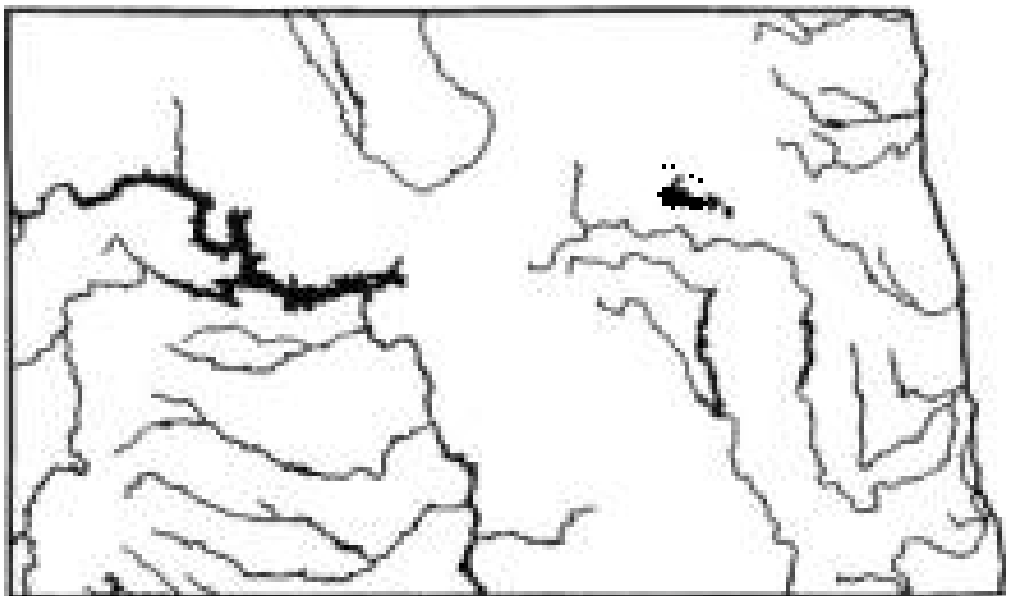

Technical Report No: ND08-10

Genetic Distribution and Diversity of the Johnny Darter (Etheostoma Nigrum,Rafinesque) in the Upper Midwest

by

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ABSTRACT

This novel study examined the existing phylogeography of *Etheostoma nigrum* (Johnny darter, Rafinesque) in the Upper Midwest. Four microsatellite loci were chosen to examine eight populations of *E. nigrum,* a non-migratory, benthic fish, from three drainage basins: the Red River of the North, the upper Missouri River, and the upper Mississippi River. These systems provide an excellent opportunity for phylogeographic studies as they have only been recently available to colonization by ichthyofauna. we applied four microsatellite loci to examine population structure within and among these major watersheds. Using AMOVA, strong evidence of a watershed effect was observed with 31% of the genetic variance among watersheds. Variance between populations within drainages accounted for 9% of the total variation. This observation was further supported using a Bayesian analysis which identified five well-supported assemblages which roughly agreed with watershed assignment. One notable exception to this pattern was observed for Lake Ida. This isolated lake is located in the Red River drainage; however, the population more closely aligned with populations from the upper Mississippi River. Because Johnny darters show considerable geographic genetic structure, they provide a good model to compare with other species that have been translocated within and among drainages.

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INTRODUCTION

Phylogeography is a powerful tool capable of revealing the influence of dispersal and evolutionary processes, such as vicariance, on the geographic and historic distribution of genetic traits within and among populations of similar species (Avise 2004). Further, a phylogeographic analysis utilizes both micro- and macroevolutionary processes to compare genealogies across genetic boundaries. Thus, organisms can be compared within and between populations of the same species, species of similar taxonomic heritage, and more complex taxonomic levels (Bermingham and Moritz 1998). Comparative phylogeography (sensu Bermingham and Moritz 1998) is used to describe the evolution of genetic landscapes and allows for the incorporation of history and geography with species population genetic structure. With this approach, it is possible to evaluate the combined effects of regional, historical, and ecological factors in shaping genetic

diversity. For instance, comparative phylogeography can be used to evaluate patterns of colonization and dispersal into newly available habitats.

Particularly, comparative phylogeography has been shown to be effective in evaluating post-Pleistocene colonization in several studies. Triantafyllidis et al. (2002) found that glacial refugia in Europe significantly influenced the genetic structure of the European catfish (*Silurus glanis,* L.). Their microsatellite data were able to reveal much more discrete levels of genetic variability than prior allozyme and RFLP analyses. These data exposed the existence of a single refugium near the Caspian Sea from which all *S. glanis* originated and spread throughout Europe. Similarly, the genetic structure of brown trout (*Salmo trutta,* L.) in Europe and North Africa was shaped by the Pleistocene glaciations which greatly impacted habitat availability, with greater genetic diversity and population structure occurring in the more southern latitudes (Bernatchez 2001). Similar patterns have been seen in North America. Heilveil and Berlocher (2006) noted that the postglacial expansions of the saw-combed fishfly (*Nigronia serricornis,* Say) substantially affected the haplotype diversity of the species throughout its range. They found that diversity decreased as latitude increased, which follows the path of glacial retreat.

The genetic relationship among populations also has important implications for identifying conservation units (Moritz and Faith 1998; Crandall et al. 2000) and for providing guidance to management programs. The identification of conservation units is imperative to the monitoring and regulation of anthropogenic effects on natural populations (Palsboll et al. 2007); however, the necessary data for species of conservation concern is often lacking. Yet, decisions regarding the habitat of the

species of concern must be made before sufficient evidence can be collected. A reasonable approach to alleviating this problem is to evaluate the genetic structure of surrogate species. Thus, by protecting surrogates, managers can also protect species of concern. These surrogates may be any of three classes: 1) flagship or charismatic mega-fauna, 2) umbrella or wide ranging species, or 3) indicator species (Caro and O'Doherty 1999; Andelman and Fagan 2000). Further, Andelman and Fagan (2000) note that for the successful use of a surrogate species, the species must spatially co-occur with a large portion of the biotic community of interest and it should also have a high likelihood of persistence in the community. Often, common species not of conservation concern fulfill both of these assumptions.

Whiteley et al. (2006) proposed a common species *Prosopium williamsoni* (Mountain whitefish, Girard) as an umbrella model to determine historic genetic structure for management and conservation strategies for other fishes located throughout its range in the Pacific Northwest. The genetic distribution of *P. williamsoni* was hierarchical for both allozyme and microsatellite data. This distribution was loosely correlated with the geographic structure of the major watersheds.

