DNA Barcoding of *Quercus sp.* at Pierce Cedar Creek Institute Using the matK Gene

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Abstract:

When identifying different species in the field or in a laboratory, morphological characteristics are often used. These distinguishable characteristics can be very similar among different species which can make the identification process very difficult. Another way to denote different species is through a DNA barcoding method. Similar to items in a store that have a universal product code (UPC), DNA barcoding is a short genetic sequence that identifies an individual organism as a member of a particular species. The species examined were *Quercus* sp. (oaks); 71 samples of the 8 different species growing at Pierce Cedar Creek were collected for DNA extraction. The samples were all preserved into herbarium specimens and DNA was extracted from all of them. The extracted DNA was then amplified using PCR and gel electrophoresis methods. The successful amplified DNA was then sequenced using the gel slab method and the capillary method. Successful sequences came from the capillary method of sequencing and these samples were then analyzed using computer software to obtain a base pair reading of the sequence code. The seven sequences that were analyzed had no variations in their base pairs supporting the idea that they are of the same species, *Quercus rubra.*

Introduction:

The purpose of this experiment was to determine if the different oak species (*Quercus sp*.) or their hybrids could be identified by using sequences of DNA from their matK gene. The samples used for this experiment were taken from the Pierce Cedar Creek Institute, located in Barry County, Hastings, Michigan. Generally there are two ways that the oak species, or any species, can be identified: using morphological characteristics or by DNA sequencing. Oak species have several similar morphological characteristics that make it more difficult to distinguish between species. *Quercus sp.* either have bristle tips or they have smooth lobes. Oak species were used as the sample species because they hybrid often and their hybrids are hard to distinguish from the parent species that combined to make the hybrid.

Pierce Cedar Creek Institute (PCCI) is located on 661 acres of land that was originally farmland and is now comprised of wetlands, prairies, and many forests. At PCCI there are six different species of oaks on the institute's propriety along with two probable hybrid species, *Q. rubra, Q. velutina, Q. alba, Q. bicolor, Q. macrocarpa, Q. muehlenbergii, Q.x. hawkinsiae,* and *Q.x. jackiana.* Of these species the *Q. velutina* and *Q. rubra* look very similar and the hybrids are morphologically indistinguishable from the two different species that bred together to make the hybrid. Because of the similarities among the different species, the best way to distinguish them is through biochemical differences, i.e. DNA barcoding.

In plant DNA barcoding there is not one gene that is used to distinguish between species. There are many different genes that are being used in an attempt to establish a universal gene for DNA barcoding. One gene in particular that has been of interest is the matK gene. Matk is located in the chloroplast of plants and not in the mitochondria like the Cytochrome Oxidase I (COI) gene that is used for DNA barcoding of animals (Hebert et. al. 2003). This gene is made up of approximately 1600 base pairs (bp) and is located in the trnK intron of most angiosperms, and it is believed to code for a maturase gene (Hilu et. al. 2003). The matK gene has a higher number of nucleotide substitutions, nonsynonymous, mutations, and indels (Hilu et. al. 2003). These factors make matK an ideal gene marker for determining the differences between dissimilar species.

Materials and Methods:

Collecting of samples

Through the entire month of May of 2009, 71 samples of 6 different species of oaks were collected at Pierce Cedar Creek Institute (PCCI) in Hastings Michigan. A minimum of 10 samples were collected from each species of oak except for the chinquapin species where only 5 samples were collected. Each tree specimen was plotted with GPS coordinates and tagged, located in Table 4. While at PCCI the samples were made into two sets of herbarium specimens, one for Aquinas College

Herbarium and one for PCCI Herbarium. A sample from each tree was ripped into small pieces and stored in a -80°C freezer to destroy parasites and for preservation.

