread prevalence through all populations such as allele 169, 181, and 185
• Allele 177 was only found in the southern states of South Carolina, Georgia, and Florida
• Allele 173 is only present in North Carolina and South Carolina
• Alleles 191 and 179 are only present in the populations along the east coast states
• Allele 193 only appears in western populations in Alabama and Arkansas
• Heterozygous and homozygous individuals were observed in most of the populations; homozygous individuals allele's were counted as two alleles when calculating the frequencies

Further Research

In future work, more microsatellite (stm#) primers, derived from *Magnolia stellata,* need to be optimized and tested on the population samples for *Magnolia acuminata*. A larger subset of regions would add more support for population genetics hypotheses about gene flow and geographic structuring. Furthermore, successfully sequencing nuclear regions such as ITS 1 and 2 may also provide support for population genetics of *Magnolia acuminata.*

Acknowledgments

I would like to thank Cristién Samper for the funding for the NHRE program, Dr. Elizabeth Zimmer for her guidance and support, and Gabriel Johnson for his insights and technical expertise. I would also like to thank the Magnolia Society for partial funding for this project and Richard Figlar for orchestrating the collection of the plant samples. Finally, I would like to thank for Virginia Power, Gene Hunt, and Liz Cottrell for their guidance and supervision over the NHRE program.

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