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Effects of Drainage Isolation in Kansas Streams on the Genetics of Fish

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ABSTRACT

Reproductive isolation in species can be caused geographically by extrinsic barriers. Isolated drainage basins within the state of Kansas could cause the fish populations to undergo this reproductive isolation. Reproductive isolation can be measured by the number of nucleotides that differ within the same gene in a species. Here genetic isolation was tested by examining the cytochrome oxidase I, (COI gene), in fish of the same species from both the northern and southern drainage basins. A small tissue sample taken from each fish, underwent PCR amplification, and DNA barcoding to determine speciation. The samples were sent off for genetic sequencing, and phylogenetic trees were constructed with the useable sequences. Based on this analysis of the phylogenetic trees, there was no significant genetic isolation within and between the fish in the northern and southern drainage basins.

Keywords: *COI gene, drainage basins, fish populations, genetic diversity, Kansas, reproductive isolation*

INTRODUCTION

Over time many methods have been used to identify the vastly diverse species that inhabit our environments. Genetic identification has increasingly been used to understand the multitude of species, and is helpful in maintaining this diversity. As evolutionary theory states random genetic mutations occur within an organism's genetic code, beneficial mutations that aid in the organism's survival will be passed to the next generation (Bynum, 2009). Accumulation of these beneficial genetic changes in reproductively isolated populations can result in speciation. Dobzhansky (Palumbi, 1994), proposed one way that populations could become reproductively isolated is through geographic separation. This allopatric speciation model limits gene flow, and if held long enough the accumulated genetic diversity will maintain reproductive isolation between the populations, even if the extrinsic barrier is diminished. (Palumbi, 1994). When determining the genetic variation of each fish species, one has to look at nucleotide polymorphisms. Nucleotide polymorphisms accumulated after speciation events can be measured as an indication of genetic diversity. The number of nucleotide polymorphisms in highly important genes, may indicate how recently the individuals speciated. Fewer nucleotide differences indicate individuals who speciated more recently.

One major type of extrinsic barrier for aquatic organisms is drainage basins, due to the inability of most fish to swim upstream for a long duration of time. Drainage basins are defined as the area of land where all the surface water drains into a central area whether it be a creek, stream, river or ocean. Kansas contains two major drainage basins. The northern streams and tributaries flow into the Missouri River, and the southern streams and tributaries flow into the Arkansas River (Cross, and Collins, 1995). According to a study done by Thornbrugh, fishes move between large or small streams but do not move through different river junctions (Thornbrugh, and Gido, 2009). This means that fish in Kansas will only remain in the drainage basin they are found in. The fish do not mix due to their inability to swim through the large river junction of the Mississippi River. Kansas contains an abundance of different species of fish throughout the drainage basins. In 1978 Dwight R. Platt published a list of rare and endangered fish in KS and updated it until 1994 at which time it contained 37 different fish (Cross, and Collins, 1995). In 2005 the *Current Status of Native Fish Species in KS* showed that an increased number of 44 different species are now endangered in KS out of the 116 native species found.

When fish are isolated based on their specific drainage basin, the fishes genetic code within that basin should be adapting to the specific environment. This would mean that genetic diversity could be found within the state of Kansas due to the separate basins. Genetic diversity is useful in comparing the same genus of fish across both drainage systems to show a better representation of the gene differences. It is also helpful in showing whether genetic diversity is occurring within the same species in a drainage basin. In a similar study done by the University of California on minnow species, little genetic variation (<.95) was found, suggesting that gene flow was still occurring within populations that had be extrinsically isolated (Avise, and Francisco, 1976), and according to a study done by the University of Kansas, Kansas shows little to no genetic variation (<.95) in fish species due to stocking decreasing species diversity in the entire state (Chapin, and Kennedy, 2006). A common gene used in eukaryotes to show the genetic differences is the cytochrome c oxidase I gene (COI). Universal primers for the COI gene at the 5' end of the mtDNA strand are typically used during DNA barcoding for fish. This specific use of DNA barcoding is effective for

nearly 200 species of fishes (Ivanova, *et al*., 2007).

By being able to measure the nucleotide polymorphism in the COI gene in the different types of fresh water fish from the different basins, we shall understand how the extrinsic barriers are affecting the gene flow within Kansas drainage basins. Kansas contains in abundance several species of the family *Centrachdae;* Sunfish, White Crappie and White Bass along with an abundance of family *Cyprinidae;* shiners and minnows. With Kansas drainage basins being an ideal mechanism for allopatric speciation to be occurring, collection of these highly populous families and determining the amount of polymorphism differences will show whether or not speciation is occurring within the drainage basins.

