Conserv Genet (2008) 9:1195–1209
DOI 10.1007/s10592-007-9441-9

RESEARCH ARTICLE

Evaluation of a remnant lake sturgeon population's utility as a source for reintroductions in the Ohio River system

Andrea M. Drauch · Brant E. Fisher · Emily K. Latch · Jennifer A. Fike · Olin E. Rhodes Jr.

Received: 2 July 2007 / Accepted: 15 October 2007 / Published online: 30 October 2007

Abstract The selection of an appropriate source population may be crucial to the long-term success of reintroduction programs. Appropriate source populations often are those that originate from the same genetic lineage as native populations. However, source populations also should exhibit high levels of genetic diversity to maximize their capacity to adapt to variable environmental conditions. Finally, it is preferable if source populations are genetically representative of historical lineages with little or no contamination from non-native or domesticated stocks. Here, we use nuclear (microsatellite) and cytoplasmic (mitochondrial control region) markers to assess the genetic suitability of a potential source population inhabiting the White River in Indiana: the last extant lake sturgeon population in the Ohio River drainage. The White River population exhibited slightly lower levels of genetic diversity than other lake sturgeon populations. However, the population's two private microsatellite alleles and three private haplotypes suggest a unique evolutionary trajectory. Population assignment tests revealed only two putative migrants in the White River, indicating the population has almost completely maintained its genetic integrity. Additionally, pairwise F_{ST} estimates indicated significant levels of genetic divergence between the White River and seven additional lake sturgeon populations, suggesting its genetic distinctiveness. These data indicate that the White River population may be the most suitable source population for future lake sturgeon reintroductions throughout the Ohio River drainage. Furthermore, the White River population appears to be a reservoir of unique genetic information and reintroduction may be a necessary strategy to ensure the persistence of this important genetic lineage.

keywords Lake sturgeon · *Acipenser fulvescens* · Reintroduction · Source selection · Remnant population

A. M. Drauch (✉)
Department of Animal Science, Meyer Hall, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA
e-mail: amdrauch@ucdavis.edu

B. E. Fisher
Atterbury Fish and Wildlife Area, Indiana Department of Natural Resources, Edinburgh, IN, USA

E. K. Latch · J. A. Fike · O. E. Rhodes Jr.
Department of Forestry and Natural Resources, 1159 Forestry Building, Purdue University, West Lafayette, IN 47907, USA

Introduction

The lake sturgeon is a slow growing, long-lived freshwater fish endemic to the Great Lakes Basin, Hudson Bay, and Mississippi River drainages in North America. This remarkable fish may achieve greater than 2 m in length and may live up to 150 years (Harkness and Dymond 1961; Scott and Crossman 1973; Becker 1983). Lake sturgeon once were widely distributed within the Ohio River watershed. Historical records document the presence of lake sturgeon in the Ohio River from western Pennsylvania to its confluence with the Wabash River in Indiana (Jordan 1878; Lachner 1956; Trautman 1981). Although lake sturgeon once were highly abundant in these waters, declines of lake sturgeon in the Ohio River system were noted as early as the turn of the nineteenth century (Trautman 1981). Declines in lake sturgeon abundance within the Ohio River mainstem have been attributed to the river's impoundment, as well as pollution, habitat destruction, and commercial overharvest (Lachner 1956;

Trautman 1981). A few isolated sightings of lake sturgeon in the Ohio River mainstem were documented as late as 1969, but it is generally thought that the species is now extirpated from most of this system (Lachner 1956; Pearson and Krumholz 1984).

Only one population of lake sturgeon is thought to remain in the Ohio River drainage: year-round river residents inhabiting the East Fork of the White River in southern Indiana. Its genetic relationship to lake sturgeon populations in North America is unknown, and it may represent a remnant lake sturgeon lineage characteristic of the Ohio River drainage. Several state management agencies have proposed reintroducing lake sturgeon from the White River to appropriate areas in the Ohio River system. However, before a source can be selected for Ohio River lake sturgeon reintroductions, genetic and demographic characteristics of the White River population must be assessed.

Ideally, a source population should occur in the same geographic region as the proposed stocking or reintroduction event to minimize the risk of outbreeding depression (Carvalho 1993; IUCN 1996; Fitzsimmons et al. 1997; Latch and Rhodes 2005; Stephen et al. 2005a, b). The extent of this region would be defined by a species' biology and the manner in which genetic variation was partitioned among populations within the species range. Source populations also should exhibit ecologically exchangeability, occupying fundamental niches and possessing adaptations to selective regimes similar to those of native organisms (Crandall et al. 2000). However, there are two additional criteria that should be considered in the selection of an appropriate source population: genetic diversity and genetic integrity.

Our first criterion, that potential source populations exhibit levels of genetic diversity comparable to those of alternative source populations, reflects a fundamental principal of all species reintroduction programs, which is to provide populations with the genetic potential to successfully establish and grow in size. Source populations that exhibit high levels of genetic diversity provide reintroduced populations with the capacity to adapt to environmental changes over time (Meffe 1995; Lacy 1997; Hughes and Sawby 2004). Selecting source populations with high levels of genetic diversity is a particularly relevant concern for fish reintroduction programs, where vulnerability of hatchery-reared fish to predation and other stressors may result in demographic bottlenecks due to high post-stocking mortality (Einum and Fleming 2001; Nickelsen 2003).

Also, it is important to examine the genetic composition of potential source stocks to ensure their genetic integrity. Supplemental stocking is a heavily utilized fisheries management strategy and mixing between resident and introduced stocks has been observed (Fritzner et al. 2001; Bartron et al. 2004; Vasemägi et al. 2005). Presumably, resident stocks that have been introgressed by non-native introduced or domesticated fish may no longer be genetically representative of historical lineages, and thus may be no more suitable to be used for reintroduction than fish from other geographic locations. Genetic integrity is a particular concern for White River lake sturgeon, for it is possible that the population may be supplemented by immigrants from recently reintroduced populations in the nearby Mississippi and Missouri Rivers.

