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GENETIC COMPARISON OF *Castanea dentata* AND *Castanea mollissima* USING RAPD ANALYSIS

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Reviewed and approved* by the following:

Sairam Rudrabhatla
Associate Professor of Biology
Thesis Supervisor
Honors Advisor

Thomas Eberlein
Associate Professor of Chemistry
Faculty Reader

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Castanea dentata, the American chestnut, is all but extinct thanks to a fungal predator brought to America by the importation of *C. mollissima* (the Chinese chestnut). *Cryphonectria parasitica* ravaged the *C. dentata* population; yet, it seemingly had no effect on the *C. mollissima* plants. This study was designed to look at the genetic diversity between American and Chinese chestnut using Random Amplified Polymorphic DNA markers employing 13 different primers. The resulting amplified DNA bands checked on agarose gels were analyzed for similarities and differences based on common band size. The results indicate distinct bands mainly around 700 and 1200 base pairs that only existed in the Chinese chestnut species. Future studies will look closely into the relevance of these differential bands and their possible a role in the development of fungal disease resistance, particularly against *C. parasitica*.

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Chapter 1: Introduction

Castanea dentata, or the American chestnut, was once a thriving tree in the United States but has since been wiped out by a fungal parasite, *Cryphonectria parasitica*, which was brought over by its Asian cousins. The American chestnut is now virtually unrecognizable from its past self, which stately stood over 100 feet tall.

The chestnut blight disease was discovered in the year 1904; within a span of 50 years, the majority of the chestnut trees on the Eastern Seaboard had been infected with *C. parasitica*. The source of the fungal parasite was discovered to be *Castanea mollissima*, the Chinese chestnut, which was brought over to the Bronx in the early 20th century. Since the onset of the chestnut blight, there have been no discoveries on how to stop the spread of the disease or how to treat it.

1.1. *Castanea dentata*: A Once Great Tree Torn Apart by a Fungal Blight Disease

Castanea dentata was once a fast-growing hardwood species that made up nearly a quarter of the trees found from Maine to Georgia. Due to the fungal blight, *C. dentata* is now found as a small shrub that is sprouting from the remains of its great ancestors.

1.1.1. Classification and Distribution of American Chestnut trees

The Global Biodiversity Information Facility (2012) classifies *C. dentata* as Kingdom Plantae, Phylum Magnoliophyta, Class Magnoliopsida, Order Fagales, and Family Fagaceae. The Fagaceae family contains as many as 900 members. These include

Quercus alba L (White Oak) and the *Fagus sylvatica* variety 'Pendula' (Weeping European Beech).

The genus is *Castanea* and the species is *dentata*. There are nine total accepted species in the genus *Castanea.* Some examples include *Castanea sativa* or the sweet chestnut, *Castanea henryi* or the Chinese chinquapin, and the *Castanea mollissima* or the Chinese chestnut. The plants in the genus *Castanea* can grow in a multitude of soil conditions and can thrive on ground that is nutritionally deficient. It is drought tolerant and can grow in areas with little to no shade. *C. dentata* can be found in the northern and eastern regions of the United States, with a range from Maine down to Georgia and Arkansas (PFAF, 2012).

1.1.2. Applications of C. dentata

Before the blight lessened it, *C. dentata* was a great source of revenue for the economy in North America. For example, the leather industry used it as a main source of its tannin (Saucier, 1973). The husks surrounding the seeds as well as the leaves produced a high level of tannin, thirteen percent and nine percent respectively. Additionally, a brown dye was produced from the bark of the tree (PFAF, 2012).

Lumber from the tree itself was very widely used in general construction and the manufacture of goods. It was a main source of wood for telephone poles, plywood, roofing shingles, and caskets (Saucier, 1973). The wood was very soft, easy to split, and durable, which made it the ideal source of firewood and fuel.

C. dentata produces edible nuts that were eaten either raw or cooked. The seed composition is seven percent fat and eleven percent protein. It was used as a powder that was added to various baked goods; it was also processed to obtain oil (PFAF, 2012). Before the occurrence of the blight, the seeds were produced in large quantities and its demand provided a great economic boost to the orchards producing them (Saucier, 1973).

1.1.3. Chestnut Fungal Blight Disease

As mentioned earlier, *C. dentata,* once a magnificent tree reaching heights of nearly 100 feed with a seven foot girth, was reduced in the twentieth century to a small shrub sprouting from the leftover stubs of diseased trees. The fungal parasite *Cryphonectria parasitica*, which was brought to the United States by the importation of Asian chestnut trees, ultimately claimed three to four billion mature *C. dentata* trees. (Newhouse, 1990).

Herman W. Merkel, from the New York Zoological Park located in the Bronx, first noticed the blight of the chestnuts in 1904. He observed necrotic depressions on the trees in the park. Within a year of discovery, many of the young trees had succumbed to the disease and many more were infected with the fungus only to die in the next decade. William A. Murrill, who was from the New York Botanical Gardens, later discovered that the fungus invaded the plant through openings in its bark tissue (Newhouse, 1990).

C. parasitica was able to spread with relative ease. By the 1950s, the entire natural habitat of *C. dentata* was infected. The fungus has the ability to live on even after its host dies. It was able to attach to nearby tree species as a parasite, albeit a weak one, or as a saprophyte on its deceased host (Newhouse, 1990).

At first, investigators were not sure how *C. parasitica* was invading the *C. dentata* population. They quickly discovered a few developments. In 1938, nurseries in Italy noticed the same fungus that was attacking *C. dentata* was living on their *Castanea sativa (*European chestnut trees). These nurseries were important because, a decade earlier, *Castenea crenata* (Japanese chestnut trees) were established in the area (Newhouse, 1990).