MATERIALS AND METHODS

Collection of genus *Cyprinidae* and *Centrarchidea* was done from June 2016 to October 2016 using purse seines. Several samples were taken from rivers within each drainage basin; (NDB= Missouri Drainage Basin, SDB= Arkansas Drainage Basin). The Turkey Creek River (SDB), was collected from July 27th, the Smoky Hill River (NDB), was collected from August 4th, and the McPherson State Fishing Lake (NDB) was collected from August 9th. Finally the last collection was done October 21st from the Smoky Hill River again. After each sample was taken, it was brought back to McPherson College, and placed into a site specific container with 95% ethanol. Identification of each individual genus and species was done using the book *Fishes of Kansas* by Cross. Samples were then put into individual bags with species identifiers and frozen until DNA barcoding.

There are several genetic technologies that can be used to measure nucleotide polymorphisms. The most common being DNA barcoding. DNA barcoding assess genetic differentiation that has occurred due to speciation events, such as adaptation to the environment, and genetic drift. DNA barcoding is the use of mtDNA to screen one or a few reference genes in order to assign individuals to species, or determine if speciation has occurred (Moritz and Cicero, 2004). The DNA barcoding was done in lab at McPherson College, using a method from DNA Learning Center, *Using DNA Barcodes to Identify and Classify Living Things* (Cold Spring Harbor Laboratory, 2014).

After being taken out of the freezer, samples were dried using paper towels. I then collected a small 10-20 mg sample and placed it in a 1.5 mL microcentrifuge tube, with label numbers 1-30 on the top of the tubes. To the samples, I added 300 μl of lysis solution using a micropipette. The solution in the tube was then ground forcefully for two minutes, using a new plastic pastel for each tube. Next I incubated the tube in a water bath at 65°C for ten minutes, with vortexing of the sample for one minute after removing from the water bath. A new 1.5 mL microcentrifuge tube was then labeled with the identification number, and 150 μL of the supernatant, or the clear solution over the debris pellet, was added to the tube. Once done I added 3 μL of silica resin to the tube and mixed well by vortexing for one minute. The mixed tubes were then allowed to sit in a 57°C water bath for five minutes. After I removed the tubes from the water bath, the tubes were centrifuged for 30 seconds, and removal of the supernatant with a fresh micropipette tip occurred in all 30 samples. The pellet leftover in each sample got an addition of 500 μL of ice cold wash buffer, and was then vortexted for around one minute. The tubes were then put through the same process of centrifuging, removal of the supernatant, wash buffer, and vortex again. After the removal of the supernatant a third time, instead of wash buffer, 100 μL of distilled water was added to the silica resin and vortexed for one minute. The tubes were again incubated in the 57°C bath for five minutes. Once out of the bath, I placed the tubes in the centrifuge to run one last time for 30 seconds. After removal from the centrifuge, new tubes containing the identification number were made and 90 μL of the supernatant in the old tube was transferred and the old tube discarded. All 30 samples were then stored in the freezer until PCR amplification.

The samples that were in the freezer were set out on the counter and allowed to thaw for use in the PCR tubes. 30 Ready-To-Go PCR Bead tubes were collected, and 22.5 μL of fish COI was put into the tubes. The tubes were allowed to sit for one minute so the primer dissolved the PCR bead. Next I added 2.5 μL of each DNA collected from the samples to the PCR tube. I then put samples into the Thermocycler, which ran for 35 cycles of the following; 94°C for 30 seconds, 54°C for 45 seconds, and 72°C for 45 seconds. Once this cycle was complete the samples stayed at a 4°C hold until removal about four hours after being placed in the Thermocycler. Once the samples were removed they were put back into the freezer until I performed the gel electrophoresis. Gel electrophoresis allows for the determination of DNA in the PCR product. This is done before sending the samples off for sequencing. I sealed off the electrophoresis trays, and put the well forming combs inside to form wells. 2 grams of agarose was mixed with 100 mL of 1x TBE and poured into the two trays. I then allowed the gels to sit for 20 minutes to set up before placing them into the electrophoresis chamber. By pouring the 1x TBE buffer over the top of the gel, this allowed the current to run properly over the top of the gels. The forming combs were then removed, and I added an additional TBE just to fill the wells left by the combs. Once complete, 20 μL of the maker pBR322/*Bst*NI is loaded into the far left well as the identification marker. In the remaining gels 5 μL of the PCR samples were added. The gels were allowed to run for 45 minutes at 130V until the dyes have moved

a sufficient amount to show the results. The gels were stained post-run with CarolinaBLU Final Stain for 20-30 minutes. Then allowed to sit in deionized water for 30-40 minutes. Once destaining was completed, they were viewed under the UV light. The visible bands that appeared showed were DNA was successful.