The overall goal of this study was to examine the genetic relationships between the remnant White River population and other Midwestern lake sturgeon populations and evaluate its suitability to be used as source population for future reintroductions into the Ohio River drainage in terms of two genetic criteria: (1) its genetic potential as a source population in terms of overall genetic diversity; (2) its genetic integrity as a lineage representative of the Ohio River drainage, not introgressed with genetic material from immigrants originating from ongoing reintroductions in associated river systems. Data from nuclear (microsatellite) and mitochondrial (control region) genomes were used to evaluate levels of genetic diversity within the White River and other lake sturgeon populations that might be considered as potential sources for reintroduction in the Ohio River watershed. Additionally, we evaluated the magnitude of genetic divergence between the White River population in the Ohio River drainage and other potential source populations in the Hudson Bay, Great Lakes Basin, and Mississippi River drainages. Finally, population assignment testing was utilized to determine if the White River population is a unique genetic stock or represents an amalgamation of native and non-native fish that have immigrated from nearby populations (e.g. reintroduced individuals from the Mississippi or Missouri Rivers).

Materials and methods

Sample collection

A total of 499 lake sturgeon were sampled from populations across four different drainages: Hudson Bay, Great Lakes Basin, and Mississippi River, and Ohio River (Fig. 1; Table 1). Populations sampled in the Great Lakes Basin included Lake Winnebago (Wisconsin; $N = 80$), Sturgeon River (Michigan; $N = 30$), and Peshtigo River (Wisconsin; $N = 43$), and populations originating from the Mississippi River drainage were the E. Fork of the Chippewa River (Wisconsin; $N = 18$), Mississippi River (Missouri; $N = 101$) and Missouri River (Missouri; $N = 48$). The White River population (Indiana; $N = 79$) was from the Ohio River drainage, and Lake of the Woods (Minnesota; $N = 100$) was the lone representative of the

Hudson Bay drainage in this study. Lake Winnebago, Sturgeon River, Peshtigo River, Lake of the Woods, Chippewa River, and White River are natural lake sturgeon populations, while the Mississippi and Missouri River populations were recently reintroduced using Lake Winnebago stock (Drauch and Rhodes 2007).

Lake sturgeon were captured in gill nets, trammel nets, and hoop nets, and tissue was collected for DNA extraction in the form of a small clip from the pectoral or dorsal fin. Sampling of White River lake sturgeon was conducted primarily in summer and fall from 1997–2002 and in 2004. Length of White River lake sturgeon captured ranged from 591–1,860 mm fork length, indicating that multiple age classes were sampled. Fin clips from the White River, Lake of the Woods, Chippewa River, Missouri River, and Mississippi River were stored in 95% ethanol at −20°C, while samples from Lake Winnebago, Sturgeon River, and Peshtigo River were dried and stored in scale envelopes at 4°C.

DNA extraction

DNA was extracted following a potassium acetate rapid isolation protocol modified from Sambrook and Russell (2001). For tissue digestion proteinase K was increased to 225 μg from 200 μg and all centrifugation was carried out at room temperature. Nucleic acid pellets were resuspended in 100 μl TLE (10mM Tris–HCl, pH 8.0, 0.1 mM EDTA). Genomic DNA was quantified by eye on agarose gels stained with ethidium bromide and diluted to a concentration of ∼10 ng/μl in dH_20.

Microsatellite genotyping

All 499 lake sturgeon were genotyped at ten disomically inherited tetranucleotide microsatellite loci: Aox 27 (King et al. 2001), Spl 120, (McQuown et al. 2000), AfuG 68b (McQuown et al. 2002), and AfuG 63, AfuG 56, AfuG 160, AfuG 195, AfuG 9, AfuG 204, and AfuG 74 (Welsh et al. 2003). Primers used to amplify AfuG 9 for all samples were redesigned to resolve unsuccessful amplification of this locus in several populations (Drauch 2006). Microsatellite PCRs contained ∼10 ng of DNA, 0.2 mM of each dNTP, and 1.0 unit of Taq polymerase (New England Biosystems), in 1× PCR Buffer (10 mM KCl, 10 mM $(NH)_4SO_4$, 20 mM Tris–HCl, 2 mM $MgSO_4$, 0.1% Triton X-100; pH 8.8). Optimized PCR conditions, including appropriate primer and $MgCl_2$ concentrations for individual loci are provided in (Table 2). Thermal profiles for microsatellite amplification consisted of a denaturation step at 95°C for 2 min, with 40 cycles of 30 s at 94°C, 30 s at T_A°C (Table 2), and 30 s at 72°C, followed by a 10 min hold at 72°C and a 45 min hold at 60°C to complete adenylation and reduce stutter. A separate touchdown profile was used for AfuG 56 and Spl 120, which consisted of an initial denaturation of 1 min at 94°C, followed by 20 cycles of 30 s at 92°C and 40 s of 70°C (decreasing by 0.5°C each cycle), ending with 20 cycles of 30 s at 92°C and 40 s at 60°C (decreasing by 0.5°C each cycle). Samples from Lake Winnebago often did not amplify successfully at AfuG 56 using the standard touchdown profile. Therefore, another touchdown protocol (modified from LI-COR, Inc. 1997; Mock et al. 2002) was substituted