For many fish species, anthropogenic activities, such as extensive translocations, may have altered historic spatial patterns of genetic diversity. This is especially true for game fish species in the upper Midwest which have been extensively translocated both within and among major drainage basins. Unfortunately, the history of such movements was poorly documented. In contrast,

some aquatic species are unlikely to have been translocated due to life history characteristics which make them unfavorable for use as game or bait fish. One such species is the Johnny darter, *Etheostoma nigrum* (Rafinesque). Furthermore, *E. nigrum* shows relatively high levels of philopatry which should result in high levels of genetic structure within and between smaller populations (Stepien and Faber 1998). Thus, the genetic structure of Johnny darter populations is more likely to reveal historic and recent landscape processes that would impede or promote gene flow. Here, we report a genetic survey for the Johnny darter for the upper Midwest using microsatellite DNA. We examined the diversity of Johnny darters within and among three major river drainages in Minnesota and North Dakota: the Red River of the North, the Missouri River, and the Mississippi River. These data provide a baseline for evaluating the genetic structure of other aquatic organisms in this area, especially those fishes historically translocated. Additionally, these data may provide insights regarding the post-Pleistocene relationship between the Red River of the North and the Mississippi River.

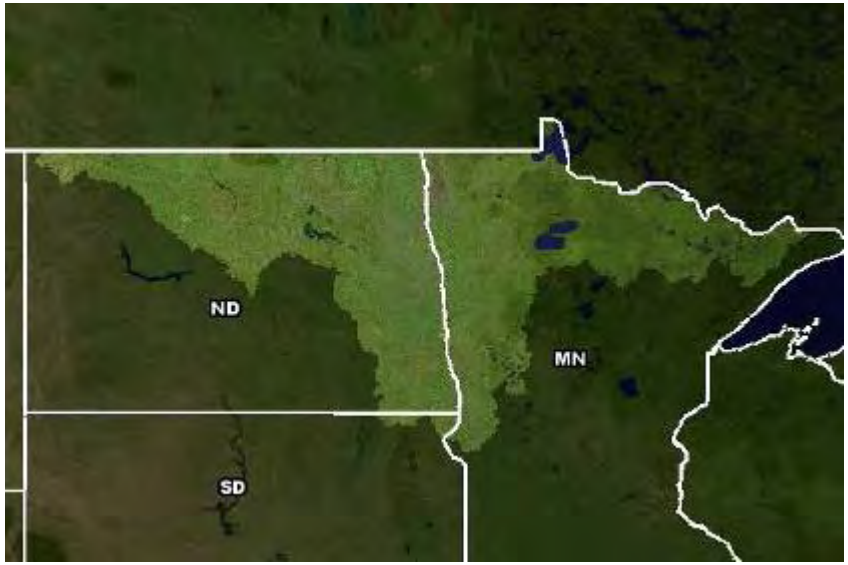
STUDY SYSTEM

The upper Midwest USA, specifically North Dakota and Minnesota, was inundated by glacial advances, with the latest covering most of northern Minnesota and central to eastern North Dakota. Presently, three major river systems drain this area: the Red River of the North (Red River), the Mississippi River and the Missouri River (Figure 1). The Red River watershed drains northwest Minnesota, a small corner of northeast South Dakota, and much of North Dakota northwards into

Lake Winnipeg, which in turn empties into the Hudson Bay. The remainder of North Dakota and much of Minnesota are drained by the Missouri River and Mississippi River, respectively. The lentic and lotic bodies in these watersheds have been isolated since the end of the Pleistocene and offer an excellent opportunity for assessing genetic divergence and gene flow among fish populations.

The relationship between populations of organisms located in the upper Mississippi and Red River, however, is not fully understood. This is particularly true for the period in which both systems were developing. What is known is that as the glaciers of the Pleistocene receded, the westernmost ice lobe uncovered what would become the southern margin of the Red River Valley and its melting glacial waters created Lake Agassiz. This lake was blocked to the north by an ice dam and water drained through several outlets including what would become the Minnesota River. At that time, fish were free to go from the Mississippi river streams to the lake and what would later become the Red river basin. Once the ice dam melted, the lake was able to drain north. At some point between when the lake drained south and then north, the Red River basin was cut off from other basins. Presently, separation between the Red River basin and Mississippi River basin (by way of the Minnesota River) is a height of land between Lake Traverse and Big Stone Lake (Underhill 1958; Teller et al. 2005). Thus, a genetic survey may help evaluate various hypotheses concerning the recent hydrological relationships among drainage basins.

A



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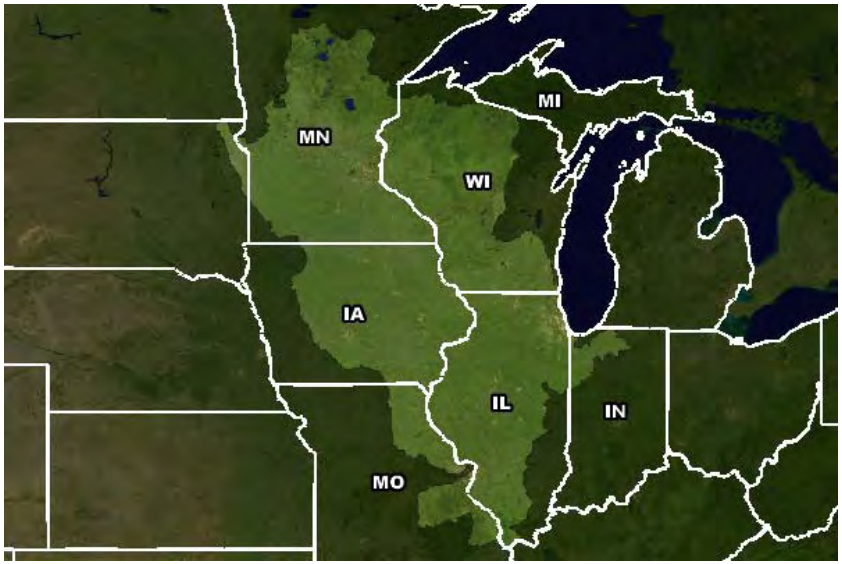

C

Figure 1. Major river drainages of the Upper Midwest. The Missouri River, the Red River of the North, and the Mississippi River watersheds are A, B, and C, respectively (“Regional Watersheds” 2005).

BACKGROUND INFORMATION

Part 1: Darter Natural History

Darters of the genera *Etheostoma* are small benthic fish in the family *Percidae* (Perches). Most fishes in this group do not exceed 10 centimeters standard length and are found throughout the United States, except for Pacific drainages. For instance, the Johnny darter (*Etheostoma nigrum*) may grow to 72 millimeters; however, it rarely exceeds 50 millimeters in standard length (Kuehne and Barbour 1983; Page and Burr 1991). The range of *E. nigrum* extends from the Hudson Bay to southern Mississippi and from Wyoming to the Atlantic coast. It is one of the most abundant and wide-ranging species of *Etheostoma* (Kuehne and Barbour 1983; Eddy and Underhill 1969).

Johnny darters prefer clear, slow flowing waters but can tolerate a broad environmental range (Kuehne and Barbour 1983). Due to their wide environmental tolerance, it is likely that *E. nigrum* was able to persist in the harsh habitats often associated with glacial maximums, which enabled its survival during Pleistocene glacial advances (Underhill 1958).

Subsequently, broad environmental tolerance would have facilitated rapid colonization of habitats as glaciers receded. The vigor of this species and its repeated displacement by Pleistocene glaciers may have led to its large dispersion and provided potential for genetic differentiation among populations after Pleistocene glaciations.