The samples that show DNA were then prepped for sendoff to Carolina Biological. The remaining 20 μL of PCR product that was left over is from the barcode was split in half and put into two separate 0.2 ml PCR tubes. These tubes were labeled with the identification numbers DW01-DW60. The PCR samples were sent off to Carolina Biological for identification of the genetic sequence. Once the genetic sequence data is returned analyzation using the online interface DNA Subway at http://dnasubway.iplantcollaborative.org was done. Determination of sequence relationships are done within the online interface by selecting Sequence Viewer and using the blue line to determine the relationships. Clicking on the BLASTIN option allows for searching of the NCBI Database for matches to the selected sequences and then selecting the MUSCLE option allows for all sequences to be aligned in a multiple alignment or phylogenetic tree. The neighbor-joining tree based of the Kimura 2-paramete (KP2) model is run by selecting PHYLIP NJ, and compared with prospected genomes.

RESULTS

I collected the following species; the Turkey Creek River contained from family *Cyprinidae*; one Red Shiner (*Cyprinella lutrensis*), and one Suckermouth Minnow (*Phenacobius mirabilis*), and from family *Centrarchidae*; one Longear Sunfish (*Lepomis megalotis*), one Bluegill Sunfish (*Lepomis macrochirus*), two Green Sunfish (*Lepomis cyanellus*), and four White Crappie (*Pomoxis annularis*). In the Smoky Hill River, I collected from family *Cyprinidae*; three Red Shiners and from family *Centrarchidae*; two White Bass (*Morone chrysops*). I collected from the McPherson State Fishing Lake from family *Cyprinidae*; three Mosquitofish (*Gambusia affinis*), and from family *Centrarchidae*; three White Crappie,