1.2. Goals and Objectives

The goal of my research was to compare the American and Chinese chestnuts (*C. dentata* and *C. mollissima* species respectively) on a genetic and molecular level. The objectives put in place to achieve this goal were to perform Random Amplification of Polymorphic DNA (RAPD) in both American chestnut and Chinese chestnut species. This was accomplished by extracting the genomic DNA of three samples each of both tree species using and performing a polymerase chain reaction (PCR) employing 15 different primers that were 10 base pairs long. During a PCR reaction, the genomic DNA (template DNA) was mixed with nucleotide base pairs (adenine, guanine, cytosine, and thymine); primers (short segments of DNA that can attach to the genomic DNA based on nucleotide base pair complementation); buffer; $MgCl_2$; and DNA polymerase. The entire mixture was alternately heated and cooled to create multiple copies of the DNA. The RAPD protocol varies greatly with each experiment, and it takes a lot of trial and error to get the optimized conditions to run a PCR. The resulting amplified DNA segments represent the presence of complementary DNA present in the genomic DNA used in a particular tree sample. Based on the presence or absence of the amplified DNA, it is possible to make a

judgment whether or not two samples are related to each other. Amplification of similar size DNA using same primers in a PCR reaction (albeit using two different genomic DNA samples from two different trees) means that they are similar to each other to a certain extent. This similarity reflects that both these trees have some common regions in their genomic DNA.

The following chapter features a review of previous research completed using RAPD analysis and both chestnut species.

Chapter 2: Literature Review

2.1. Current Chestnut Research

Fungal isolates from America, Japan, China, and Europe were compared for similarities. Tests showed a resemblance in both virulence and morphology in all species. It was also documented that *Castanea* species were established in China and Japan long before other parts of the world (Newhouse, 1990).

2.1.1. Cryphonectria parasitica: Cause of American Chestnut Blight

Previous studies explored the different responses of the two different chestnut species with regards to the chestnut blight. Barakat *et al.* (2009) performed a transcriptome analysis of both American and Chinese chestnuts on healthy stem tissue as well as tissue that was infected with the *C. parasitica* fungus. Through the 250 million base pairs that were generated, they were able to isolate between 30-40 thousand unigenes (similar genes) for each species. The genes are currently known to be present in biological processes - many of which are used in defense against various pathogens. They concluded that some of the genes isolated may be responsible for providing the resistance to the fungal blight in the Chinese chestnut tree.

Barakat (2012) and his group analyzed the genes, and they were able to identify the defense related genes in the infected tissue and the healthy tissue. These genes were activated in response to salicylic acid, ethylene, abscissic acid, etc. The genes identified in this response were determined to be a candidate for the resistance to the *C. parasitica.*

Studies were also done on the fungal parasite that causes the chestnut blight, *C. parasitica*. Rigling and van Alfen (1993) worked on two fungal strains: one dsRNA free and one infected by the blight fungus. They worked to clarify the laccase production by *C. parasitica*. A liquid fungal culture was grown and homogenized and was put through a filtered culture which determined the laccase activity. They found that the infected strain had a greater laccase production than the non-virulent strain.

2.1.2. Plant Growth Regulators

William Cooper and Lynne Rieske (2008) worked with jasmonic acid to see if it affected the level of tannin in the leaves and stems of both the American and Chinese chestnut. The jasmonic acid was added to the leaves of the chestnut species before the tannins were extracted and purified. The extracted tannins were analyzed, which revealed that the jasmonic acid had an increased effect on the American chestnut. It was observed that there were increased levels of tannin in the tissue treated with the jasmonic acid only in the American chestnut. The Chinese chestnut had no change.

D' Amico, Horton, Maynard, and Powell (2011) tested oxalate oxidase gene transgenic American chestnut trees to see if a difference existed in ectomycorrhizal associations when compared with wild type American chestnut plants. Each type of plants was grown in soil to bait the fungus. Their root tips were harvested and their RNA was compared on a molecular level. Researchers found that there was an expression of oxalate oxidase in the transgenic plants but not in the wild type plants. However, there was no difference in the mycorrhizal associations formed.

2.1.3. Chestnut Breeding Studies

The morphological traits of American and Chinese chestnuts were examined in crosses of the two species. Hubard (1994) cross pollinated American and Chinese chestnut trees. On the mature crossed plants, the twig hairs' stem color and the stipule (the hair like structures on the stems of plants) size was analyzed. He found that the twig hairs on the Chinese chestnuts were found to be controlled by two dominate genes. As well, the red stem color was found to be caused by two complementary genes and the stipule size on both the species appeared to be caused by the same gene. The differences in each species are due to different dominances between the two species.

2.1.4. Analysis of the Random Amplification of Polymorphic DNA

Molecular markers are sequences of DNA that can be easily detected and passed onto future generations and are based on the polymorphisms that occur naturally between closely related living organisms, including trees. A good molecular marker is codominate, which is frequently occurring and highly reproducible. Restriction Fragment Length Polymorphism (RFLP) is another type of a molecular marker. In RFLP analysis, the DNA sample is broken into fragments (digested) by restriction enzymes and the resulting restriction fragments are separated according to their lengths by gel electrophoresis. The RFLP exploits the variations in homologous DNA sequences. It refers to differences between samples of homologous DNA molecules that come from differing locations of restriction enzyme sites. The resulting DNA fragments could be illustrated by gel electrophoresis. The RFLPs are highly reproducible, but a large quantity of purified DNA is required for each analysis; therefore, it is not a commonly used

molecular marker. The RAPD is a PCR-based molecular marker with the main advantages of being quick to use and only requires a small, nanogram amounts of DNA sample per reaction. RAPDs, however, have a low reproducibility and must have a highly standardized procedure (Kumar, Gupta, and Misra *et al.* 2009).

In their experiment analyzing the genetic difference in *Sphaeropsis sapmea* isolates, Wu, Xiong, and Wang (2007) detail the RAPD protocol. Cluster analysis split isolates into three groups: Chilean, which was separated from the rest of the group the most; Chinese; and 20 most similar samples comprising of the American, Chinese, and South African species. These differences point to genetic differences among isolates originating from different countries.

Goulão, Valdiviesso, Santana, *et al.* (2001) used the RAPD PCR protocol to compare the phenotypic characterization of cultivated chestnut. Their results show a 90.5% similarity between samples that were phenotypically alike. However, the UPGMA showed differences in sample clusters.