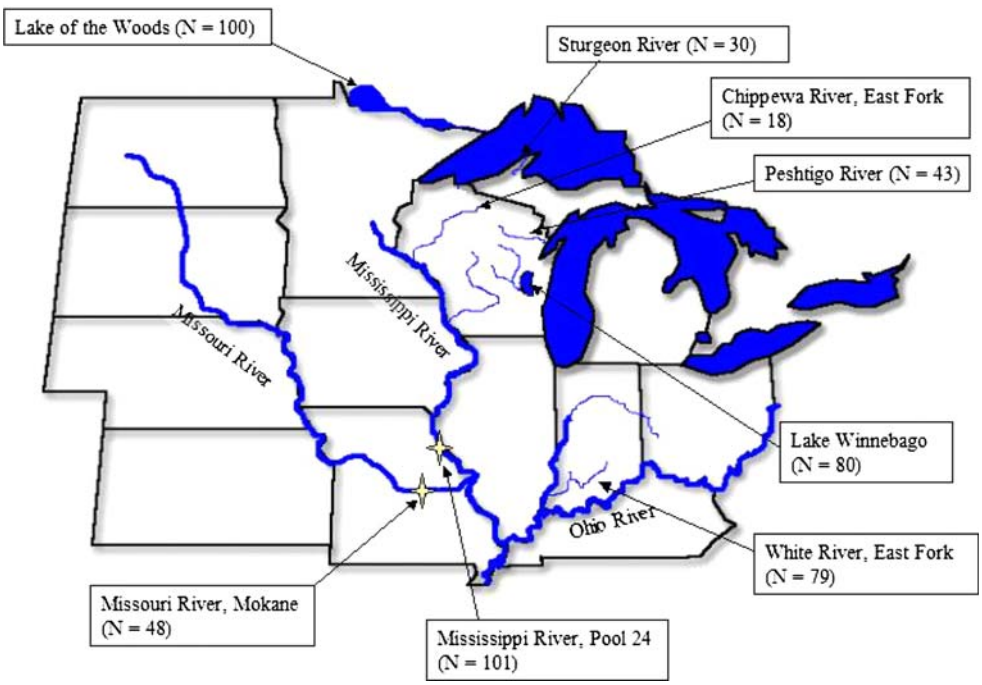

Fig. 1 Locations and sample sizes of lake sturgeon populations included in this study. Stars denote sampling locations on the Mississippi and Missouri Rivers. Populations from the Mississippi River drainage include the White, Chippewa, Mississippi, and Missouri Rivers. Lake Winnebago, Sturgeon River and Peshtigo River belong to the Great Lakes drainage, while Lake of the Woods is part of the Hudson Bay drainage system

Table 1 Populations, sample size, drainage of origin, and sampling details for eight lake sturgeon populations

Population	*N*	Drainage	Sample collection
White River	79	Ohio River	Adult and subadult
Lake of the Woods	100	Hudson Bay	Adult and subadult
Lake Winnebago	80	Great Lakes Basin	Spawning adults
Mississippi River	101	Mississippi River[a]	Adult and subadult
Missouri River	48	Mississippi River[a]	Adult and subadult
Peshtigo River	43	Great Lakes Basin	Spawning adults
Sturgeon River	29	Great Lakes Basin	Spawning adults
E. Fork Chippewa River	18	Mississippi River	Spawning adults

[a] Populations reintroduced from Lake Winnebago stock

for amplification of AfuG 56 in individuals from these populations.

Electrophoresis was performed on an ABI Prism® 377 genetic analyzer (Applied Biosystems) with GeneScan® 400 HD Rox™ size standard (Applied Biosystems) in 5% polyacrylamide gels (Long Ranger Singel Pack; Cambrex). Fragment analysis was conducted with ABI Prism® GeneScan Analysis Software, Version 3.1 (Applied Biosystems) and size-calling was performed in Genotyper® Software, Version 2.5 (Applied Biosystems).

Allelic standards comprised of mixtures of known alleles were amplified prior to genotyping and included in every twelfth lane on each genotyping gel to facilitate comparisons among multiple gels, and ambiguous genotypes were re-amplified and re-scored to reduce genotyping error. Random rescoring of approximately 20% of the dataset was conducted by independent parties as another means of quality control. Of the 1,000 genotypes that were evaluated, seven genotyping errors were detected (0.7% error) and corrected through re-amplification and re-scoring.

Mitochondrial control region sequencing

PCR was performed with primers H740X (sequence extended from Brown et al. 1993) and STURGD1F2X (extended from Scribner 2002) to amplify 585 base pairs of the mitochondrial control region (Table 2). Each PCR (16.6 µl volume) included ~20 ng of DNA, 2.6 mM $MgCl_2$, 0.25 mM of each dNTP, and 1.5 units of Taq polymerase (Eppendorf) in 1X reaction buffer (500 mM KCl, 100 mM Tris–HCl pH 8.3, 15 mM $MgCl_2$). The thermal profile for control region amplification consisted of a 2 min denaturation at 94°C, with 40 cycles of 45 s at 94°C, 1 min at 50°C, and 1 min at 72°C, followed by 1 min at 50°C and a 5 min hold at 72°C. PCR products were quantified in a 2% agarose gel stained with ethidium bromide, and subsequently purified by a wash with 0.12 mM $NaC_2H_3O_2$ in 100% ethanol, followed by precipitation with 70% ethanol and resuspension in 20 µl of dH_2O.

To maximize our confidence in the data, both STURGD1F2X (forward) and H740X (reverse) primers were used to sequence 382 bases of the mitochondrial control region for a total of 414 lake sturgeon samples. Sequencing reactions included ~30 ng of cleaned PCR product, 5 pmol of STURGD1F2X or H740X, 1.5 µl of ABI 5X Sequencing Buffer (400 mM Tris–HCl, 10 mM $MgCl_2$, pH 9.0), 1.5 µl Big Dye, version 3.1 (Applied Biosystems), diluted to a total volume of 10 µl with dH_2O. The sequencing thermal profile consisted of an initial denaturation at 98°C for 10 min, with 25 cycles of 30 s at 98°C, 15 s at 50°C, and 4 min at 60°C.

Sequence products from both forward and reverse primers were purified using the protocol described above.

