Report as of FY2006 for 2006ND120B: "Molecular Phylogeography of Etheostoma nigrum (Rafinesque) in the upper Midwest"

Publications

Project 2006ND120B has resulted in no reported publications as of FY2006.

Report Follows

MOLECULAR PHYLOGEOGRAPHY OF *ETHEOSTOMA NIGRUM* (RAFINESQUE) IN THE UPPER MIDWEST

DESCRIPTION OF CRITICAL STATE OR WATER PROBLEM TO BE INVESTIGATED

The geologic history and abundant potential study sites of the upper Midwest provide a unique opportunity for the assessment of spatial genetic diversity. The Johnny Darter, *Etheostoma nigrum* (Rafinesque), with its large range and abundant populations, is an excellent species to study to answer phylogeographic questions about North Dakota and Minnesota. I will examine the genetic diversity of *E. nigrum* by using microsatellite PCR primers designed initially for other species of *Etheostoma* and recently optimized for *E. nigrum*. This information will provide not only the inferred gene flow among the darters but will also provide a baseline against which to evaluate gene flow for other fish species located in the same water bodies. For instance, many game fish are stocked and transferred within and among watersheds with no genetic monitoring. By studying a benthic fish with a small home range, it will be possible to uncover the phylogeographic structure among the various watersheds of the upper Midwest. In turn, this information can be used by managers for conserving genetic diversity within and among watersheds.

SCOPE AND OBJECTIVES

With the advent of molecular techniques, the field of ecology has become dramatically dynamic. It is now possible to use these new techniques to answer a wide variety of ecological questions. I am particularly interested in using molecular genetic markers to address these questions. My time with the Stockwell Lab at NDSU will help me develop the skills I need to become a better scientist and learn the techniques that are the future of ecological work. It is with these goals in mind that I have began to examine the genetic relationships among *Etheostoma nigrum* (Rafinesque), Johnny Darter, populations in the North Dakota and Minnesota.

Many of the watersheds of the northern Midwest have been isolated since the end of the Pleistocene and offer great opportunity for assessing the recent (evolutionarily speaking) genetic divergence and gene flow among fishes in the upper Midwest (Underhill 1958). *Etheostoma nigrum* is a small, benthic fish in the perch family. It is commonly found in the lakes and streams of North Dakota and Minnesota. *E. nigrum*, found from the Hudson Bay to southern Mississippi and Colorado to the Atlantic coast, is one of the most abundant and wide-ranging species of *Etheostoma* genus (Eddy and Underhill 1978 and Kuehne 1983). Prior observations have noted significant morphological differences among these fish in the lakes and streams of the upper Midwest (Dr. Jim Grier, personal communication). However, despite its abundance and range, little work has been conducted to better understand this species. *E. nigrum* is a non-migratory fish with movements confined only locally before and after the spawning season (NatureServe 2005 and Winn 1958). In addition, because of their benthic habits, the fish are unlikely to be transferred via an angler's bait-bucket. Due to these two facts, fine-scale structure is

likely to exist among populations of *E. nigrum*. It is because of these reasons that I have chosen to use *E. nigrum* as a model species to study genetic diversity and gene flow among the lentic and lotic systems of North Dakota and Minnesota.

Polymerase chain reaction (PCR)-based analysis of microsatellites is one of the most popular techniques for determining the genetic diversity of established populations (Avise 2004). Microsatellites are a type of co-dominant DNA marker inherited in a Mendelian fashion (DeWoody and Avise 2000). That is, an individual fish will have one allele from the female parent and another from the male parent. Each population is likely to contain alleles that are unique to the population. Thus, using PCR to amplify microsatellites, it will be possible to examine the genetic uniqueness of each *E. nigrum* population and what, if any, gene flow is occurring among populations.

The first step in performing a microsatellite analysis involves creating the PCR primers. Once primers are designed, it is then possible to screen large numbers of individuals (Avise 2004). This work has already been accomplished for two species of *Etheostoma*. Published literature provides nine different PCR microsatellite primers for *E. virgatum* and *E. olmstedi*, the latter belonging to the same subgenus as *E. nigrum* (Porter et al. 2002 and DeWoody et al. 2000). Once optimized for *E. nigrum,* these primers can be used to PCR-amplify DNA that can then be used with an automated fragment analysis program to determine the genetic diversity and gene flow within and among populations of *Etheostoma nigrum* in the major watersheds of North Dakota and Minnesota. Similar success has been achieved in the Stockwell Lab with congeners of *Cyprinodon tularosa*, a threatened fish of New Mexico (Jones et al. 1998, Stockwell et al. 1998, and Iyengar et al. 2004).