Huang, Dane, and Kubisiak (1998) analyzed the genetic diversity of wild populations of American chestnut using RAPDs. The RAPD analysis grouped chestnuts into four groups: Southern, South-Central, North-Central Appalachian, and North Appalachian mountains. Due to the results shown, a conservation strategy was developed.

The RAPD analysis of *Juniperus* that was completed by Kasaian, Behraven, and Hassany (2011) indicated that two different species, *J. communis* and *J. oblonga* had a similarity that ranged from .19 to .69 from other species. Subspecies exceisa and polycarpos were most similar and formed their own grouping.

Hepsibha, Premalakshmi, and Sekar, (2010) who completed the analysis of the genetic diversity of *Azima tetracantha,* showed a wide genetic variation that matches the level of diversity within *A. tetracantha*.

Chapter 3: Materials And Methods

3.1. Plant Material

The *C. dentata* and *C. mollissima* samples used during this experiment were obtained from mature trees grown at Pine Grove Farms in Central Pennsylvania.

3.2. Molecular Analysis

A RAPD analysis was chosen for this experiment due to its short reaction time and the low amount of genomic DNA needed. A standardized procedure was already in place when experiments began. The following section outlines the RAPD process used.

3.2.1. DNA Extraction

The plant material from all five samples (Table 1) was placed in a 1.5 ml microcentrifuge tube. All the samples used were first frozen in liquid nitrogen and stored in a freezer set at 80°C. The plant material was crushed into a fine powder using a sterile pestle; extra care was taken to make sure the plant material remained frozen to avoid the degradation of the genomic DNA. Then, 300 µl of DNAzol was added to the microcentrifuge tube which was mixed well with the powdered plant material. The tubes were then centrifuged at 14000 RPM for 10 minutes.

The supernatant from the tubes was transferred to a new 1.5 ml microcentrifuge tube that was then mixed with 225 µl of 100% ethanol. The tube was allowed to sit at 25ºC for five

minutes and was then centrifuged at 5000 RPM for five minutes. The supernatant was then removed, reserving the pellet.

At this point, 300 µl of DNAzol wash (.25 ml DNAzol to 1 ml of 100% ethanol) was added to the pellet. The tube was vortexed for a few seconds to mix the tube, then it was centrifuged at 5000 RPM for four minutes.

The supernatant was then removed without disturbing the pellet and 300 µl of 75% ethanol was added to the tube. After 30 seconds of vortexing, the tube was centrifuged at 5000 RPM for four minutes. This step was repeated one more time. The tubes were then taken into a sterile laminar flow hood to allow for the excess ethanol to evaporate. Then, 100 µl of sterile distilled water was added to the tubes and was frozen in the -80ºC freezer.

Table 1: Plant Material

Plant Name	Origin
A0430-1	Pine Grove, Pennsylvania
A0430-2	Pine Grove, Pennsylvania
C0401-1	Pine Grove, Pennsylvania
C0401-2	Pine Grove, Pennsylvania
C0401-3	Pine Grove, Pennsylvania

3.2.2. Polymerase Chain Reaction

The DNA was quantified and its purity was estimated using the nanodrop machine. Each of the DNA samples was diluted to a concentration of 1 µg/µl. A 10 ng diluted sample was aliquotted and kept on ice under further use. A 96 well plate was also placed on ice along with the 13 primers (Table 2) used, Promega GoTaq Green Mastermix 1x, and the 0.1 dilution Taq polymerase (Table 3).

Table 2: Primers used for RAPD analysis

Primer Name	Primer Sequence (5'-3')
Primer 1	5'-AAGACGACGG
Primer 2	5'-AATCCGCTGG
Primer 3	5'-AGTCGGCCCA
Primer 4	5'-AACAGGGCAG
Primer 5	5'-TGGAAGCACC
Primer 6	5'-AGGCAGCCTG
Primer 7	5'-GATGCGACGG
Primer 8	5'-CCAGATGGGG
Primer 9	5'-GGGGGCTTGA
Primer 11	5'-GAAGGCTCCC
Primer 12	5'-GGGAGCGCTT
Primer 14	5'-AACTGGCCCC
Primer 15	5'-TGGTCATCCC

Table 3: PCR Reaction Set Up

Order Solution Is Added	Volume of Solution
1x Master Mix	7.5 µl
0.1 Taq	1 µl
Primer (1-15)	3.4 µl
10 ng of DNA	4 µl
Final Reaction Volume	17 µl

The 96 well plate was then sealed and loaded into the PCR machine. The cycle chosen to run the PCR was similar to the earlier reported protocol (Vollmaan, Grausgruber, and Stify, et al. 2005): 94°C for one minute; 36°C for 30 seconds; and 72°C for one minute. This cycle was repeated for 40 cycles with a final extension at 72°C for five minutes. After the reaction was complete, the 96 well plate was stored at 4°C until it was ready to run the PCR samples using gel electrophoresis to separate out the resulting DNA bands.

3.2.3. DNA Gel Electrophoresis

The first thing that was done was to make a 50x TAE buffer. The 242g of Tris base was weighed out and mixed with 57.1 ml of Acetic acid. To this mixture, 100 ml of .5M EDTA was added and the final volume was changed to 1 L by adding distilled water. The buffer then was given a pH of 8.5. Using the 50x TAE buffer, a 1L volume of 1x TAE buffer was made.

The 1x TAE buffer was used to make a one-percent agarose gel. For each volume of 125 ml of the agarose mixed with the 1x TAE buffer, 7.5 µl of ethidium bromide was added after heating, then the mixture was poured into a gel casting tray that would fit a 20 well comb. Once the gel was completely solid (after approximately 15 minutes), the gel was carefully removed from the casing and placed in a gel tank. The comb was removed gently and the samples that were run through the PCR machine were loaded into the wells made by the comb. Between each primer was a 1 kb ladder (Table 4). The gels were run at 80 V until all the solutions were moved out of the wells. After which, the voltage was increased to 120 V.