Extraction, Amplification, and Cleanup

The MoBio PowerPlant® DNA Isolation kit was used for extraction of DNA for the 71 leaf samples that were being preserved. After DNA was extracted the Quiagen® Fast Cycling PCR kit (200) was used for DNA amplification. There were four sets of primers that were used for amplification that are listed in Table 1. The only primer set that was used continuously was the 3F_KIM and 1R_KIM. The denaturation temperature was 96°C for 5 seconds, the annealing temperature was 57.2°C for 5 seconds, and the extension temperature was 68°C for 38 seconds. The samples were then run through gel electrophoresis with 5µl of sample each well along with 5µl of ladder in each row. When there was successful amplification those samples were then cleaned up using the Quiagen QIAquick® PCR Purification Kit (50).

Sequencing

The cleaned up samples had to be put through a sequence reaction provided by the ABI BigDye® Terminator v3.1 Cycle Sequencing kit to create a single strand of DNA. These samples were then washed using a 80% and 70% ethanol wash. The ABI Prism® 377 DNA sequencer requires a slab gel plate be created for each sequence run. The software that was used for running the sequencer was ABI Sequencing Analysis® Software version 3.4 that was compatible on a Macintosh. The samples were loaded into the gel and the sequencer was run for 7-9 hours. The gel image was analyzed to discover if there were samples successfully sequenced. The data from the computer was exported onto another Apple Macintosh. This information was analyzed by hand to see if there were similarities between sequences.

Samples that had cleaned up using the Quiagen QIAquick® PCR Purification Kit (50) were also sent to Davis Sequencing® in California. These sequences were compared using the program DNA Baser®, which compares chromatograms and makes a contig for comparison. Samples that had been

PCR purified were also sent to the University of Michigan DNA Sequencing Core in Ann Arbor. These sequences were also analyzed using the DNA Baser® program.

Results

The samples that were amplified and a comparison of the success of each primer are displayed in Table 3. Only the samples that were amplified using the primer sets cox1 and cox 42F along with 3F_KIM and 1R_KIM were attempted to be sequenced. The samples 005.A, 006.A, 008.A, 010.A, 062.A, 063.A, 064.A, and 066.A were sequenced. The results of the sequencing are in Table 2.

Table 1: Results of amplification of oak species. Percentage of oaks amplified was calculated by taking the amount of species successfully amplified divided by the amount of species attempted .

	Protocol A	Protocol B	Protocol C	Protocol D
Primer set	matK 2.1af, matK 3r	cox 1, cox 42F	trnH, psbA	3F_KIM, 1R_KIM
Denaturation Temperature	96°C	96°C	96°C	96°C
Denaturation time	5 seconds	5 seconds	5 seconds	5 seconds
Annealing temperature	47	55	55	57.2°C
Annealing time	5 seconds	5 seconds	5 seconds	5 seconds
Extension temperature	68°C	68°C	68°C	68°C
Extension time	15 seconds	18 seconds	18 seconds	38 seconds
Number of Samples tested	22	23	7	71
Percentage of oaks amplified	0%	13.64%	14.29%	26.76%

Table 2: Results of samples amplified, sequenced and average sequence length of the samples sequenced.

Species Collected	**Samples Collected**	**Amplified**	**Sequenced**	**Average Length of Sequence**
Red Oaks	**17**	**17**	**7**	**650 bp**
Black Oaks	**12**	**3**	**1**	**700**
White Oaks	**15**	**2**	**0**	**NA**
Swamp Oaks	**11**	**1**	**0**	**NA**
Bur Oaks	**9**	**1**	**0**	**NA**
Chinquapin Oaks	**5**	**0**	**0**	**NA**

Table 3: List of samples collected, along with what primers were attempted. Successful and unsuccessful amplification is listed. Sample number 004.A did not have a leaf sample and therefore did not have DNA extraction.