Part 2: *Etheostoma nigrum* as a model

E. nigrum, a non-migratory fish with movements confined locally before and after the spawning season, is commonly found in the lakes and streams of North Dakota and Minnesota (Winn 1958; Eddy and Underhill 1969; Kuehne and Barbour 1983; Hammerson 2005). Despite its abundance and range, little work has been conducted in the upper Midwest to better understand this species (Winn 1958; Hammerson 2005). Indeed, a search of the literature revealed few articles that directly address the biology of the Johnny darter (Winn 1958; Chapleau and Pageau 1985; Propst and Carlson 1989; Parrish and Heins 1991; Leidy 1992). Of these studies, the closest in geographic location to North Dakota or Minnesota were conducted by Winn (1958) near Ann Arbor, Michigan and Leidy (1992) in Wyoming.

Due to the life history and biology of *E. nigrum*, fine-scale genetic structure is likely to exist among populations. It is because of this reason that we chose *E. nigrum* as a model species to study the phylogeography, genetic diversity, and gene flow among lentic and lotic systems of Minnesota and North Dakota. We applied microsatellite markers to evaluate genetic distribution and diversity of *E. nigrum* within and among the Mississippi, Red River, and Missouri drainages.

Part 3: Microsatellites and Molecular Ecology

Microsatellites

Microsatellites are co-dominant DNA markers inherited in a Mendelian fashion and consist of simple tandem repeats averaging 2-5 base pairs (bp) in

length (Levinson and Gutman 1987; Schlotterer 1998). Total repeat size is typically under 200 bp in length (Schlotterer 1998). Because most microsatellites are non-coding neutral markers, they evolve more rapidly than functional genes. The hypervariability of microsatellites makes them especially useful for recently isolated populations.

Microsatellite allele frequencies may also change due to genetic drift. Over time, alleles accumulate in a population. These alleles can be used to determine the genetic uniqueness of populations. Further, microsatellites are useful for the evaluation of gene flow, parentage, and kinship (Queller et al. 1993).

Molecular Ecology

Each population is likely to contain microsatellite alleles that are unique to the population. Slatkin (1993) and DeWoody and Avise (2000) surveyed several studies and concluded that, at neutral markers, genetic differentiation increases with geographic distance. Thus, using microsatellites, it is possible to determine the genetic uniqueness of each *E. nigrum* population and assess gene flow among populations.

We chose to examine nine published microsatellite primers for the genus *Etheostoma*. The primers were developed for *E. virgatum* (Jordan) and *E. olmstedi* (Storer), the latter belonging to the same subgenus as *E. nigrum* (DeWoody et al. 2000; Porter et al. 2002, respectively). By attaching a fluorescent dye to the forward oligonucleotide of each of the nine published primers, we were able to use modern sequencing technology to both genotype individuals and reveal the genetic

structure of the populations of *E. nigrum* sampled throughout North Dakota and Minnesota.

BASIC APPROACH

Part 1: Study Area and Collection Sites

Study Area

Samples were taken throughout North Dakota and Minnesota. Eight different collection sites were identified within the boundaries of the Missouri, Red River, and Upper Mississippi River drainages in both North Dakota and Minnesota. Sites were chosen based on stream properties and the habitat preferences of *Etheostoma nigrum*. The length of each site varied due to accessibility and suitable habitat; but none were longer than 300 meters.

Collection Sites

A total of 212 fish were caught by seining, backpack electrofishing, or snorkeling during ice-free months of 2005 and 2006. Within the Missouri River basin, fish were collected in Beaver Creek and Pipestem Creek (Figure 2).

Within the Red River basin, fish were collected from the Turtle River and the Forest River. These two sites were both in the main Red River watershed and 215 stream-kilometers apart (Figure 3).

Three collection sites were identified in the Mississippi River basin; the Shell River, the Fishhook River, and Mississippi River at Coffeepot landing. The distance between these sites varied from 30 to 686 river-kilometers (Figure 4).

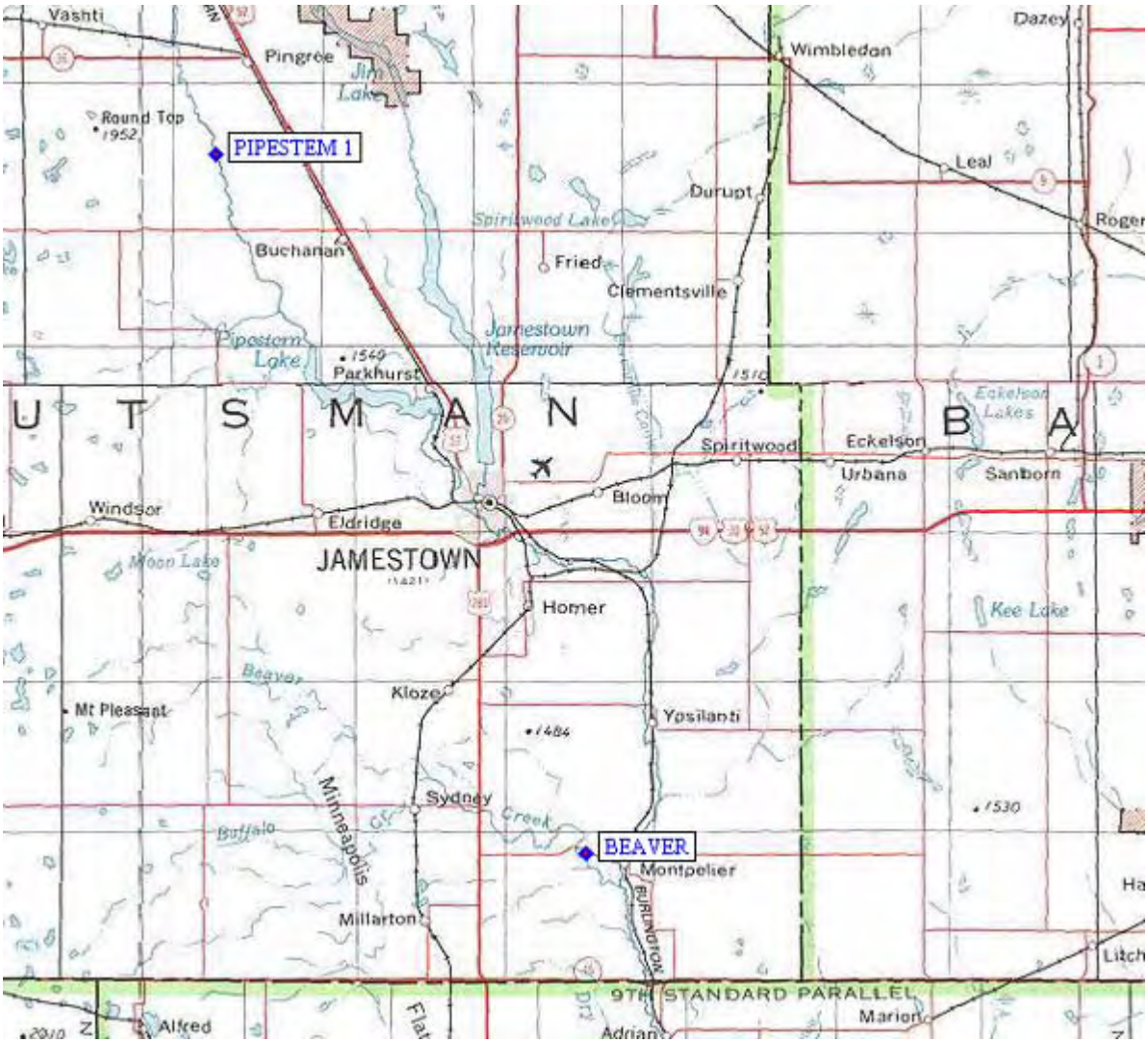

Figure 2. Missouri River basin collection sites. Beaver Creek fish were collected at stream crossing with 49th Street SE, west of Montpelier, ND. Pipestem Creek fish were collected at stream crossing with 21st Street SE, northwest of Buchanan, ND.