Table 4: Composition of 1kb Ladder

1x Ladder Mixture
0.5 µl of 1kb ladder
5 µl of 10x loading dye
4.5 µl nuclease free water
Final volume: 10 µl

The resulting gels were analyzed for any common or uncommon bands that the two species might share. The band analysis was then used to create a dendrogram.

Chapter 4: Results And Discussion

4.1. Results

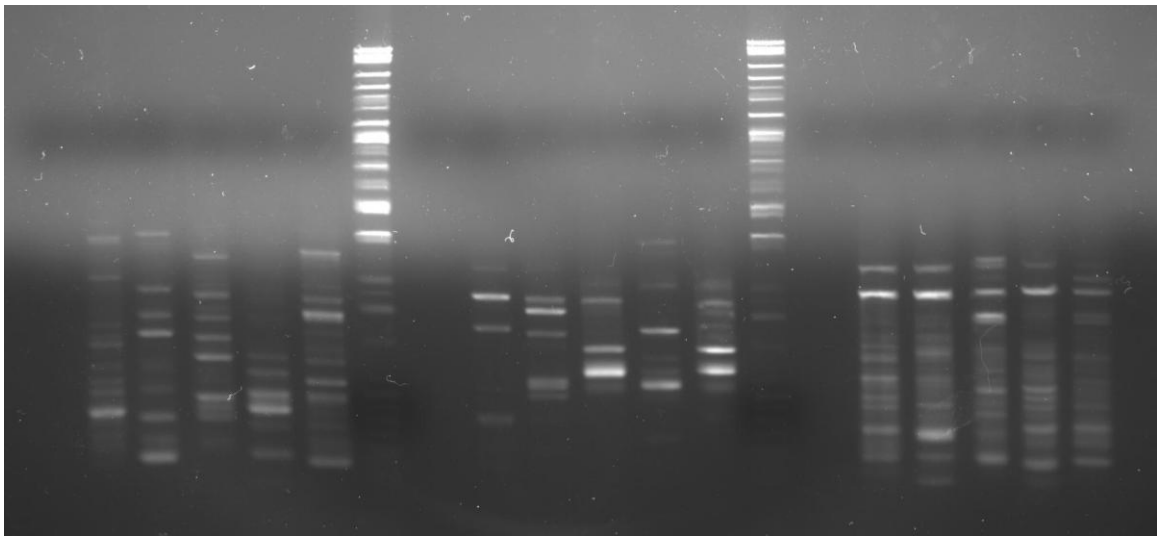

Figure 1: RAPD Analysis of American and Chinese chestnut trees using primers 4, 5, and 6. Samples are loaded (from left): A0430-1, A0430-2, C0401-1, C0401-2, and C0401-3

Table 5: Data showing number of bands present per chestnut sample

Primer Name	A0430-1	A0430-2	C0401-1	C0401-2	C0401-3
Primer 1	7	7	7	8	6
Primer 2	0	2	0	0	1
Primer 3	10	8	8	9	0
Primer 4	7	7	6	5	7
Primer 5	4	5	5	6	6
Primer 6	7	6	6	6	5
Primer 7	2	2	2	2	3
Primer 8	2	4	6	5	5
Primer 9	2	2	5	4	4
Primer 11	6	0	9	6	5
Primer 12	1	8	8	4	9
Primer 14	3	2	8	0	7
Primer 15	3	2	5	3	4

Table 6: Band sizes in base pairs present in various chestnut samples

Primer Name	A0430-1	A0430-2	C0401-1	C0401-2	C0402-3
Primer 1	500, 1000, 1600, 1000 3000, 4800	500, 1000, 1600, 2000, 3000, 4000	500, 750, 1000, 1600, 2000, 3000, 4000,	400, 500, 750, 1000, 1600, 2000, 3000, 4000	300, 750, 1000, 1600, 2000, 3000
Primer 2	0	1600, 2000	0	0	1600
Primer 3	344, 500, 600, 750, 900, 1000, 1400, 1600, 2000, 2800	344, 500, 600, 750, 1000, 1400, 1600, 2000	344, 500, 600, 750, 1000, 1400, 1600, 2000	344, 500, 600, 900, 750, 1000, 1400, 1600, 2000	0
Primer 4	900, 1000, 1100, 1200, 1500, 2000, 3000	500, 750, 1000, 1500, 1600, 2000, 3000	1000, 1300, 1500, 1600, 2000, 2800	500, 900, 1000, 1200, 1300	500, 1000, 1100, 1300, 1600, 1700, 2800
Primer 5	800, 1600, 2000, 2500	1000, 1300, 1600, 1800, 2000	1000, 1200, 1400, 1700, 2000	500, 1000, 1200, 1600, 2000, 3000	1000, 1200, 1400, 1600, 1700, 2000
Primer 6	400, 500, 900, 1000, 1400, 2000, 2800	500, 800, 1000, 1400, 2000, 2800	500, 1200, 1400, 1600, 2000, 2800	500, 800, 1200, 1400, 2000, 2800	500, 800, 1400, 1600, 2000
Primer 7	500, 1600	800, 1000	500, 1000	500, 1000	300, 500, 800
Primer 8	300, 1400	200, 300, 400, 750	200, 300, 400, 500, 750, 1000	300, 400, 500, 600, 1000	200, 300, 400, 500, 750
Primer 9	200, 500	400, 500	220, 300, 450, 500, 600	300, 400, 750, 1000	200, 300, 500, 750
Primer 11	1100, 1000, 1600, 1700, 2800, 3000	0	500, 1100, 1500, 1600, 1700, 2000, 2800, 3000, 4000	500, 1000, 1200, 1700, 2000, 2800	500, 1100, 1600, 2000, 2800
Primer 12	2000	500, 600, 750, 1000, 1300, 1400, 1600, 3000	600, 1000, 1200, 1400, 1600, 2800, 3000, 4000	1000, 1200, 1400, 3000	500, 750, 1000, 1200, 1400, 1600, 2000, 3000, 4000
Primer 14	1300, 1600, 2800	1000, 1500	1500, 1550, 1600, 2000, 2200, 2500, 2800, 3000`	0	1100, 1500, 1600, 1800, 2000, 2200, 2800
Primer 15	200, 750, 1000	500, 750	500, 7500, 800, 1000, 1600	500, 600, 750	500, 750, 1000, 1600