Sample number	species	matK 2.1 af, matk 3r	trnH, psbA	cox1, cox 42F	trnH, psbA	3F_KIM, 1R_KIM
001.A	*Q. bicolor*	not amplified				not amplified
002.A	*Q. bicolor*	not amplified				not amplified
003.A	*Q. macrocarpa*					**amplified**
005.A	*Q. rubra*	not amplified				**amplified**
006.A	*Q. rubra*	not amplified				**amplified**
007.A	*Q. rubra*	not amplified				**amplified**
008.A	*Q. rubra*	not amplified				**amplified**
009.A	*Q. velutina*	not amplified				**amplified**
010.A	*Q. velutina*	not amplified				**amplified**
011.A	*Q. velutina*	not amplified				not amplified
012.A	*Q. macrocarpa*	not amplified				not amplified
013.A	*Q. macrocarpa*	not amplified				not amplified
014.A	*Q. rubra*					not amplified
015.A	*Q. alba*				not amplified	not amplified
016.A	*Q. alba*				not amplified	not amplified
017.A	*Q. velutina*			not amplified		not amplified
018.A	*Q. macrocarpa*					not amplified
019.A	*Q. rubra*			not amplified		not amplified
020.A	*Q. macrocarpa*					not amplified
021.A	*Q. macrocarpa*					not amplified
022.A	*Q. rubra*	not amplified		not amplified		not amplified
023.A	*Q. velutina*					not amplified
024.A	*Q. rubra*	not amplified		not amplified	not amplified	not amplified
025.A	*Q. rubra*			not amplified		not amplified
026.A	*Q. rubra*	not amplified		not amplified	not amplified	**amplified**
027.A	*Q. velutina*					**amplified**
028.A	*Q. rubra*	not amplified		not amplified		not amplified
029.A	*Q. alba*					not amplified
030.A	*Q. rubra*			not amplified		**amplified**
031.A	*Q. velutina*					not amplified
032.A	*Q. velutina*					not amplified
033.A	*Q.x. hawkinsiae*					not amplified
034.A	*Q.x. hawkinsiae*			not amplified		not amplified
035.A	*Q. rubra*			not amplified		not amplified
036.A	*Q. macrocarpa*					not amplified
037.A	*Q. bicolor*					not amplified
038.A	*Q. bicolor*					not amplified
039.A	*Q. bicolor*					**amplified**
040.A	*Q. bicolor*			not amplified		not amplified
041.A	*Q. bicolor*	not amplified				not amplified
042.A	*Q. macrocarpa*	not amplified		not amplified		not amplified
043.A	*Q. velutina*			**amplified**		**amplified**
044.A	*Q. velutina*	not amplified				not amplified
045.A	*Q. macrocarpa*					not amplified
046.A	*Q. alba*					not amplified
047.A	*Q. alba*					**amplified**
048.A	*Q. velutina*					not amplified
049.A	*Q. alba*	not amplified	not amplified	not amplified		not amplified
050.A	*Q. alba*					not amplified
051.A	*Q. alba*					not amplified
052.A	*Q. velutina*					not amplified
053.A	*Q. alba*					not amplified
054.A	*Q. alba*					**amplified**
055.A	*Q. muehlenbergii*					not amplified
056.A	*Q. muehlenbergii*					not amplified
057.A	*Q. muehlenbergii*			not amplified		not amplified
058.A	*Q. muehlenbergii*					not amplified
059.A	*Q. muehlenbergii*			not amplified		not amplified
060.A	*Q. alba*					not amplified
061.A	*Q. alba*					not amplified
062.A	*Q. rubra*			amplified		**amplified**
063.A	*Q. rubra*			not amplified		**amplified**
064.A	*Q. rubra*	not amplified	**amplified**	**amplified**	**amplified**	**amplified**
065.A	*Q. alba*			not amplified		not amplified
066.A	*Q. rubra*			not amplified	not amplified	**amplified**
067.A	*Q. alba*				not amplified	not amplified
068.A	*Q. bicolor*	not amplified				**amplified**
069.A	*Q. bicolor*			not amplified		not amplified
070.A	*Q. bicolor*					not amplified
071.A	*Q. alba hybrid*	not amplified				not amplified
072.A	*Q. bicolor*					not amplified

Table 4

Date samples were collected, sample numbers, Latin and common name, authority of whom named species, GPS location and description of area where collected.