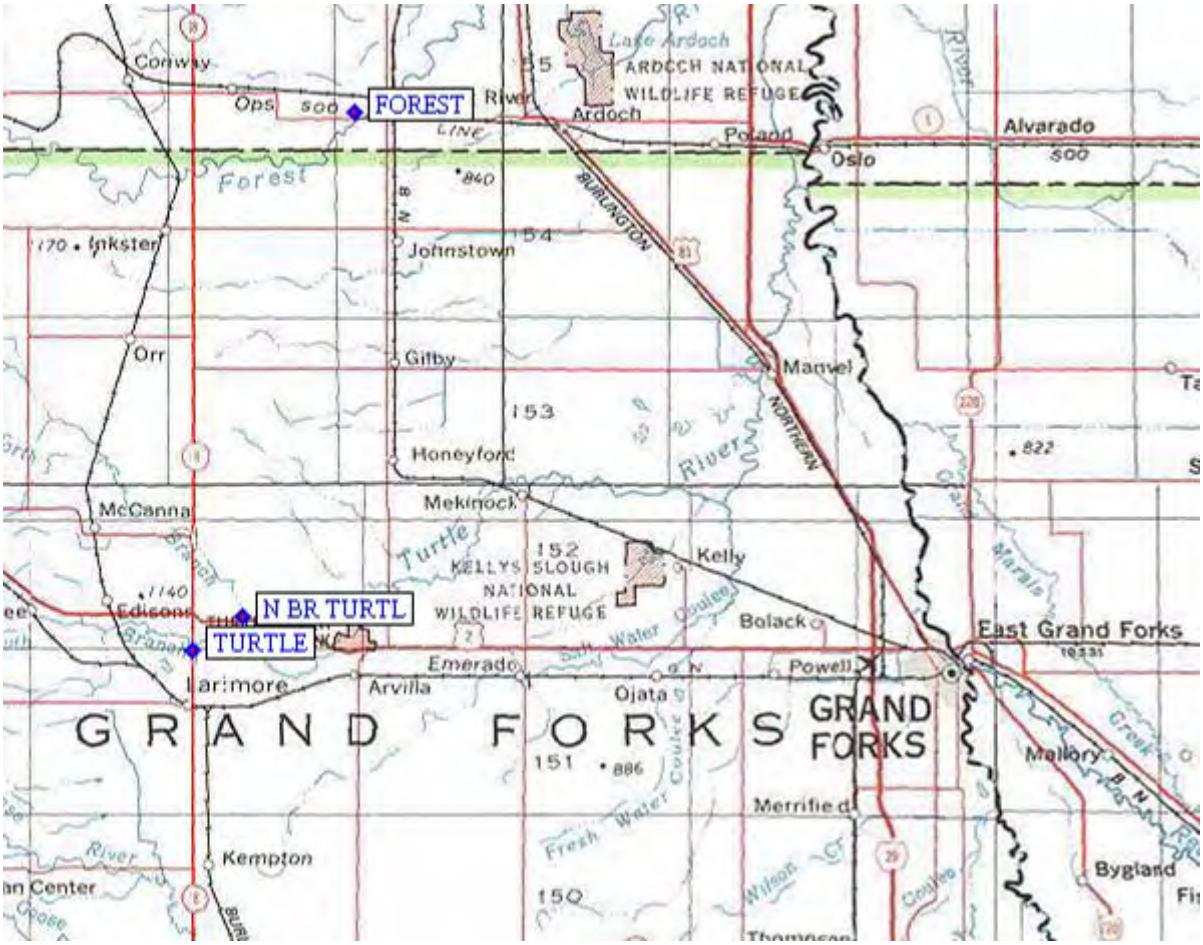

Figure 3. Red River basin collection sites. Forest River fish were collected at stream crossing with County Highway 19, northwest of Grand Forks, ND. Turtle River fish were collected from two locations: the first at main branch stream crossing with State Highway 18 and the second at north branch stream crossing with U.S. Highway 2, both east of Grand Forks, ND.