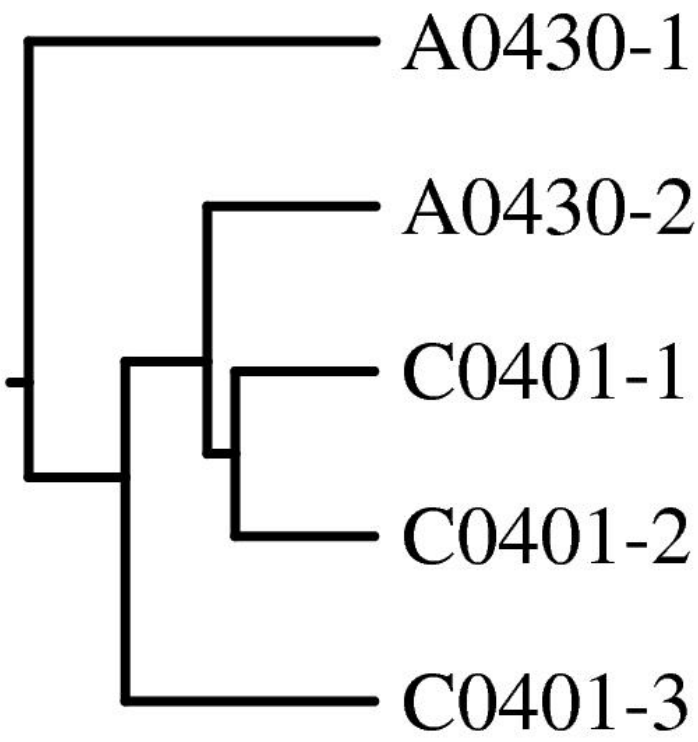

Figure 2: Dendrogram: Summarized results of RAPD analysis using 13 different primers

Table 7: Similarity matrix

Sample Name	A0430-1	A0430-2	C0401-1	C0401-2	C0401-3
A0430-1	1	0.722583	0.77325	0.712545	0.645417
A0430-2		1	0.751	0.79	0.626
C0401-1			1	0.80925	0.716692
C0401-2				1	0.668417
C0401-3					1

Table 8: Grouping trends between different chestnut samples

	Number of occurrences	Primers where trend shows
A0430-1 and A0430-2	2	1, 6
C0401-1 and C0401-2	2	2, 12
A0430-2 and C0401-3	4	2, 12, 14, 15
C0401-2 and A0430-2	1	3
C0401-1and C0401-3	4	4, 5, 7, 11
C0401-2 and C0401-3	2	6, 9
A0430-1 and C0401-2	2	7, 11
A0430-2 and C0401-1	2	8, 9
Grouped A0430-2 and C0401-1 with A0430-1	1	9
Grouped C0401-3 and A0430-2 and Grouped C0401-1 and C0401-2	1	12

4.2. Discussion

The bands present in each gel, Figure 1 and Appendix, were scored on a binary scale with a "1" marking a band present and a "0" for no band. Then the number of bands for each sample was counted. The results are seen in Table 5. The number of bands present for each sample does not show a direct correlation between species type and the number of bands present per species. There are instances where only one plant per species shows any bands (primer 2) or the number of bands is disproportional to the rest of those in the species (A0430-1 and A0430-2 in Primer 12). This indicates no clear distinction between the number of bands and the apparent resistance of the Chinese chestnut.

Table 6 shows the sizes of each band present per sample. Amplification of DNA using primer 1 showed a unique band present only in the Chinese variety located at around 750 base pairs. Results using primer 4 showed a band only seen in the American species located at 300 base pairs and another present in the Chinese chestnuts at 1300 base pairs. The use of primer 5 resulted in two unique bands at the 800 and 1300 base pair size for the American chestnuts and showed a 1200 base pair band only present in the Chinese chestnut. The Chinese species also showed unique bands when primer 8 (500 base pair) was used. Primer 9 (300 base pair), primer 11 (500 and 2000 base pair), and primer 12 (1200 base pair) resulted in different size bands. Any one of the unique bands that were present in the Chinese chestnut could code for a gene that could help it express resistance to *C. parasitica*.

The RAPD analysis showed many unique bands that were shown for only one or two members of the Chinese species. Results from PCR using primer 4 had a 2800 base pair band for C0401-1 and C0401-3 which also showed a band at the 1400 and 1600 base pair in primer 5 and 1600 base pair in primer 6. Using primer 14 also showed unique bands around the 2000 and 2200 base pair mark for C0401-1 and C0401-3. C0401-1 and C0401-2 showed common bands in primer 6 (1200 base pair) and prier 8 (1000 base pair). The inconsistency between plants in the species could be due to a spontaneous mutation in the genetic code, the effect of the environment the trees were growing in, or the blight itself. The bands present in two of the three samples from the Chinese chestnut could code for the gene present in the resistance that the Chinese chestnut could have to the fungus *C. parasitica*.

The dendrogram constructed using the results from PCR reactions using 13 different primers is shown in Figure 2. Two primers did not result in any bands. The grouping shows that C0401-1 and C0401-2 are most closely related genetically. The two Chinese samples are also closely related to A0430-1 and A0430-2. C0401-3 is the most different from all the other samples. Each individual primer's dendrogram was analyzed for grouping trend shown in Table 8. A0430-2 and C0401-2 were grouped four times in four different primers as were C0401-1 and C0401-3.

This indicates that these samples show more similarities than the other samples. Other trends include a grouping of A0430-2 and C0401-1, A0430-1 and C0401-2, A0430-1 and A0430-2, and C0401-1 and C0401-2 with each having two groupings.

4.3. Conclusion

The DNA was extracted from five mature chestnut tree samples. This DNA was then amplified using 13 different primers in separate PCR reactions. The resulting DNA fragments from PCR reactions using each primer was used to analyze whether or not American or Chinese chestnuts were genetically similar and to see if a common sequence from the genomic DNA that is responsible for providing blight resistance could be found. The unique bands seen only in the Chinese chestnut could play a part in the apparent resistance against *C. parasitica*.