Date Collected	Species number	Species	Authority	Common Name	Trail Location	Description
15-May-09	001	*Q. bicolor*	Willd.	Swamp white oak	N42.53478, W085.29635 Blue Trail	moist habitat, behind muscle wood
15-May-09	002	*Q. bicolor*	Willd.	Swamp white oak	N42.53478, W085.29635 Blue Trail	moist habitat, behind 001
15-May-09	003	*Q. macrocarpa*	Michx.	Bur oak	N42.53478, W085.29635 Blue Trail	south of 002, moist habitat, cherry and autumn olive nearby
15-May-09	005	*Q. rubra*	L.	Red oak	N42.51352, W085.29551 Blue Trail	top of ridge before sharp turn, in beech grove, mature
15-May-09	006	*Q. rubra*	L.	Red oak	N41.53152, W085.29551 Blue Trail	top of ridge before sharp turn, in maple grove among other oaks
15-May-09	007	*Q. rubra*	L.	Red oak	N41.53152, W085.29551 Blue Trail	directly next to 006, young
15-May-09	008	*Q. rubra*	L.	Red oak	N41.53152, W085.29551 Blue Trail	5 meters from 007, young, among maple/oak grove
15-May-09	009	*Q. velutina*	Lam.	Black oak	N41.53152, W085.29551 Blue Trail	1 meter from 008, young
19-May-09	010	*Q. velutina*	Lam.	Black oak	N42.52942, W085.29833 White Trail	Beginning of white trail, young oak grove, orange fissures in trunk
19-May-09	011	*Q. velutina*	Lam.	Black oak	N42.52879, W085.29815 White Trail	midway up first hill, ironwood and cherry present, split trunk
19-May-09	012	*Q. macrocarpa*	Michx.	Bur oak	N42.52704, W085.30117 White Trail	beginning of loop, sandy open area, mature tree
19-May-09	013	*Q. macrocarpa*	Michx.	Bur oak	N42.52704, W085.30117 White Trail	10 meters from 012, in prairie area along loop
19-May-09	014	*Q. rubra*	L.	Red oak	N42.52581, W085.30105 White Trail	Eastside of loop, near creek, young trees
19-May-09	015	*Q. alba*	L.	White oak	N42.52580, W085.30057 White Trail	Eastside of loop, near creek, on upward slope to Y intersection
19-May-09	016	*Q. alba*	L.	White oak	N42.52579, W085.30003 White Trail	20 meters from 015 at top of hill/ridge
19-May-09	017	*Q. velutina*	Lam.	Black oak	N42.52694, W085.30054 White Trail	100 meters from Y intersection, sandy, green area
21-May-09	018	*Q. macrocarpa*	Michx.	Bur oak	N42.54009, W085.29524 Red Trail	just north of Batt's Cottage, greenish area, young cherry near
21-May-09	019	*Q. rubra*	L.	Red oak	N42.54009, W085.29524 Red Trail	North of Batt's Cottage, across from 018, open area, mature trees
21-May-09	020	*Q. macrocarpa*	Michx.	Bur oak	N42.54039, W085.29484 Red Trail	Eastside of trail, mature tree, sandy soil, lots of autumn olive
21-May-09	021	*Q. macrocarpa*	Michx.	Bur oak	N42.54170, W085.29265 Red Trail	Northside of downward slope, open area with red oaks
21-May-09	022	*Q. rubra*	L.	Red oak	N42.54262, W085.29100 Red Trail	Northside of open area on hill, red oaks and cherries present
21-May-09	023	*Q. velutina*	Lam.	Black oak	N42.54275, W085.29072 Red Trail	Before bend in trail, wooded area, maples, cherries and ironwoods
21-May-09	024	*Q. rubra*	L.	Red oak	N42.54309, W085.29081 Red Trail	At westward turn in trail, narrow trunk, westside of trail
21-May-09	025	*Q. rubra*	L.	Red oak	N42.53882, W085.30378 Red Trail	West entrance of trail, 20 meters into prairie