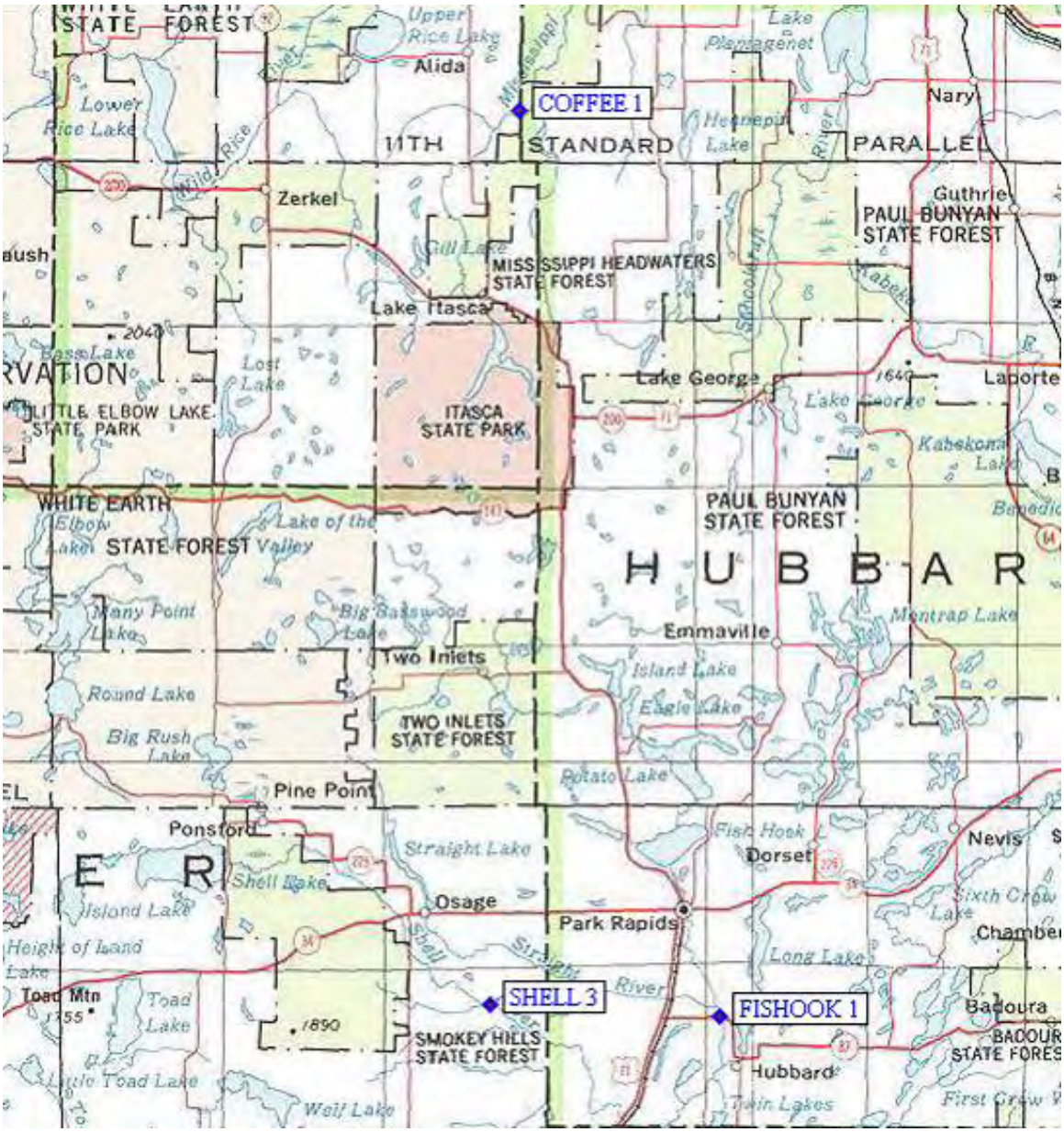

Figure 4. Mississippi River basin collection sites. Shell River fish were collected at stream crossing with County Highway 125, southwest of Osage, MN. Fishhook River fish were collected from stream crossing with State Highway 87, southwest of Park Rapids, MN. Mississippi River fish were collected at Coffeepot Landing near County Highway 9, north of Itasca State Park, MN.

One hydrologically isolated site was identified at Lake Ida (Figure 5). This site is geographically and hydrologically located within the Red River drainage basin.

At each site up to 30 fish were collected, sequestered in a live-well, and administered a lethal dose of anesthetic (500mg/L MS-222). Once sacrificed, fish were placed into labeled bags on ice, and transported to the lab, where they were placed into 1.5 or 15 mL centrifuge tubes, and stored at -80°C.

Part 2: Laboratory Techniques and Analysis

Laboratory Techniques

Genomic DNA was extracted from fin clips taken in the laboratory using a DNeasy kit (Qiagen®, Valencia, California, USA) as per the manufacturer's instructions, including the optional RNAse A step for removal of RNA. Once samples were processed through the final elution, each was checked on an agarose gel to ensure that the DNA extraction was successful.

Each sample was amplified using the Polymerase Chain Reaction (PCR) with one of four different fluorescent-dye labeled primers. PCR conditions were modified from the published literature and optimized for Beckman Coulter fluorescent dye chemistry.

The PCR products, representing four loci for each fish, were then analyzed using a Beckman Coulter CEQ8000 automated DNA Sequencer and its automated fragment analysis program as per manufacturer's instructions with the following alterations. The amount of 600 size-standard was reduced from 0.5 µL to

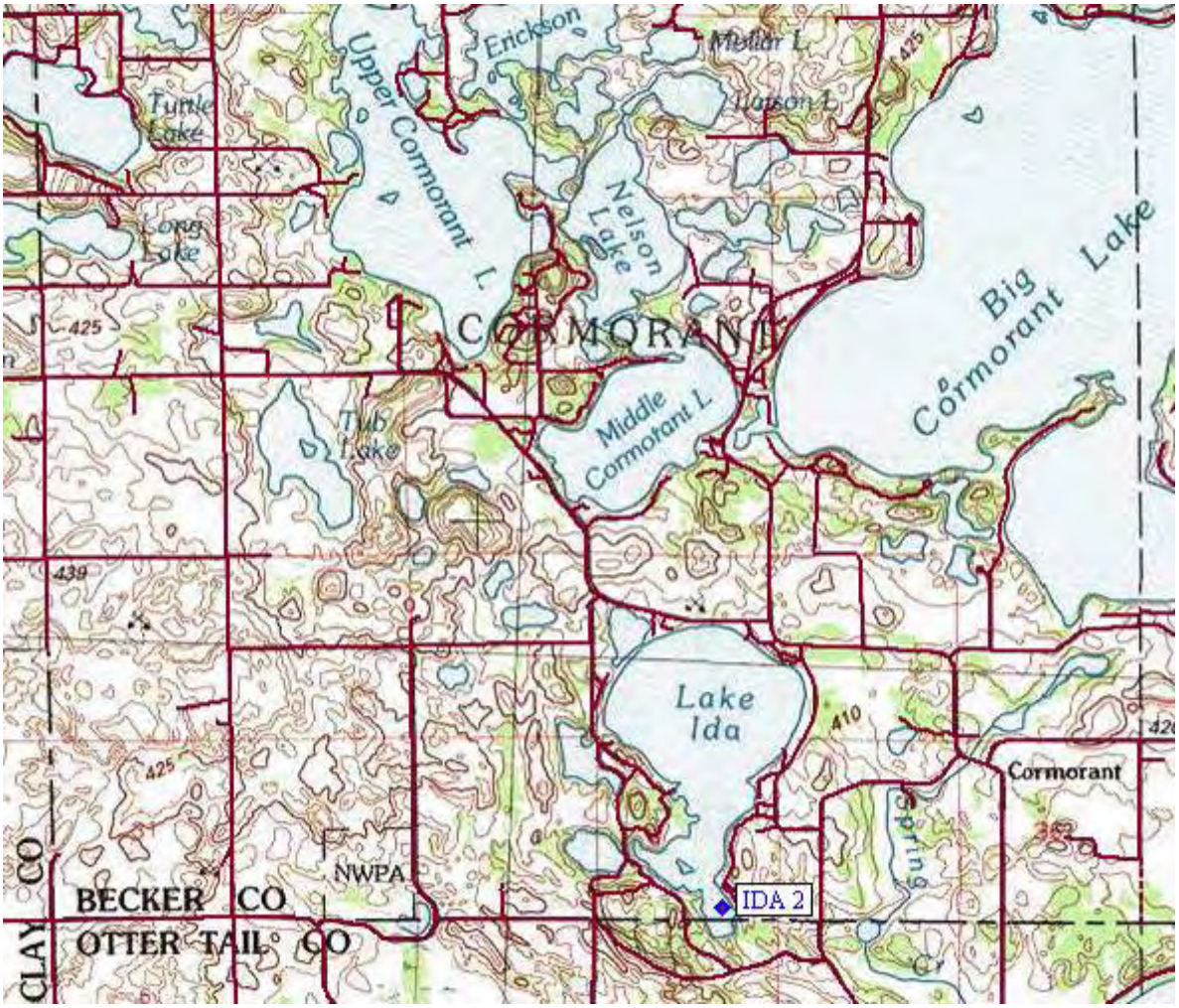

Figure 5. Hydrologically isolated Lake Ida collection site. Fish were collected along a high point offshore, just north of the Becker County line and south of Lake Park, MN.