Table 2 Annealing temperatures (T_A), $MgCl_2$ concentration, and primer volume for microsatellite loci and mitochondrial control region amplification

Locus	Accession number	T_A (°C)	$MgCl_2$ Conc. (mM)	Primer (pmol)
AfuG 9	AF529447	58	1.5	4.0
AfuG 56	AF529472	70	1.5	4.0
AfuG 63	AF529475	52	1.5	4.0
AfuG 68b	AF529480	53	1.25	10.0
AfuG 74	AF529483	52	1.5	4.0
AfuG 160	AF529526	52	1.75	4.0
AfuG 195	AF529548	55	1.5	4.0
AfuG 204	AF529551	55	1.25	10.0
Aox 27	AF067812	52	1.5	4.0
Spl 120	AF276189	70	1.5	8.0
Control region	U32309	50	2.6	6.0

A total of 15 µl of cleaned sequence product from each reaction was submitted to the Purdue University Genomics Core Facility for sequencing on an ABI Prism® 3730 Genetic Analyzer. Sequences were aligned and trimmed using Sequencher®, Version 4.1 (GeneCodes Corporation). To reduce the incidence of sequencing error, forward and reverse sequences within individuals were scanned for discrepancies, and re-sequencing was conducted to confirm all unique haplotypes.

Data analysis

Microsatellite data

Genetic diversity Microsatellite genotype data were converted to appropriate input formats for population genetic analysis software with the program CONVERT (version 1.2; Glaubitz 2004). Deviations from Hardy–Weinberg equilibrium (HWE) for each population × locus combination were evaluated in GENEPOP 3.3 (1,000 batches, 1,000 iterations/batch; Guo and Thompson 1992) and Bonferroni correction was employed to compensate for multiple comparisons. To investigate the presence of null alleles at each microsatellite locus, randomization tests were used to test for heterozygote deficiency in the program FSTAT (Goudet 2001).

Allelic richness (A_T), number of private alleles (A_P), and observed (H_O) and expected (H_E) heterozygosities were calculated for all sampled lake sturgeon populations using the program HP-Rare (Kalinowski 2005). Rarefaction was employed to account for bias in allelic richness induced by unequal sample sizes among populations (Hurlbert 1971; Leberg 2002). Comparisons of rarefacted allelic richness (A_R) and expected heterozygosity (H_{ER}) were made between the White River and each natural lake sturgeon population sampled with Wilcoxon signed rank tests, pairing locus-specific data (Zar 1999; Mock et al. 2004). Additionally, F_{IS} was estimated in FSTAT to look for evidence of non-random mating within populations.

Population structure An overall exact test of allelic differentiation was conducted in GENEPOP 3.3 (100,000 iterations; Raymond and Rousset 1995) to estimate levels of genetic divergence among all sampled lake sturgeon populations. FSTAT was used to estimate θ between all pairs of populations (Weir and Cockerham 1984) and randomization tests were conducted to evaluate significance of pairwise θ. A Bonferroni correction was employed to account for multiple comparisons. To illustrate differences among lake sturgeon populations, an unrooted neighbor-joining tree was generated with the program PHYLIP v3.67 (Felsenstein 1989) using the Nei (1972) genetic distance estimate. Bootstrap values were generated in PHYLIP using the SEQBOOT executable (10,000 iterations).

Assignment testing In order to assess the genetic composition of the White River population, microsatellite data were used in the population assignment program GENECLASS2 (Piry et al. 2004) to detect the presence of immigrants in all sampled lake sturgeon populations. The Rannala and Mountain (1997) criterion was used to identify first generation migrants using the L_home/L_max likelihood ratio (Paetkau et al. 2004). Although this is the most powerful analysis within the GENECLASS2 program, it assumes that all possible sources of migration have been sampled, which may not be the case in this study. It has been shown that the presence of immigration from unsampled populations can bias genetic estimates of population interactions (Waples and Gaggiotti 2006). We accounted for this by conducting a second GENECLASS2 analysis using the Rannala and Mountain (1997) criterion and the L_home likelihood ratio, which can identify potential migrants whose source has not been sampled (Paetkau et al. 2004; Piry et al. 2004). For both analyses, a Monte Carlo resampling method was used to determine critical values for assignments (Paetkau et al. 2004). In this resampling method, 10,000 individuals are simulated for each population by uniting gametes created from observed allele frequencies. This preserves admixture linkage disequilibrium and approximates natural mating schemes better than methods that generate multilocus genotypes on a per locus basis (Paetkau et al. 2004). Next, allele frequencies are calculated from the 10,000 resampled individuals and a genotype likelihood distribution is created for each population. *P*-values for genotype likelihoods of real individuals are derived from this likelihood distribution. To reduce the incidence of type II error, the *P*-value threshold for assignments was set at 0.01.

Mitochondrial DNA

Genetic diversity For mitochondrial control region data, consensus sequences were exported from Sequencher into PAUP* version 4.0b10 (Swofford 2000) to determine the number of informative variable sites in the mitochondrial control region for all sequenced lake sturgeon. The total number of haplotypes (H_T) and numbers of private haplotypes (H_P) within populations were quantified in NETWORK 4.2.1 (Fluxus Technology Ltd 2004). Haplotype diversity within populations (H_d; Nei and Tajima 1981; Nei 1987) and the average number of pairwise

nucleotide differences among haplotypes within populations (k; Tajima 1983) were estimated in DnaSP version 4.0 (Rozas et al. 2003) to provide additional measures of genetic diversity at this locus.

Population structure Population structure at the control region locus was examined using a measure of differentiation that considered not only haplotype frequency differences among populations but evolutionary differences among haplotypes. Population pairwise F_{ST} values were calculated in the program Arlequin (Schneider et al. 2000) using the Kimura 2-Parameter correction to account for differences in the substitution rates for transition and transversion mutations (Kimura 1980). To illustrate evolutionary relationships between haplotypes, a haplotype network was generated in NETWORK 4.2.1 using a median-joining algorithm in which gaps were treated as a fifth state (Bandelt et al. 1999; Posada and Crandall 2001).

Results

Microsatellite data

Genetic diversity

In general, all loci in all populations (with the exception of AfuG 68b) were found to be in HWE with application of the Bonferroni correction. The only inconsistencies observed in the data occurred within the reintroduced populations. Loci AfuG 63, 68b, and 160 were found to be out of HWE in the Mississippi and Missouri River reintroduced populations without a multiple test correction. Because these two populations were initiated by multiple reintroductions and have not yet reproduced, there was no a priori expectation for them to be in HWE and subsequently no loci were discarded from analyses. Null alleles were not detected at any of the ten loci utilized in this study.