21-May-09	026	*Q. rubra*	L.	Red oak	N42.53940, W085.30369 Red Trail	Prairie area, near trail post, next to cherry tree
21-May-09	027	*Q. velutina*	Lam.	Black oak	N42.54037, W085.30378 Red Trail	Prairie area near end of trail going north, cherry and blackrasberries
21-May-09	028	*Q. rubra*	L.	Red oak	N42.54162, W085.30093 Red Trail	Along prairie edge, north past several bends, between cherry and walnut
21-May-09	029	*Q. alba*	L.	White oak	N42.54251, W085.29842 Red Trail	Well into west to east stretch in forest, maple and ironwood nearby
21-May-09	030	*Q. rubra*	L.	Red oak	N42.54280, W085.29642 Red Trail	Just past branch trail to lake, northside of trail, near autumn olive and cherry
26-May-09	031	*Q. velutina*	Lam.	Black oak	N42.53556, W085.29959 Green Trail	Labeled black oak, along prairie edge, eastside of trail
26-May-09	032	*Q. velutina*	Lam.	Black oak	N42.53565, W085.29915 Green Trail	Southside of trail, open area with oak saplings, young tree
26-May-09	033	*Q.x. hawkinsiae*	Sudw.	Red x Black	N42.53247, W085.30407 Blue Trail	Heading from visitor center, in woods up hill, along deer path
26-May-09	034	*Q.x. hawkinsiae*	Sudw.	Red x Black	N42.53247, W085.30407 Blue Trail	Right next to 033
26-May-09	035	*Q. rubra*	L.	Red oak	N42.53213, W085.30392 Blue Trail	Heading from visitor center, right off main trail in woods, woodbine nearby
28-May-09	036	*Q. macrocarpa*	Michx.	Bur oak	N42.53478, W085.29635 Blue Trail	In marsh area off of path, directly behind 001
28-May-09	037	*Q. bicolor*	Willd.	Swamp white oak	N42.53478, W085.29635 Blue Trail	Behind 036 in marsh area
28-May-09	038	*Q. bicolor*	Willd.	Swamp white oak	N42.53478, W085.29635 Blue Trail	Left of 001 in marsh area
28-May-09	039	*Q. bicolor*	Willd.	Swamp white oak	N42.53478, W085.29635 Blue Trail	Behind 002 in marsh area
28-May-09	040	*Q. bicolor*	Willd.	Swamp white oak	N42.53478, W085.29635 Blue Trail	7 meters from 038 , marsh area, cherry and musclewood nearby
28-May-09	041	*Q. bicolor*	Willd.	Swamp white oak	N42.53446, W085.29640 Blue Trail	10 meters from 040, behind american elm, marsh area
28-May-09	042	*Q. macrocarpa*	Michx.	Bur oak	N42.53478, W085.29635 Blue Trail	Across from large cedar tree, marsh area, about 10 meters from 003
28-May-09	043	*Q. velutina*	Lam.	Black oak	N41.53152, W085.29551 Blue Trail	Directly behind 008 in maple grove
28-May-09	044	*Q. velutina*	Lam.	Black oak	N41.53152, W085.29551 Blue Trail	Diagnolly across from 009, top of ridge, among other oaks and maples
29-May-09	045	*Q. macrocarpa*	Michx.	Bur oak	N42.52704, W085.30117 White Trail	Across from 012, dry prairie area, near large cherry
29-May-09	046	*Q. alba*	L.	White oak	N42.52580, W085.30057 White Trail	Near creek, infront of 015
29-May-09	047	*Q. alba*	L.	White oak	N42.52580, W085.30057 White Trail	Near creek, between 015 and 046
29-May-09	048	*Q. alba*	L.	White oak	N42.52580, W085.30057 White Trail	Across from creek, before 046 on trail, among other white oaks
29-May-09	049	*Q. alba*	L.	White oak	N42.52580, W085.30057 White Trail	Behind 048, near creek