0.25 µL and the amount of PCR product was increased from 0.25 µL to 0.5 µL per sample. The alleles of each individual were scored and compared to others from its own population and geographically distant populations. PCR and automated fragment analysis were standardized by running the same two individuals with each primer and population across runs for both PCR and sequencer protocols.

Analysis

Arlequin (version 3.1, Excoffier et al. 2005) was used to evaluate linkage disequilibrium, heterozygosities (expected and observed), Hardy-Weinberg equilibrium, allele frequency divergence, F-statistics, and AMOVA. A hierarchical analysis of genetic structure within and among populations with Wright's F-statistics (Weir and Cockerham 1984) was conducted treating populations and drainages as two distinct levels in the hierarchy.

Further, STRUCTURE (version 2.0, Pritchard et al. 2000) was used to conduct genotypic population assignment (MCMC = 100,000 generations; burnin = 17,000; and iterations = 3). This program uses Bayesian methods to assign individuals to groups based on their haplotype and disregards any geographic origin. These data were then evaluated to determine the Bayesian likelihood of population assignment for each individual.

RESULTS

Part 1: Primer Optimization, Linkage Disequilibrium, and Hardy-Weinberg Equilibrium

All *E. nigrum* samples were screened for variation at each of nine published primers. Of these primers, one failed to amplify (CV24), one was monomorphic (EO7), and one had a high frequency null allele (EO9). Two others failed to provide consistently reproducible results (EO12 and CV12; Table 5). Thereafter, four primers were selected for this study: EO4, E06, and D1 AND CV09.

Linkage disequilibrium was not observed among any of the loci examined for any of the populations. Thus, all markers were assumed to be unlinked for further analysis. After Bonferroni correction, only 1 of 26 allele frequencies revealed significant departure from Hardy-Weinberg Equilibrium; CV09 in the Fishhook River population.

Analysis of Molecular Variance (AMOVA) revealed 60% of the total genetic variation occurred within populations, 31% occurred among watersheds and 9% occurred among populations within watersheds (Figure 6). Much of the among watershed variation was due to the extreme differentiation of Missouri basin populations. Once these were removed from analysis, the variance was partitioned as: 74% within populations, 14% among watersheds, and 12% among populations within watersheds (Figure 7).

A hierarchical analysis of genetic structure within and among populations with Wright's F-statistics indicated that all population pairs were significantly

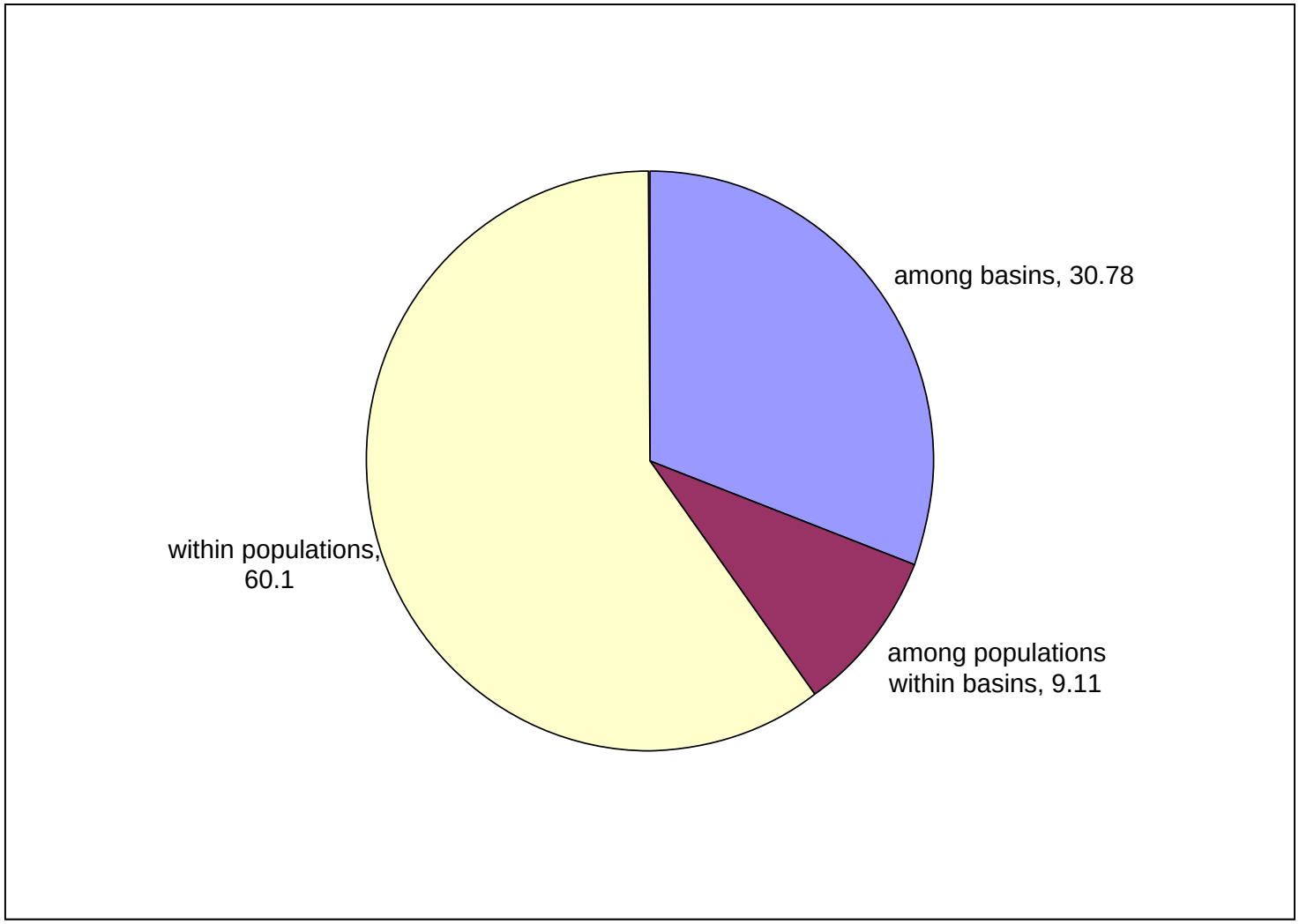

Figure 6. AMOVA within and among the three watersheds.