The total number of microsatellite alleles detected across loci within populations ranged from 33–47, with the fewest total number of alleles observed in the White River population and the greatest in Lake Winnebago (Table 3). When rarefaction was utilized to account for unequal sample sizes among populations, the number of alleles within populations ranged from 29.8 (White River) to 37.8 (Lake Winnebago). Wilcoxon signed rank tests revealed significant differences in rarefacted allelic richness between lake sturgeon from the White River and both Lake Winnebago ($P = 0.02$) and the Peshtigo River ($P = 0.02$). The numbers of private alleles ranged from 0 to 5 across populations, and more private alleles were generally observed in populations with the largest sample sizes (Table 3). Private allelic frequencies ranged widely from 0.005 to 0.345 (Appendix 1). The presence of unique alleles at high frequencies (0.345) is likely due to genetic differentiation and not sampling artifacts (Appendix 1). Values of H_{ER} ranged from 0.419 (White River) to 0.537 (Sturgeon River; Table 3), and a significant pairwise difference in H_{ER} was found only for the comparison between fish from the White and Sturgeon Rivers ($P = 0.01$). Observed heterozygosities ranged from 0.439 (White River) to 0.561 (Chippewa River), but there was not significant departure from expected levels, as indicated by non-significant F_{IS} values within all populations (Table 3). A non-significant F_{IS} also indicates that mating within the White River population does not deviate from random expectations and there is currently no evidence suggesting that inbreeding is occurring in this population.

Population structure

Tests of overall microsatellite allelic differentiation indicated significant levels of genetic divergence among

Table 3 Sample sizes (N), observed allelic richness (A_T), rarefacted allelic richness (A_R), number of private alleles (A_P) and expected (H_{ER}) and observed (H_o) heterozygosities averaged over ten microsatellite loci for all sampled lake sturgeon populations

	N	A_T	A_R	A_P	H_{ER}	H_o	F_{IS}^a
White River	79	33	29.8	2	0.419	0.439	− 0.047
Lake of the Woods	100	42	33.5	5	0.454	0.457	− 0.007
Lake Winnebago	80	47	37.8	1	0.512	0.521	− 0.017
Mississippi River	101	47	37.0	1	0.486	0.507	− 0.044
Missouri River	48	41	35.6	0	0.498	0.521	− 0.046
Peshtigo River	43	41	37.4	0	0.473	0.449	0.053
Sturgeon River	30	39	37.5	2	0.537	0.512	0.047
Chippewa River	18	35	35.0	0	0.526	0.561	0.070

F_{IS} values are also provided

[a] F_{IS} values not significantly different from zero ($P \geq 0.05$)

populations ($P < 0.001$). Generally, pairwise θ values indicated that the highest levels of genetic divergence existed among drainages (Table 4). The Lake of the Woods population appeared to be the most genetically divergent of all populations surveyed (Table 4). Nearly all pairwise F_{ST} values were significant, with the exception of the comparisons between the reintroduced Mississippi and Missouri River populations and their source population, Lake Winnebago (Table 4, Fig. 2). A more detailed analysis of the genetic relationships between the Lake Winnebago and the reintroduced Mississippi and Missouri River populations is provided elsewhere (Drauch and Rhodes 2007).

Population assignment

Using the L_home/L_max likelihood ratio, GENECLASS2 identified nine possible migrants among the eight lake sturgeon populations. Two of these individuals were sampled in the White River (Table 5). One White River individual was excluded from the White River population ($P = 0.0001$) and assigned to the Mississippi River population while a second individual was excluded ($P = 0.0012$) and assigned to the Chippewa River. Interestingly, one migrant identified in the Lake of the Woods populations appeared to originate from the White River. In the L_home likelihood analysis, only three potential migrants were identified across populations, one each from the White River, Lake of the Woods, and Peshtigo River populations. All of these migrants also were identified in the L_home/L_max likelihood analysis and were assigned to a sampled population, indicating that immigration from unsampled populations was not a concern in this dataset.

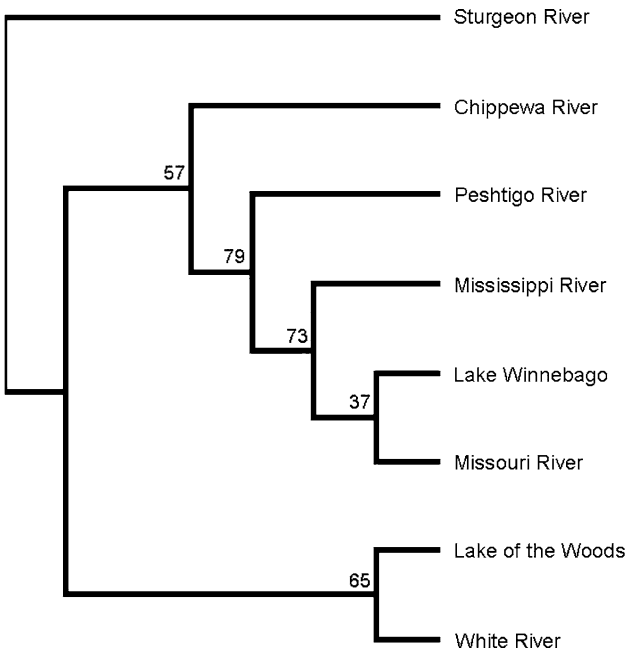

Fig. 2 An unrooted neighbor-joining tree generated with Nei's (1972) genetic distance for microsatellite data. Bootstrap values provided show percent support for each node based on 10,000 iterations

Mitochondrial data

Genetic diversity

A total of 382 bases within the mitochondrial control region were aligned in 414 lake sturgeon and 23 variable sites were identified, 19 of which were parsimony informative. Twenty-two haplotypes were detected across the eight populations surveyed, with numbers of haplotypes within populations ranging from two in the Chippewa