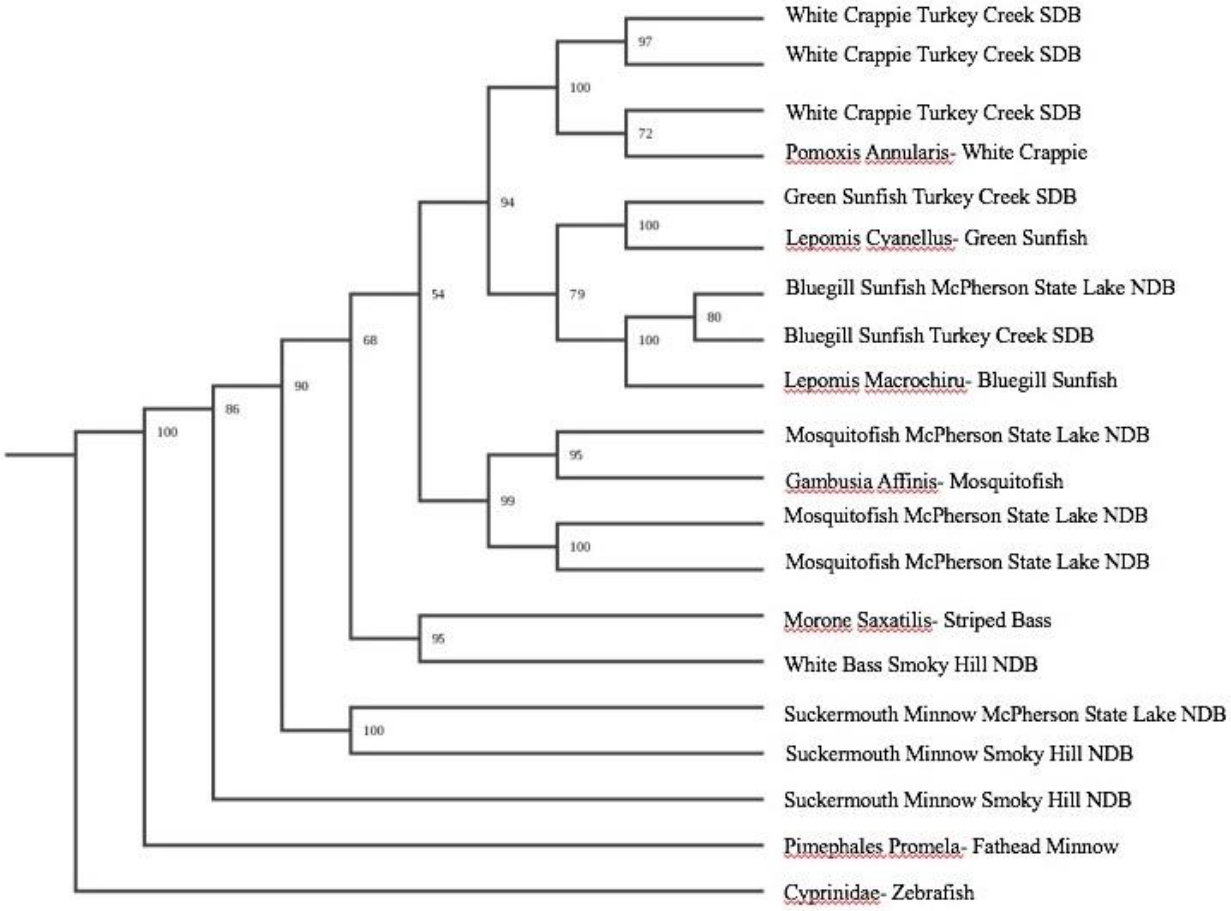

Figure 1. Neighbor joining phylogenetic tree. Samples collected are identified first by the common name and then by what drainage system they were found in. Other samples are scientific species, used as species identifiers. On analysis, drainage systems seem to be overlapping showing little genetic variation.

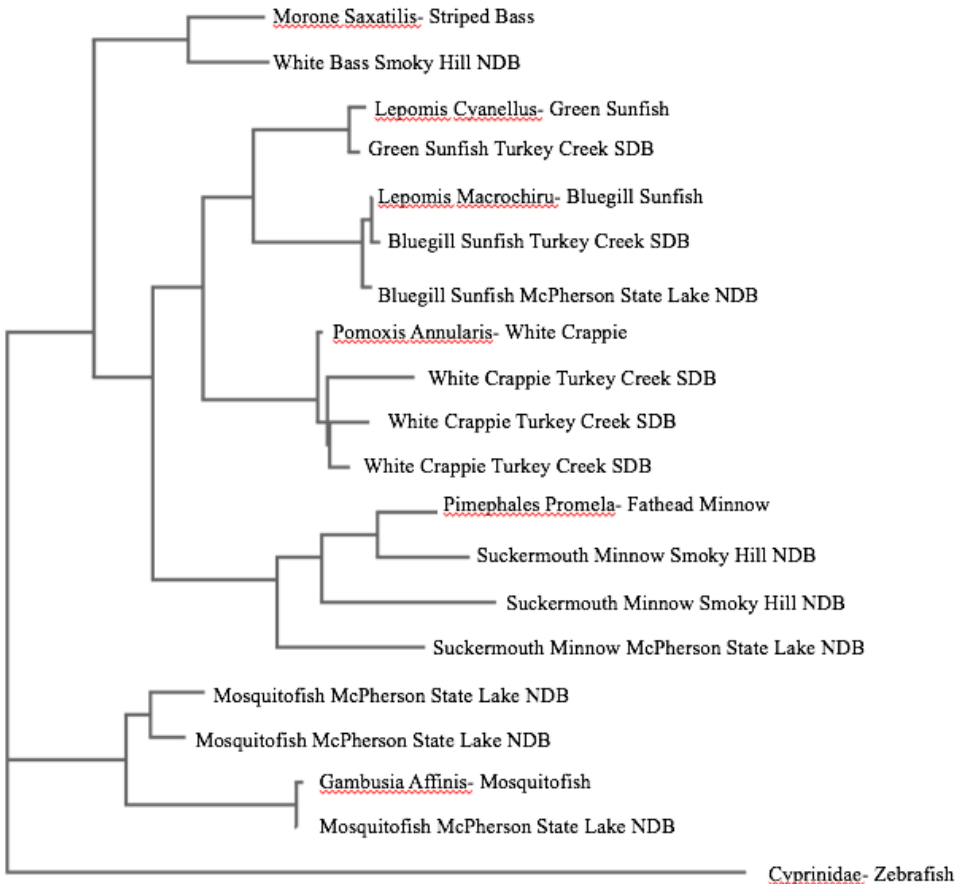

Figure 2. Maximum likelihood phylogenetic tree. Same identification method was used as in Figure 1. It is seen that, species from the same family *Centrarchidae* all fall within one evolutionary line.

one Green Sunfish, and one White Bass. Finally, from the second sample in the Smoky Hill River I collected from family *Cyprinidae*; three Red Shiners, and from family *Centrarchidae*; two White Bass. This came to 30 fish samples that I could have used for sequencing in the tree.

The sequence data for 13 of the 30 fish was collected and phylogenetic trees were created. The matched NCBI sequences, along with the paired sequences from the 13 samples are shown in both the neighbor joining tree and maximum likelihood trees.