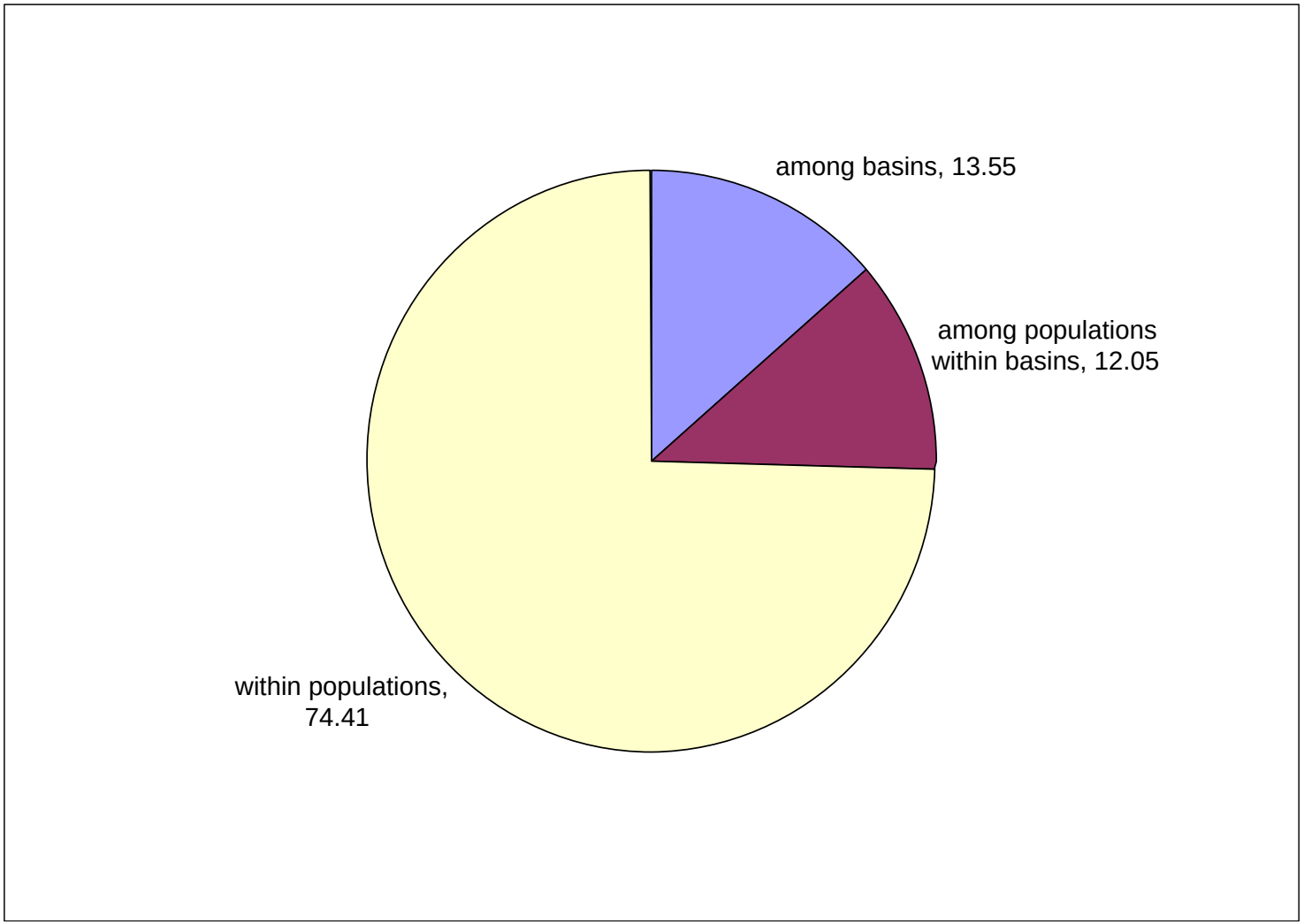

Figure 7. AMOVA within and among the Red River and Mississippi River watersheds.

different with only two exceptions. Beaver Creek was not significantly different from Pipestem Creek. Further, the Lake Ida and Fishhook River populations were not significantly different (Table 8). The number of effective migrants is inversely proportional to genetic divergence as measured by F_{st}: ($N_em = (\frac{1}{Fst} - 1) / 4$). Thus, gene flow appears to be relatively high for these two relationships; N_em was 11.4 and 12.8, respectively (Table 9).

Part 2: STRUCTURE Analysis

STRUCTURE analysis revealed five distinct groups which were essentially organized by geographic location. Bayesian genotypic assignment testing correctly assigned virtually all individuals to their population of origin (Table 10).The single exception to this was the grouping of the Fishhook River out of the Mississippi River complex with fish from Lake Ida. Lake Ida is hydrologically isolated and located within the geographical boundaries of the Red River watershed (Table 11), yet it identifies genetically with the Fishhook River of the Mississippi River watershed.

Table 1. F_{st} values between all eight populations. Significant values are bold, non-significant values are in italics.

	Beaver	Pipestem	Forest	Turtle	Lake Ida	Shell	Fishhook	Coffeepot
Beaver	0							
Pipestem	*0.02137*	0						
Forest	0.41	0.4689	0					
Turtle	0.60343	0.63574	0.21795	0				
Lake Ida	0.48222	0.51667	0.21016	0.40701	0			
Shell	0.56211	0.60209	0.28296	0.32696	0.23935	0		
Fishhook	0.48374	0.5202	0.18324	0.33357	*0.01903*	0.14189	0	
Coffeepot	0.50149	0.55048	0.27268	0.36301	0.16616	0.06192	0.10258	0

Table 2. N_{em} values between all eight populations. Significant values are bold, non- significant values are in Italics.

	Beaver	Pipestem	Forest	Turtle	Lake Ida	Shell	Fishhook	Coffeepot
Beaver	0							
Pipestem	**11.44864**	0						
Forest	*0.359756*	*0.283163*	0					
Turtle	*0.164298*	*0.143243*	*0.897052*	0				
Lake Ida	*0.268436*	*0.233868*	*0.93957*	*0.364236*	0			
Shell	*0.194753*	*0.16522*	*0.633517*	*0.51462*	*0.794496*	0		
Fishhook	*0.266807*	*0.230584*	*1.114331*	*0.499468*	**12.88715**	*1.511928*	0	
Coffeepot	*0.248514*	*0.204149*	*0.666826*	*0.438686*	*1.254574*	*3.787468*	*2.187122*	0

Table 3. STRUCTURE inferred population assignment for all eight populations. Five significantly different groups were assigned for the three watersheds and isolated lake.

Basin of Origin	Population	Inferred Population					Number of Individuals
		1	2	3	4	5	
Missouri River	Beaver Creek	**0.934**	0.022	0.024	0.012	0.008	20
Missouri River	Pipestem Creek	**0.957**	0.013	0.012	0.012	0.007	27
Red River	Forest River	0.052	**0.741**	0.085	0.101	0.021	28
Red River	Turtle River	0.043	0.070	**0.810**	0.046	0.031	26
Isolated	Lake Ida	0.075	0.190	0.056	**0.628**	0.052	22
Mississippi River	Shell River	0.038	0.100	0.150	0.129	**0.583**	30
Mississippi River	Fishhook River	0.035	0.224	0.099	**0.525**	0.117	30
Mississippi River	Coffee Pot	0.029	0.071	0.073	0.213	**0.614**	29

Table 4. STRUCTURE inferred population assignment excluding Missouri River watershed populations. Four significantly different groups were assigned for two watersheds and the isolated lake.