Table 4 Pairwise F_{ST} calculated among lake sturgeon populations based on 382 bases of mitochondrial control region sequence (above diagonal) and pairwise θ estimates for ten microsatellite loci (below diagonal)

	White River	Lake of the Woods	Lake Winnebago	Mississippi River	Missouri River	Peshtigo River	Sturgeon River	Chippewa River
White River	–	0.849[a]	0.636[a]	0.474[a]	0.561[a]	0.595[a]	0.684[a]	0.812[a]
Lake of the Woods	0.159[a]	–	0.260[a]	0.263[a]	0.373[a]	0.303[a]	0.487[a]	0.513[a]
Lake Winnebago	0.086[a]	0.207[a]	–	0.077	0.092	0.157[a]	0.259[a]	0.023
Mississippi River	0.094[a]	0.216[a]	0.006	–	−0.015	0.082	0.156[a]	0.166
Missouri River	0.104[a]	0.230[a]	0.006	0.005	–	0.108	0.191[a]	0.204
Peshtigo River	0.138[a]	0.231[a]	0.050[a]	0.050[a]	0.045[a]	–	0.010	0.271[a]
Sturgeon River	0.112[a]	0.160[a]	0.083[a]	0.092[a]	0.094[a]	0.066[a]	–	0.432[a]
Chippewa River	0.099[a]	0.172[a]	0.054[a]	0.056[a]	0.063[a]	0.073[a]	0.052[a]	–

Bonferroni corrections were employed to account for multiple tests

[a] Statistically significant comparisons (table-wide $\alpha = 0.002$)

Table 5 Potential immigrants identified within the White, Mississippi, Missouri, and Chippewa River populations by GENECLASS2

Population	−log(L_h/L_max)	*P* value	Proposed origin
White River	3.024	0.0000	Mississippi River
	2.549	0.0008	Chippewa River
Lake of the Woods	1.125	0.0007	White River
Lake Winnebago	2.453	0.0079	Missouri River
	3.118	0.0025	Mississippi River
Peshtigo River	2.848	0.0039	Chippewa River
	3.171	0.0022	Missouri River
Sturgeon River	2.475	0.0069	Peshtigo River
Chippewa River	2.057	0.0045	Missouri River

A Monte Carlo resampling method (10,000 iterations) was implemented to determine critical values for assignments (threshold *P*-value = 0.01). *P*-values indicate the likelihood that an individual originated from the population in which it was sampled (Paetkau et al. 2004)

River to nine in the Peshtigo River (average = 5.2; Table 6). Most haplotypes were found at low frequencies across the populations surveyed, although Haplotype A was observed at an overall frequency of 0.408 and was detected in all populations except the White River (Appendix 2). In the Lake of the Woods population, Haplotype A was the predominant haplotype observed and was found in 94% of individuals sampled. The number of private haplotypes (H_P = 4) was greatest in Lake Winnebago, with the Peshtigo and White Rivers each possessing three private haplotypes. Two of the three private haplotypes in the White River were rare, but one was observed at a frequency of 0.259 (Appendix 2).

Haplotype diversity (H_d) across all populations was 0.771, ranging widely within populations from 0.109 (Lake of the Woods) to 0.776 (Peshtigo River; Table 6). The overall average number of pairwise differences between haplotypes (k) within populations was 3.55. The most closely related suite of haplotypes was observed in the Lake of the Woods population, largely because most individuals shared the same haplotype (k = 0.40). Haplotypes in the White River population also were closely related (k = 1.09) and haplotype diversity (H_d = 0.488) in the White River population was low relative to all lake sturgeon populations sampled except Lake of the Woods (Table 6).

Population structure

As observed for the microsatellite data, pairwise F_{ST} values derived from control region data generally indicated that the highest levels of genetic divergence existed among basins (Table 4). High levels of genetic divergence were found between the White River population and all other sampled lake sturgeon populations. No significant genetic divergence was observed between the Sturgeon and Peshtigo River populations, and the Chippewa River population was not significantly differentiated from the Lake Winnebago, Mississippi River, and Missouri River populations. This may be explained by the small sample size in the Chippewa River population (N = 17) and the fact that the two haplotypes detected in that population were among the most common haplotypes observed across populations.

The haplotype network clustered the control region haplotypes into four groups (Fig. 3). The most common haplotypes observed, A and G, were found to be closely related. All individuals from the White River population fell into a single group, with the exception of one individual that had been identified as a possible immigrant in the White River population (see above). Of the 70 individuals found in the White River haplotype group, 80% were sampled from the White River population.

Discussion

In this research we put forth the idea that in order to be considered as a good source for reintroduction programs, a

Table 6 Sample sizes (N), total number of haplotypes (H_T), number of private haplotypes (H_P), Nei's haplotype diversity (H_d) and the average number of nucleotide differences among haplotypes (k) within each of eight lake sturgeon populations are provided

Population	N	H_T	H_P	H_d	k
White River	58	5	3	0.488	1.09
Lake of the Woods	88	2	0	0.109	0.40
Lake Winnebago	66	8	4	0.725	3.09
Mississippi River	81	8	2	0.735	4.27
Missouri River	37	5	0	0.716	4.24
Peshtigo River	41	9	3	0.776	2.92
Sturgeon River	26	3	1	0.591	2.21
Chippewa River	17	2	0	0.529	1.06

Sample sizes differ from Tables 1 and 3 because mitochondrial data could not be collected from all individuals