Future research is going on in the Central PA Laboratory for Biofuels comparing proteome levels. It is also proposed to further enlarge the study with a large set of primers.

References:

B Thendral Hepsibha, V Premalakshmi, & T Sekar. (2010). Genetic diversity in azima tetracantha (lam) assessed through RAPD analysis. *Indian Journal of Science and Technology,3*(2), 170

Barakat, A., Powell, W., Schuster, S. C., Wheeler, N., Abbott, A., Carlson, J. E., . . . Baier, K. (2012). Chestnut resistance to the blight disease: Insights from transcriptome analysis. *BMC Plant Biology, 12*(1), 38-38. doi: 10.1186/1471-2229-12-38

Barakat, A., DiLoreto, D. S., Zhang, Y., Smith, C., Baier, K., Powell, W. A., . . . Carlson, J. E. (2009). Comparison of the transcriptomes of American chestnut (castanea dentata) and Chinese chestnut (castanea mollissima) in response to the chestnut blight infection. *BMC Plant Biology, 9*(1), 51-51. doi: 10.1186/1471-2229-9-51

Carvalho, L. C., Goulão, L., Oliveira, C., Gonçalves, J. C., & Amâncio, S. (2004). RAPD assessment for identification of clonal identity and genetic stability of in vitro propagated chestnut hybrids. *Plant Cell, Tissue and Organ Culture, 77*(1), 23-27. doi: 10.1023/B:TICU.0000016482.54896.54

Castanea dentata American sweet chestnut PFAF plant database. (n.d.). *Plants For A Future*. Retrieved December 30, 2012 from http://www.pfaf.org/user/Plant.aspx?LatinName=Castanea+dentata

Castanea: The plant list. (n.d.). *The Plant List*. Retrieved December 30, 2012, from http://www.theplantlist.org/browse/A/Fagaceae/Castanea/

Cooper, W. R., & Rieske, L. K. (2008). Differential responses in American (castanea dentata marshall) and Chinese (C. mollissima blume) chestnut (falales: Fagaceae) to foliar application of jasmonic acid. *Chemoecology, 18*(2), 121-127. doi: 10.1007/s00049-008-0399-y

Dagla, H. R. (2012). Plant tissue culture: Historical developments and applied aspects. *Resonance, 17*(8), 759-767. doi: 10.1007/s12045-012-0086-8

D'Amico, K., Horton, T., Maynard, C., & Powell, W. (2011). Assessing ectomycorrhizal associations and transgene expression in transgenic castanea dentata. *BMC Proceedings,5*(Suppl 7), O54-O54. doi:10.1186/1753-6561-5-S7-O54

Family fagaceae - beeches, chinkapins, and oaks. (n.d.). *North American Insects and Spiders / Tree Encyclopedia*. Retrieved December 30, 2012, from http://www.cirrusimage.com/trees_Fagaceae.htm

Goulão, L., Valdiviesso, T., Santana, C., & Oliveira, C. M. (2001). Comparison between phenetic characterisation using RAPD and ISSR markers and phenotypic data of cultivated chestnut (castanea sativa mill). *Genetic Resources and Crop Evolution, 48*(4), 329-338. doi: 10.1023/A: 1012053731052

Hebard, F. V. (1994). Inheritance of juvenile leaf and stem morphological traits in crosses of Chinese and American chestnut. *The Journal of Heredity, 85*(6), 440.

Huang, H., Dane, F., & Kubisiak, T. (1998). Allozyme and RAPD analysis of the genetic diversity and geographic variation in wild populations of the American chestnut (fagaceae). *American Journal of Botany, 85*(7), 1013-1021. doi: 10.2307/2446368

Kärkönen, A., Santanen, A., Iwamoto, K., & Fukuda, H. (2011). Plant tissue cultures. *Methods in Molecular Biology (Clifton, N.J.), 715*, 1.

Kasaian, J., Behravan, J., Hassany, M., Emami, S. A., Shahriari, F., & Khayyat, M. H. (2011). Molecular characterization and RAPD analysis of juniperus species from iran. *Genetics and Molecular Research : GMR, 10*(2), 1069-1074. doi:10.4238/vol10-2gmr1021

Kumar, P., Gupta, V., Misra, A., Modi, D., & Pandey, B. (2009). Potential of molecular markers in plant biotechnology. *Plant Omics*, *2*(4), 141-162.

Murashige, T, & Skoog, F. (1962). "A revised medium for rapid growth and bioassays with tobacco tissue cultures." *Physiologia Plantarum* 15: 473–497.

Newhouse, J. R. (1990). Chestnut blight. *Scientific American, 263*(1), 106-111. doi: 10.1038/scientificamerican0790-106

Quiroz-Figueroa, F. R., Rojas-Herrera, R., Galaz-Avalos, R. M., & Loyola-Vargas, V. M. (2006). Embryo production through somatic embryogenesis can be used to study cell differentiation in plants. *Plant Cell, Tissue and Organ Culture, 86*(3), 285-301. doi: 10.1007/s11240-006-9139-6

Radzan, M. K. (2003). *Introduction To Plant Tissue Culture* (2nd ed.). Enfield, NH: Science Publishers, Inc.

Rigling, D., & Van Alfen, N. K. (1993). Extra and intracellular laccases of the chestnut blight fungus, cryphonectria parasitica. *Applied and Environmental Microbiology, 59*(11), 3634-3639.

Saucier, J. (1973). American chestnut: An American wood. *The Food Productions Laboratory*. Retrieved December 29, 2012, from www.fpl.fs.fed.us/documnts/usda/amwood/230chest.pdf

Species: Castanea dentata (Marshall) Borkh. (n.d.). *Global Biodiversity Information Center*. Retrieved December 30, 2012, from http://data.gbif.org/species/5333230/

Thorpe, T. (2007). History of plant tissue culture. *Methods in Molecular Biology (Clifton, N.J.), 877*, 9.