29-May-09	050	*Q. alba*	L.	White oak	N42.52580, W085.30057 White Trail	Heading up the hill, 4 meters past 046, near creek, young tree
29-May-09	051	*Q. alba*	L.	White oak	N42.52580, W085.30057 White Trail	Behind 050, near creek, young tree
29-May-09	052	*Q. velutina*	Lam.	Black oak	N42.52580, W085.30057 White Trail	Across white trail from 050 on hill, near creek, among other oaks
29-May-09	053	*Q. alba*	L.	White oak	N42.52596, W085.30048 White Trail	Near top of smaill hill on white trail, 4 meters away from 051, young tree
29-May-09	054	*Q. alba*	L.	White oak	N42.52596, W085.30048 White Trail	Behind 053, near creek, young tree
1-Jun-09	055	*Q. muehlenbergii*	Engelm.	Chinkapin	N42.54369, W085.27449 Little Grand Canyon	Near prairie/forest edge off of trail to Little Grand Canyon, heading east
1-Jun-09	056	*Q. muehlenbergii*	Engelm.	Chinkapin	N42.54369, W085.27449 Little Grand Canyon	Right next to 055 on prairie/forest edge
1-Jun-09	057	*Q. muehlenbergii*	Engelm.	Chinkapin	N42.54369, W085.27449 Little Grand Canyon	Farther into forest, thick vegetation, behind 055
1-Jun-09	058	*Q. muehlenbergii*	Engelm.	Chinkapin	N42.54369, W085.27449 Little Grand Canyon	Furthest into forest, among 055, 056 and 057
1-Jun-09	059	*Q. muehlenbergii*	Engelm.	Chinkapin	N42.54369, W085.27449 Little Grand Canyon	Furthest into forest, among 055, 056 and 057
1-Jun-09	060	*Q. alba*	L.	White oak	N42.54369, W085.27449 Little Grand Canyon	In forest, left of 059, thick vegetation
1-Jun-09	061	*Q. alba*	L.	White oak	N42.54369, W085.27449 Little Grand Canyon	Amid grove of possible chinquapins, next to 060, mature tree
1-Jun-09	062	*Q. rubra*	L.	Red oak	N42.54407, W085.27378 Little Grand Canyon	Right after trail turns into woods from field edge, dense woods, eastside
1-Jun-09	063	*Q. rubra*	L.	Red oak	N42.54407, W085.27378 Little Grand Canyon	Right after trail turns into woods from field edge, dense woods, eastside
1-Jun-09	064	*Q. rubra*	L.	Red oak	N42.54407, W085.27378 Little Grand Canyon	Right as trail turns east and down into canyon, near hickory, 3 part trunk
1-Jun-09	065	*Q. alba*	L.	White oak	N42.54444, W085.27165 Little Grand Canyon	At end of trail and up the ridge, behind bramble bushes, near maples
1-Jun-09	066	*Q. rubra*	L.	Red oak	N42.54403, W085.27207 Little Grand Canyon	At end of trail and up the ridge, by field, near elm, walnut, poison ivy, mature
1-Jun-09	067	*Q. alba*	L.	White oak	N42.54403, W085.27207 Little Grand Canyon	Right near 066, mature
1-Jun-09	068	*Q. bicolor*	Willd.	Swamp white oak	N42.53323, W085.30181 Yellow Trail	South of the field tree line, behind education building, amid red oaks
1-Jun-09	069	*Q. bicolor*	Willd.	Swamp white oak	N42.53323, W085.30181 Yellow Trail	Next to 068, going west on tree line
1-Jun-09	070	*Q. bicolor*	Willd.	Swamp white oak	N42.53323, W085.30181 Yellow Trail	Next to 068, going west on tree line
1-Jun-09	071	*Q. x jackiana*	*Schneid*	Swamp & white hyb	N42.53323, W085.30181 Yellow Trail	Next to 070, next to red oak and walnut, mature tree
1-Jun-09	072	*Q. bicolor*	Willd.	Swamp white oak	N42.52781, W085.29984 White Trail	Next to white oak and red maple, going south before loop, mature tree