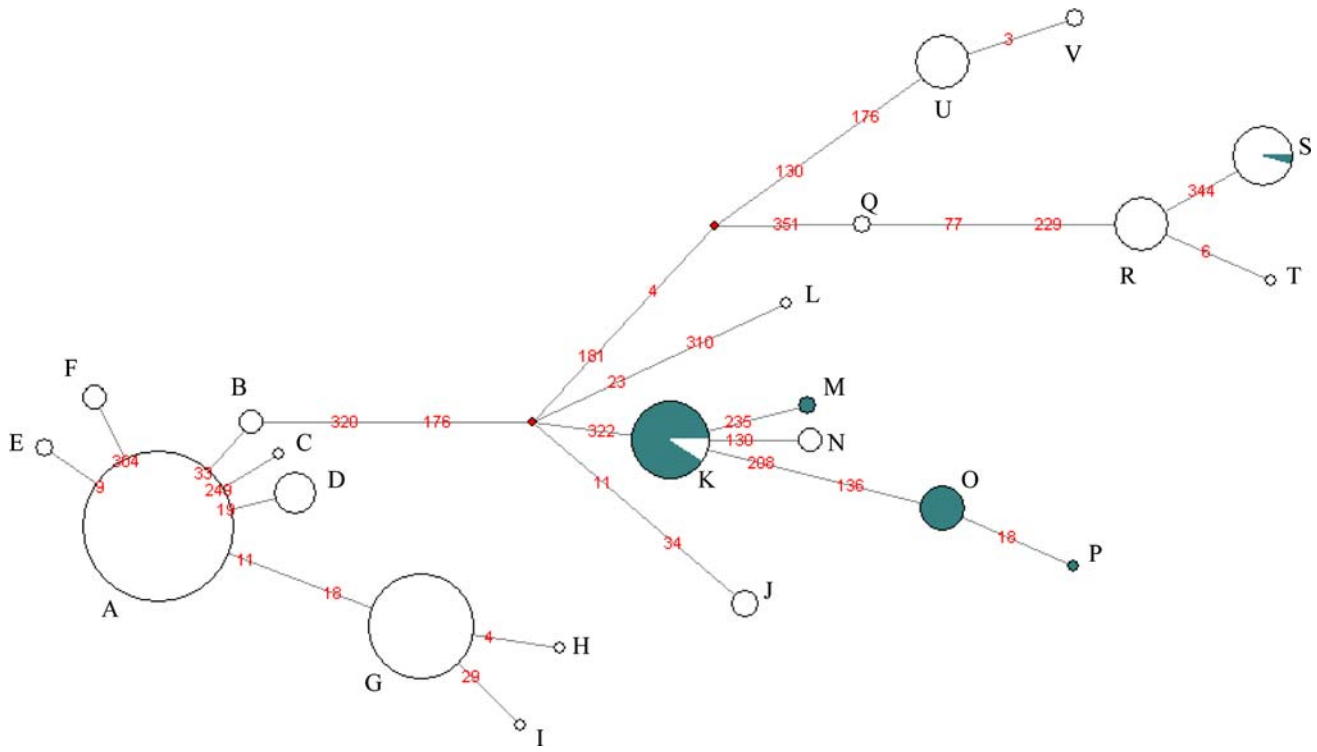

Fig. 3 A median-joining haplotype network generated from 382 bases of the mitochondrial control region sampled in eight lake sturgeon populations. Circle size reflects the relative frequency of each haplotype across all eight populations. Shading indicates the proportion of individuals with a particular haplotype that originate from the White River. Each number in the network indicates the position of a mutation in the control region sequence. Diamonds indicate the presence of a missing ancestral haplotype

population should exhibit two important genetic criteria: (1) it should exhibit high levels of genetic diversity comparable to those of alternative source populations and (2) it should be not be introgressed by gene flow from non-native stocks. We have formulated these criteria based on the fact that reintroduction programs for fish and wildlife species exhibit a unique combination of challenges relative to the conservation of genetic diversity. In many reintroduction programs, much emphasis is placed on maximizing genetic diversity within reintroduced populations at the expense of other genetic criteria for source selection (Mock et al. 2002; Stockwell and Leberg 2002; Arrendal et al. 2004). However, it is important that other genetic considerations, such as the genetic integrity of potential sources, not be overlooked. While this is a concern for all reintroduced species, it is particularly relevant for species that have the ability to disperse long distances from their point of initial reintroduction. The potential for mixing of native and reintroduced stocks exists for fish species that inhabit connected drainages. Evidence of substantial mixing of individuals from existing reintroduced populations with extant native populations can negate the advantages of using such stocks as founding populations.

In the current study, the White River population did exhibit somewhat lower levels of genetic diversity than other lake sturgeon populations surveyed in terms of both allelic richness and expected heterozygosity; on the whole, however, these differences were relatively minor. The number of haplotypes (5) detected in the White River population was similar to the average number observed within eight lake sturgeon populations sampled in this study and the numbers of unique haplotypes observed in the White River population was high relative to most other populations surveyed.

Our identification of few migrants for any population indicates that the movement of lake sturgeon between drainages is low. The two migrants identified in the Lake Winnebago population appeared to originate from two populations reintroduced using Lake Winnebago as a source and therefore likely did not represent actual immigration between those populations. The individual sampled in the Lake of the Woods population that assigned to the White River also may not be a true immigrant. This individual possesses a genotype at locus Afu 204 (141/145) that is unusual for the Lake of the Woods population (all homozygous for 141/141). It is uncertain whether this represents gene flow from another lake sturgeon population or simply a mutation event. Interestingly, the potential migrant in the White River that assigned to the Chippewa River population also was the only individual sampled

from the White River that exhibited Haplotype S (Appendix 2). Haplotype S is found in the reintroduced Mississippi and Missouri River populations and is not exhibited by any individuals sampled from the Chippewa River, suggesting that the individual may have originated from the Mississippi or Missouri River populations. Regardless of their populations of origin, the presence of only two putative immigrants in the White River population suggests it is generally uninfluenced by non-native stocks.