Vargas, V. M., De-la-Peña, C., Galaz-Ávalos, R. M., & Quiroz-Figueroa, F. R. (2008). Plant tissue culture. (pp. 875-904). Totowa, NJ: Humana Press. doi: 10.1007/978-1-60327-375-6_50

Vollmann, J., Grausgruber, H., Stift, G., Dryzhyruk, V., & Lelley, T. (2005). Genetic diversity in camelina germplasm as revealed by seed quality characteristics and RAPD polymorphism. *Plant Breeding, 124*(5), 446-453. doi:10.1111/j.1439-0523.2005.01134.x

Wu, X., Xiong, D., & Wang, Y. (2007). RAPD analysis of genetic relationships among sphaeropsis sapinea isolates. *Frontiers of Forestry in China, 2*(1), 78-81. doi:10.1007/s11461-007-0012-5

Zhuravlev, Y. N., & Omelko, A. M. (2008). Plant morphogenesis in vitro 1. *Russian Journal of Plant Physiology, 55*(5), 579-596. doi: 10.1134/S1021443708050014

Appendix:

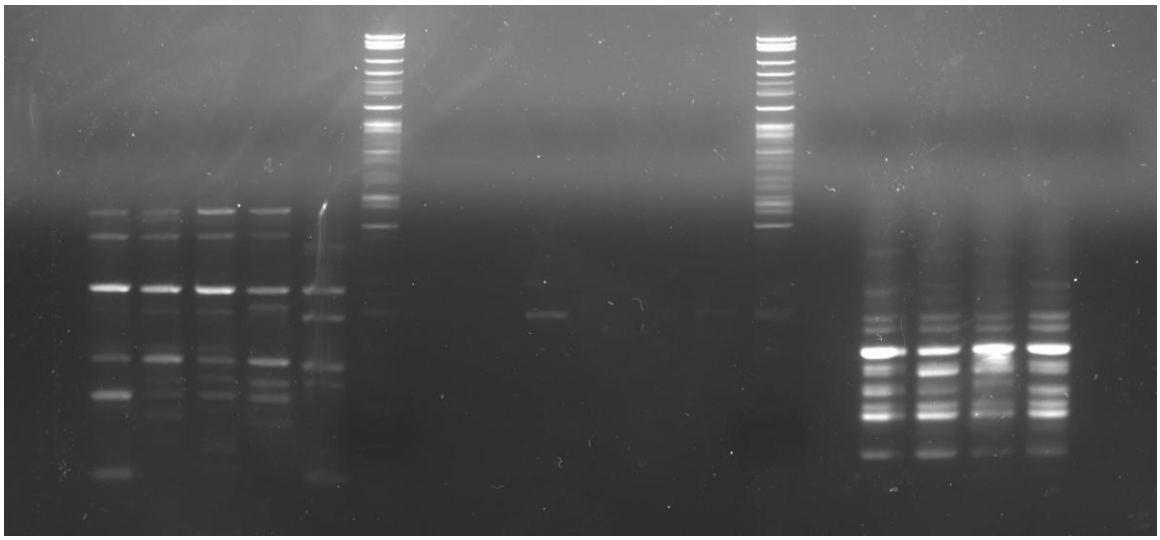

Figure 3: RAPD Analysis of American and Chinese chestnut trees using primers 4, 5, and 6. Samples are loaded (from left): A0430-1, A0430-2, C0401-1, C0401-2, and C0401-3

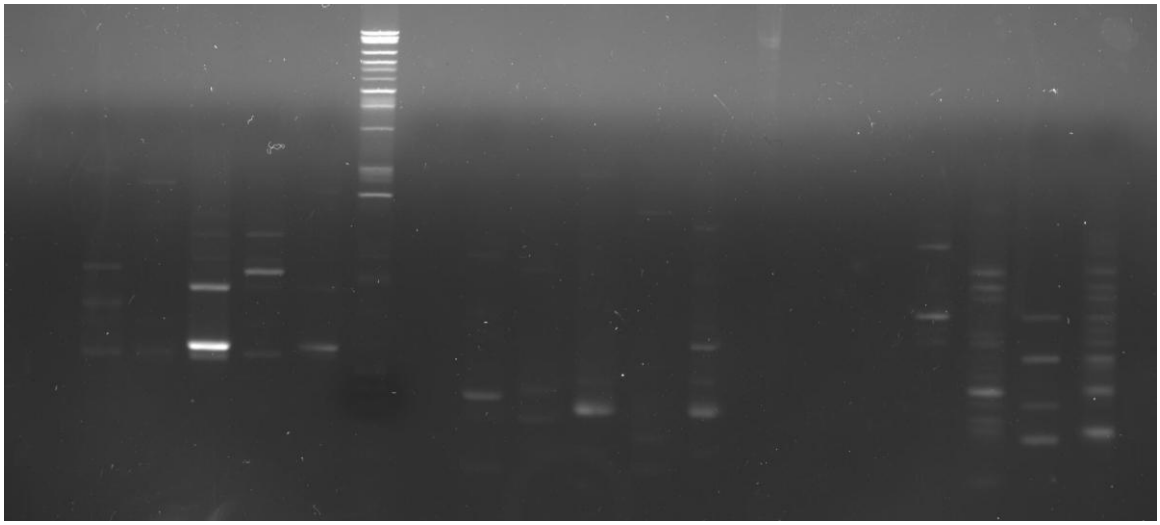

Figure 4: RAPD Analysis of American and Chinese chestnut trees using primers 7, 8, and 9. Samples are loaded (from left): A0430-1, A0430-2, C0401-1, C0401-2, and C0401-3