Discussion

Recent advancements in the development of a DNA barcoding gene for plants have enabled scientists to discover many new characteristics of the plant genome that help further more research. In this study 71 samples of *Quercus sp.,* that are listed in Table 4, were collected for DNA amplification and sequencing to determine if the samples were different species and if there were hybrids present in the samples.

The results indicated that of the eight samples that were sequenced seven of the samples were the same species while one of the samples was different. The one sample that was different was sequenced using the ABI sequencer which gave a sequence of around 400 (bp) which is not long enough to analyze The seven identical samples were identified morphologically as *Quercus rubra* while the other sample was *Quercus velutina*. There were some similarities between the individual sample and the other seven samples but there was a 5% difference that allowed for that sample to be a different species. The seven identical samples did not have a difference between DNA sequences greater than 5% so those samples are of the same species, *Quercus rubra.* Only 8 samples were sequenced during this project but there were 10 more samples that could still have been sequenced for comparison.

There are many areas of study that can still be explored for the matK gene. MatK has many different aspects that don't allow for it to be used as an universal barcode at the moment. The matK gene is an appropriate gene to use because it is very specific to a region on DNA. When the matK gene is amplified and sequenced the ability to distinguish between species is a lot easier than with other genes that have a higher amplification rate than matK. The matK gene has more areas that are distinguishable while the trnH gene has less. If the matK gene had one primer that worked for all species then the ability to use the matK gene as a universal barcode would be more eminent. The matK gene currently does not have one primer that works for all species of plants. A primer must be made for

each genus or even species.

During the course of this research there was difficulty in amplifying the oak species. Of all the oak species the *Q. chinquapin* was the only species that did not amplify. When attempts were made to amplify the samples of *Q. bicolor* that had previously been amplified, these samples were not amplified again. This showed that the *Quercus sp.* had difficulties with amplification. This could have been caused by many different variables. The PCR process could have been the main problem to the unsuccessful amplification or the oak species themselves could contain particles that interfered with the amplification.

There are several avenues that could be taken to further research on *Quercus sp*. The areas of growth at PCCI could be examined more thoroughly. The location of where each sample was collected from was plotted using GPS coordinates. The *Q. chinquapin* species grows in soil that contains lime and that the majority of this species grows farther south (citation). The collected species location could be studied further to determine if previous farming of the PCCI land had any effect upon the growth of the oak species. There are some *Q. bicolor* growing on a ridge in the yellow prairie that is uncharacteristic of the growth patterns of this species. When this area was examined further it was discovered that the ridge floods with water after heavy rains which would give this area the type of habitat that *Q. bicolor* desires to grow in.

Another area to examine further would be the surface area of oak leaves relative to the area on the tree. While collecting leaf samples it was observed that the oak leaves closer to the top of the tree had less surface area while the leaves closer to the base of the tree had more surface area. This observation had an effect on collecting samples because the leaves toward the bottom of the trees were easier to collect, but these leaves did not have characteristics similar to only one species of oak. If this was examined further it might help with further collecting of oak species and determining if hybrid species are being collected or not.

In this study the matK gene showed that it can be used as a gene for identification for oak

species. It was inconclusive whether the matK gene could be used to identify hybrid species from the oak samples. More investigation into this area may prove that the matK gene can distinguish hybrid species from other oak species. Further research on the matK gene being used as a DNA barcode for plant species should be done along with further research on the *Quercus. sp* at PCCI.

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