The neighbor joining tree shows clusters of each of the 13 species that were originally identified, and how they fall closely to the NCBI genetic sequence. It can be seen in the Bluegill Sunfish, which contains both the NDB and the SDB, that the two Bluegill are more closely related to each other than the NCBI gene sequence. It can also be seen that both the Bluegill and Green Sunfish are right next to each other on the tree showing their close lineage. The family *Centrarchidae* also falls closely related to each other with the Bluegill, Green, and White Crappie coming off of the same branch.

The maximum likelihood tree shows the most likely evolutionary tree to be formed by each species. The tree shows how each species has diverged from one another over an estimated period of time. It shows that the *Centrarchidae* family falls within the same evolutionary line. The Bluegill Sunfish is the only one that contains information from both drainage basins, allowing a look into speciation events. The *Cyprinidae* family seems to fall within two different evolutionary lines with the Mosquitofish evolving before the Suckermouth Minnow.

DISCUSSION

The phylogenetic trees were a small representation of the fish seen in Kansas. Out of the 30 samples sent off for sequencing only 13 sequences came back with useable sequence data within the limit of traceable nucleotides. These sequences fell within the limit of over 500 contiguous read length, and over 24 quality score. The useable sequences were matched, paired, and mapped in the tree. If speciation was occurring throughout drainage basins it would be expected that fish from the same drainage basin are within the same lineage, and separate from the other drainage basin.

It would be seen that fish from the NDB would be clustered together, while fish from the SDB would be together.

From the tree it is interpreted that speciation is not occurring within the different drainage basins, or within the basins themselves. In the Bluegill Sunfish the close relation of the NDB and SDB disproves that a large enough accumulation of nucleotide polymorphisms is happening. It is inferred that since members of the *Centratchidae* family are all within the same lineage, and closely related to the NCBI gene matches that drainage basins do not have a large enough effect on fish populations in Kansas.

In a study done by Hänfling on freshwater fish in different drainage basins in Bavaria, genetic variation within the populations was significant (Hanfling, and Brandl, 1998). This significance contrasts what was found within the drainage systems in Kansas. This lack of speciation could be effected by a multitude of factors within the drainage basins. Fish stocking within the lakes could cause both drainage basins to remain close to the same genetic codes between fish species. According to the Kansas Department of Wildlife, Parks, and Tourism over 85 lakes are stocked statewide (Kansas Department of Wildlife, Parks, and Tourism, 2017). With those lakes being located within both the NDB and SDB. It's also probable that the drainage basins have not been maintained long enough to have a large enough accumulation of nucleotide polymorphisms for speciation to be seen in fish. In conclusion, the fish in Kansas are more than likely not undergoing speciation.

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LITERATURE CITED

Avise, J and A Francisco. 1976. Genetic differentiation in speciose versus depauperate phylads: evidence from the California minnows. Evolution. 30:46-58.

Bynum, W. 2009. The origin of species by means of natural selection: or, the preservation of favored races in the struggle for life. Journal of Science Education. 14:204-208.

Cold Spring Harbor Laboratory. 2014. Using DNA Barcodes to Identify and Classify Living Things Lab Manual. DNA Learning Center, Cold Harbor Spring, NY.

Chapin, B and R Kennedy. 2006. Regional stream classification frameworks and biotic homogenization in streams of the Midwest: An evaluation using landscape-scale patterns in fish communities. ProQuest Information and Learning Company, Ann Arbor, MI. pp 28-49.

Cross, F and J Collins. 1995. Fishes in Kansas. University Press of Kansas, Lawrence, KS. pp 10-78.

Hänfling, B and R. Brandl. 1998. Genetic variability, population size and isolation of distinct populations in the freshwater fish Cottus gobio L. Molecular Ecology 7:1625-1632.

Ivanova, N, T Zemlak, R Hanner, and P Hebert. 2007. Universal primer cocktails for fish DNA barcoding. Molecular Ecology Notes 7: 544-548.

Kansas Department of Wildlife, Parks, and Tourism. Feb. 2017. Fish Atlas. http://ksoutdoors.com/Fishing/Fish-Stocking-Records (23 Jan, 2017).

Moritz, C and C Cicero. 2004. DNA barcoding: promise and pitfalls. Public Library of Science Biology. 2:1529-1531.

Palumbi, S. 1994. Genetic divergence, reproductive isolation, and marine speciation. Annual Review of Ecology and Systematics. 25:547-572.

Thornbrugh, DJ and K. Gido. 2009. Influence of spatial positioning within stream networks on fish assemblage structure in the Kansas River basin. Canadian Journal of Fisheries and Aquatic Sciences. 67:143-156.