METHODS, PROCEDURES, AND FACILITIES

During ice-free months, fish will be collected from sites chosen among the streams and lakes of North Dakota and Minnesota. Sampling sites will be located within the watersheds of the upper Missouri, Red River of the North, and upper Mississippi Rivers. Ten study sites will be identified in North Dakota and Minnesota. Each site will measure approximately 300 meters in length. Sites will be chosen based on the habitat preferences of *Etheostoma nigrum.* Multiple populations will be sampled within various river systems, possibly the Pipestem, Turtle, and Ottertail Rivers, to evaluate fine scale genetic structure. Future plans include sampling the James, Wild Rice, and upper Missouri Rivers and Devil's Lake in North Dakota. In Minnesota, samples will be taken from hydrologically isolated and interconnected lakes in the Lakes Region (western MN) as well as the Buffalo and Otter Tail Rivers. Fish will be collected with seines or snorkeling equipment. Captured fish will be sequestered in a living-well to first anesthetize and then sacrifice the organisms. Fish will be collected under collecting permits issued to Dr. Craig Stockwell by the states of North Dakota and Minnesota.

Once received in the lab, a fin clip will be taken from each of the voucher specimens. The fin clips can then be used in the lab for DNA extraction. The DNA will then be amplified using PCR and each of the nine optimized primers. Each primer flanks a different

microsatellite repeat. The PCR amplified samples, now representing nine loci for each fish, will then be ran on a Beckman Coulter CEQ8000 automated DNA sequencer using an automated fragment analysis program. The alleles of each individual will be scored and compare to others form its own population and geographically distant populations.

Data will be analyzed using Genetic Data Analysis (GDA). Each population will be evaluated for herterozygosities (expected and observed) allelic diversity, percent loci polymorphism, and Hardy-Weinberg equilibrium. I will also evaluate genetic structure within and among populations with Wright's F-statistics (Weir and Cockerham 1984). A hierarchical analysis will be conducted treating populations, rivers, and drainages as three distinct levels in the hierarchy. This will allow me to partition variance among populations within rivers, rivers and lakes within drainages, and between drainages.

ANTICIPATED RESULTS AND BENEFITS

The genetic evaluation of *E. nigrum* populations may have management implications. As stated above in the research description, most of the fish populations in the upper Midwest have been isolated since the end of the Pleistocene. However, many of the streams and lakes are hydrologically connected. As a result, managers often transfer and stock game fish from one water body to another with little to no regard for the genetic structure of the systems. This practice is based upon the idea that gene flow will occur in systems that are hydrologically connected; but in actuality, gene flow is largely influenced by the migratory habits of individual species. Species with small home ranges may have little gene flow between closely located populations. Over time, these populations develop a unique genetic identity, often adapting to local conditions. When fish transfers are planned without consideration of this diversity, populations become genetically homogenous. This results in a loss of genetic variation among populations and perhaps even outbreeding depression (Leberg 1992). This is especially important if populations are locally adapted. Understanding the current diversity and gene flow of *E. nigrum* in the watersheds of North Dakota and Minnesota will aide in the establishment of management and conservation units as well as help managers plan for the transfer and stocking of fishes. *E. nigrum* are particularly useful for evaluating phylogeographic structure because they are non-migratory and also unlikely to be accidentally transferred due to their use of benthic habitats.

PROGRESS TO DATE

Preliminary work with *Etheostoma nigrum* and the published primers is promising. All nine primers amplify DNA in all *E. nigrum* individuals tested. To date, the primers are assumed to amplify microsatellites. This is due to the fact that the allele sizes from *E. nigrum* are similar to the alleles in the primer-specific congeners. Future plans including sequencing the nine loci amplified to determine if the amplified samples are, in fact, microsatellites. However, for the purpose of *E. nigrum* phylogeography, a primer that amplifies diagnostic fragments can be used to determine genetic diversity and gene flow among the Mississippi River, Red River of the North, and Missouri River watersheds.

As stated earlier, 408 fish have been collected and are currently stored in the Stockwell Lab. The sample locations include the Pipestem River, Beaver Creek, Turtle River, and Forest River in North Dakota. In Minnesota, fish have been collected from Felton Creek, Hay Creek, Fishhook River, Shell River, Mississippi River, Lake Christina, and Lake Ida (a hydrologically closed system). Research is progressing as scheduled. In addition to sequencing the *E. nigrum* microsatellites, future work will include the completion of DNA extraction from collected fish, amplification of the 9 microsatellites in all 408 individuals, automated fragment analysis, and genetic data analysis.

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