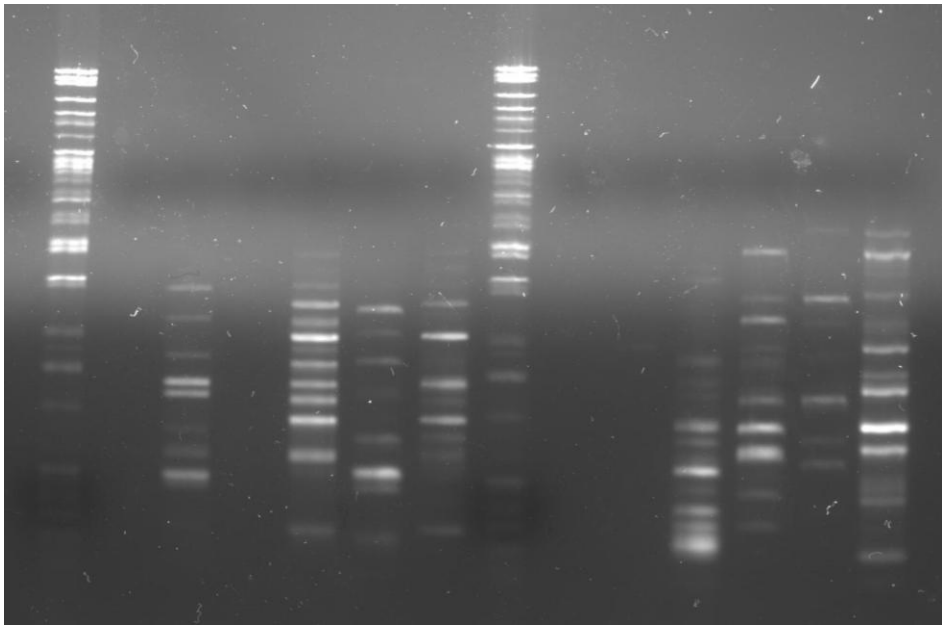

Figure 5: RAPD Analysis of American and Chinese chestnut trees using primers 11, and 12. Samples are loaded (from left): A0430-1, A0430-2, C0401-1, C0401-2, and C0401-3

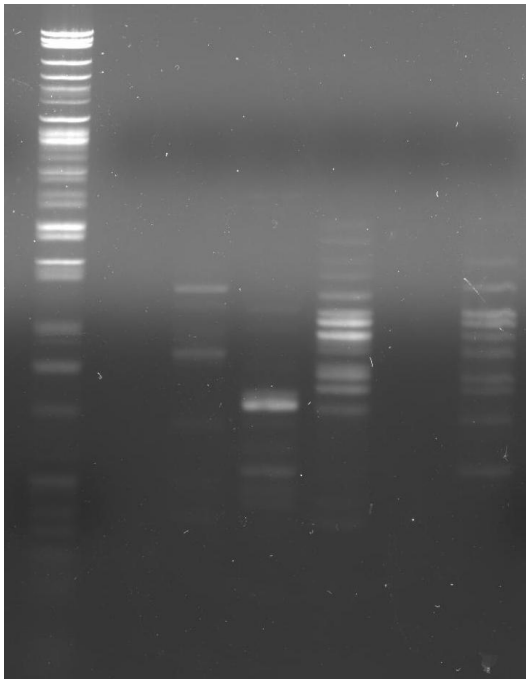

Figure 6:RAPD Analysis of American and Chinese chestnut trees using primer 14. Samples are loaded (from left): A0430-1, A0430-2, C0401-1, C0401-2, and C0401-3

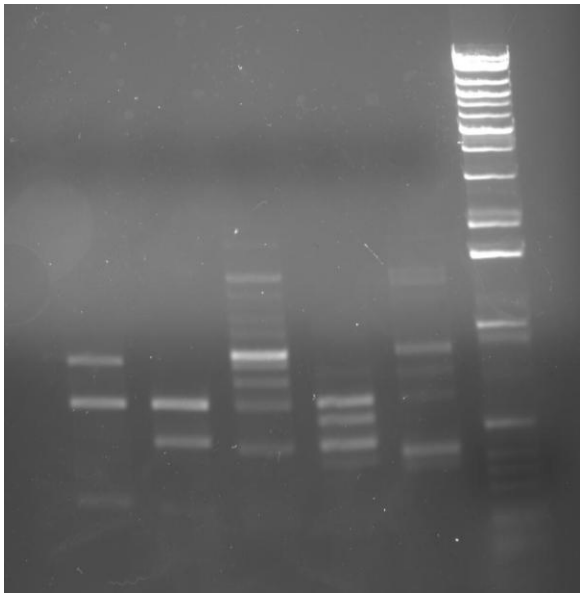

Figure 7: RAPD Analysis of American and Chinese chestnut trees using primer 15. Samples are loaded (from left): A0430-1, A0430-2, C0401-1, C0401-2, and C0401-3

Academic Vita

Nicole Briley
PO BOX 61743
Crooked Hill Road
Harrisburg, PA 17106
Nib5050@psu.edu

EDUCATION

THE PENNSYLVANIA STATE UNIVERSITY, SCHREYER HONORS COLLEGE Harrisburg, PA
B.S. in Science, Life Science Option
Honors in Biology
Fall 2013

RESEARCH/ INTERNSHIPS

Central PA Laboratory for Biofuels, Penn State Harrisburg Harrisburg, PA
Undergraduate Research Assistant, Supervised by Dr. Sairam Rudrabhatla Fall 2011-Present
Genetic Engineering of tomato with nutritionally enriched genes
Tissue culture and genetic engineering of American chestnut for Disease Resistance
Thesis: Genetic Comparison Of *Castanea dentata* And *Castanea mollissima* Using RAPD Analysis

Penn State College of Medicine, Nephrology Department Hershey, PA
Research Intern, Supervised by Dr. W. Brian Reeves Summer 2012
The Association of Rac1 and $TRPM_2$ in Ischemic Kidney Injury

RESEARCH PRESENTATIONS

N. Briley, Penn State Harrisburg, S. Potlakayala, M. Reitzel, K. Vescio, M. Zaidi, A. Shuler, and S. Rudrabhatla; Genetic Comparison of *Castanea dentata* and *Castanea mollissima* Using RAPD Analysis; **SIVB *In Vitro* Biology Meeting**; June 18, 2013

Nicole Briley, W. Brian Reeves, Guofung Gao; The Association of Rac1 and TRPM2 in Regards to Ischemic Kidney Injury; **Summer Undergraduate Research Symposium**; August 2012