Basin of Origin	Population	Inferred Population				Number of Individuals
		1	2	3	4	
Red River	Forest River	**0.670**	0.095	0.210	0.025	28
Red River	Turtle River	0.083	**0.813**	0.067	0.037	26
Isolated	Lake Ida	0.284	0.065	**0.594**	0.056	22
Mississippi River	Shell River	0.122	0.154	0.146	**0.575**	30
Mississippi River	Fishhook River	0.280	0.106	**0.491**	0.123	30
Mississippi River	Coffee Pot	0.107	0.081	0.210	**0.602**	29

DISCUSSION

This multi-locus dataset represents the first of its kind that attempts to describe the genetic structure of fishes within and between populations of the upper Missouri River, upper Mississippi River, and Red River watersheds and has important consequences for the management and conservation of genetic diversity of the upper Midwest. The high F_{st} values among populations support the genotypic assignment of groups. These groups strongly correlate with the hydrogeography of the area. Both the traditional and Bayesian analyses largely agreed that this species is highly structured, especially at the scale of drainage basin.

The genetic structure among these groups is distinct and indicates that any historical translocations have not impacted *E. nigrum*. This is especially true for the populations of the Missouri River basin and the Red River basin. All four of those sites are located in North Dakota; however, the two basins have distinctly separate genetic structure (F_{st} 0.41 to 0.64). The relationships among the populations of Minnesota, although distinct, are not as discrete as in North Dakota (F_{st} 0.06 to 0.36). This is most likely a result of the historic formation of the two basins as Pleistocene glaciers retreated and their relationship at the time of Lake Agassiz.

Johnny darter populations in the Missouri watershed showed no significant genetic differentiation (F_{st} 0.02). The two Missouri River sites were located approximately 89 river km apart. The lack of differentiation suggests relatively high gene flow: approximately 11 migrants per generation (N_{em}; Table 9). This result was expected for two reasons: the habitats between these two sites are

rather favorable for Johnny darters, which would allow for dispersal; and both of these populations are located along the periphery of the species' range and may be result of relatively recent colonization events. Peripheral populations are more susceptible to loss of genetic diversity due to isolation, genetic drift, and natural selection (Lesica and Allendorf 1994). Others have also reported less genetic variation in peripheral populations than in central populations [e.g. Sonoran topminnow (*Poeciliopsis occidentalis*, Baird and Girard), Vrijenhoek et al. 1985; Clammy Campion (*Lychnis viscaria,* L.), Lammi et al. 1999; and Eastern Collard Lizard (*Crotaphytus collaris collaris*, Say), Hutchison 2003]. In addition to this, Lammi et al. (1999) found no difference in the level of fitness between the two groups, which further reinforces the value of peripheral populations to conservation of species genetic diversity (Lesica and Allendorf 1994; Garcia-Ramos and Kirkpatrick 1997).

The two populations sampled in the Red River watershed were significantly different from one another (F_{st} 0.22). This may be a result of a lack of suitable habitat between the sites; the unfavorable habitat being the main channel of the Red River. The Red River is highly turbid with a majority of its substrate being mud and/or clay and therefore is unlikely to have much suitable habitat for *E. nigrum* (Kuehne and Barbour 1983). Additional sampling of tributaries to the Red River would be useful for finer-scale assessment of genetic structure in this river system. Large genetic distances among tributaries would be consistent with the hypothesis that the Red River serves as functional barrier to gene flow for *E. nigrum* similar to observations of the influence of connectivity to genetic structure in Lahontan

cutthroat trout (*Oncorhynchus clarki henshawi*, Gill and Jordan) by Neville et al. (2006).

The populations sampled in the Mississippi River watershed were assigned into two distinct groups. This result may suggest a local case of genetic drift as there are no obvious barriers to fish movement between these sites. Alternatively, the result may reflect an un-recorded transfer of fish. This hypothesis is supported by the alignment of the Fishhook River with Lake Ida, raising the possibility of a historic transfer of fish between these sites. Additional data will be useful to better characterize the relationships among populations within the Mississippi River.

It is interesting to note the assignment results of the hydrologically isolated Lake Ida. Although located within the geographical confines of the Red River basin, its genetic signature is similar to the Fishhook River population in the Mississippi River drainage (F_{st} 0.02). This result may be the consequence of the hydrologic history of the Red River basin. As glacial ice melted and river paths were altered, fish populations that were formerly hydrologically connected were separated (Underhill 1958; Teller et al. 2005). Lake Ida may be a remnant of the historic connection between the Red River and the Mississippi River. These data are consistent with this hypothesis and demonstrate the value of genetic data in testing phylogeographic hypotheses concerning past hydrologic and biologic connections. As stated earlier, however, these results may also represent a more recent transfer of fish.

Collectively, my data support the hypothesis of a molecular signature of post-glacial dispersal of *E. nigrum*. This result complement work in other regions of

showing genetic structure coinciding with the historic Pleistocene glaciations, both in North America and Europe that have likewise dissected post-glacial dispersal patterns (Stepien and Faber 1998; Bernatchez 2001; Triantafyllidis et al. 2002; Heilveil and Berlocher 2006; Soltis et al. 2006). Similar to Triantafyllidis' et al. (2002) findings, the relatively high diversity in the Mississippi River and the Red River basins could be a result of the retention of alleles from the ancient population while the more distant Missouri River populations lost alleles. This study only further supports the hypothesis that post glacial dispersion greatly influenced the haplotype diversity of *E. nigrum* throughout its northern range.

Management Implications

Fish transfers in the upper Midwest have not been well documented, but both bait fish and game fish have been extensively translocated within and among drainages throughout Minnesota and North Dakota since the time the first fish was shipped. North Dakota Game and Fish Department personnel have noted that the long history of translocations between the drainages in ND and the effects of drought and severe floods have persuaded the department to continue stocking and moving fish between drainages without regard to the genetic structure and historic drainage patterns of many species (NDGF personal communication). Thus, historic drainage patterns may no longer be apparent in game and bait species. In contrast, Johnny darters provide an excellent model for gaining insights as to how genetic structure may have existed for other fishes within and among these drainages.

These data have important management implications as potential hydrological connections among drainages have been proposed. In fact, recent management considerations include the construction of an outlet from Devil's Lake, an isolated body in the Missouri River drainage, into the Red River and possible water transfer from the main Missouri River into the main Red River. Such management actions are likely to result in introduced gene flow between historically isolated drainages and may be of concern if local populations are locally adapted. The introduction of new alleles into established and/or non-existent populations can create populations with less genetic diversity than founder populations and may not always guarantee long term success of the species (Leberg and Ellsworth 1999; Mock et al. 2004).

The genetic evaluation of *E. nigrum* populations also has important conservation and management implications as it will provide a baseline to evaluate population structure of other fishes. Most of the fish populations in the upper Midwest have been isolated since the end of the Pleistocene. Many of the streams and lakes are now 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 as this study shows, gene flow is largely influenced by the dispersal habits of individual species. When fish transfers are planned without consideration of this diversity, populations become genetically homogeneous. These transfers may result in a loss of genetic variation among populations and perhaps even out breeding depression (Leberg

1993; Mock et al. 2004). 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 aid in the establishment of management and conservation units as well as help managers plan for the transfer and stocking of fishes.

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