Significant levels of genetic divergence were observed between the White River fish and lake sturgeon from all other sampled populations at both nuclear and mitochondrial markers, suggesting that some degree of subdivision exists between these populations (Table 5). High values for pairwise F_{ST} calculated from these data suggest that it is unlikely that differences between populations are due to sampling error, but rather, from historical isolation and genetic drift among populations. Pairwise estimates of genetic divergence suggest that the greatest levels of structure exist among populations originating from different major drainages, concordant with previous findings (McQuown et al. 2003). Significant genetic differentiation was observed among several populations within drainages, including comparisons between fish from the Sturgeon River and Peshtigo River (Great Lakes Basin), as found by DeHaan et al. (2006). This indicates that for lake sturgeon, the selection of a within-drainage source population for reintroductions might be preferable to reduce the possibility of outbreeding depression.

Our data suggest that the White River lake sturgeon population is (1) a genetically unique remnant stock that has either survived mass extirpations in the Ohio River system or (2) the result of a recent recolonization event. The nearest source for recolonization outside the Ohio River drainage would have been the middle Mississippi or lower Missouri Rivers. However, lake sturgeon were extirpated in the middle Mississippi and lower Missouri Rivers in the late 1800's and early 1900's due to commercial overharvest and habitat degradation (Carlson and Pflieger 1981). Reintroductions of lake sturgeon in these waters by the Missouri Department of Conservation began in 1984, too recently to have produced many of the large adult sturgeon sampled in the White River (Drauch and Rhodes 2007; B. E. Fisher, unpublished data). It seems most parsimonious to assume that White River lake sturgeon population is indigenous to the Ohio River drainage and that it simply escaped harvest and severe habitat destruction possibly due to its location in a rural Indiana landscape.

All evidence suggests the White River population may represent the most appropriate source for lake sturgeon reintroductions in the Ohio River system. The benefits derived from utilizing the White River population for Ohio River system reintroductions are twofold. Lake sturgeon from the White River may be better adapted to environmental conditions of the Ohio River system and reintroducing this stock would increase the probability of establishing viable lake sturgeon populations in this drainage. In addition, increasing the representation of White River stock in the Ohio River system would help preserve the unique genetic information characteristic of this lineage by minimizing the chance that it will be eliminated through stochastic environmental or demographic events.

Due to possible logistical concerns surrounding the initiation of a breeding program for the White River population, other lake sturgeon stocks may initially appear to be attractive alternate sources for reintroduction in the Ohio River drainage. The large and genetically diverse Lake Winnebago population has been used as a source for many reintroductions, not only in the Mississippi and Missouri Rivers, but also in the St. Louis River (Schram et al. 1999), Menominee River (Runstrum et al. 2002), Tennessee River (E. Scott, Jr., personal communication), and Coosa River (C. Marion, personal communication), among others. There are some apparent advantages to using Lake Winnebago as a source for reintroductions in the Ohio River drainage. For example, reproductive behavior of lake sturgeon in the Lake Winnebago system has been well-characterized and spawning adults are very accessible for gamete collection (Bruch and Binkowski 2002). However, over-representation of Lake Winnebago stock across the species' range may have negative consequences for the long-term recovery efforts for this species. For example, populations established from the same source will share similar susceptibilities to disease and inabilities to adapt to certain environmental conditions. Thus, it may be desirable to increase the number of genetic strains represented in lake sturgeon stocking in United States and Canada to decrease the likelihood of a single epidemic or catastrophic event eliminating the species over a large portion of its range.

The Chippewa River population also may appear to be a suitable source for reintroductions into the Ohio River system at first glance. The Chippewa River stock appears to be characterized by high levels of genetic diversity and some historical physical connectivity existed between it and the White River population (both the White and Chippewa Rivers drain into the Mississippi River). In addition, this population has not been utilized for any documented lake sturgeon stocking efforts and therefore concerns about over-representation of this stock are minimal at this time. However, the Chippewa River population is significantly differentiated from the White River at both microsatellite and mitochondrial loci, suggesting some amount of evolutionary independence between these populations.

Additionally, a more intensive sampling effort within the Chippewa River population would be needed to comprehensively evaluate the genetic characteristics of this stock.

The long-term persistence of lake sturgeon in the Ohio River system is dependent upon the conservation of the White River population and initiation of reintroduction programs in other reaches of the Ohio River watershed. The genetic integrity of the Ohio River stock may be reliant upon the exclusive reintroduction of White River lake sturgeon in that system. A single reintroduction of lake sturgeon from a non-native source could permanently alter the gene pool of the Ohio River stock. Ultimately, the use of White River lake sturgeon for repopulation of the Ohio River drainage will depend upon a number of factors, not the least of which will be the logistics of a spawning program providing fish to reintroduce. Additionally, genetic broodstock management will be required to reduce inbreeding and family over-representation in reintroduced populations (Kincaid 1993). In the meantime, it is clear that management agencies involved in the reintroduction of lake sturgeon into the Ohio River drainage should focus their efforts on protecting the White River population and developing appropriate strategies to reintroduce lake sturgeon from this source into other portions of the Ohio River drainage.

Acknowledgments Financial support for this project came from the Wildlife Diversity Section of the Indiana Department of Natural Resources' Division of Fish and Wildlife (State Wildlife Grants T-3 and T-4) and Purdue University. The authors would like to express sincere appreciation to all those involved in lake sturgeon sample collection, including Travis Moore, Craig Gemming, Dennis Topp, Chris Wilson, Kyle Piller, Kim Scribner, and Trent Sutton. We also would like to thank Andrew DeWoody and Trent Sutton for helpful comments on earlier drafts. The manuscript benefited greatly from comments from Robin Waples and two anonymous reviewers. The authors also thank the members of the Wildlife Genetics Lab and Genomic Variation Lab for help with various and sundry analysis questions.

Appendices

Appendix 1 Microsatellite allele sizes and frequencies observed in eight lake sturgeon populations

Locus	Size (bp)	WH (79)	ON (100)	LW (80)	MS (101)	MO (48)	PS (43)	SR (30)	EFC (18)
Aox 27	*N*	79	100	80	101	48	43	28	18
	130	1.000	0.990	0.856	0.886	0.927	0.919	0.857	0.833
	134	–	0.010	0.031	0.025	–	0.023	0.036	0.111
	138	–	–	0.112	0.089	0.073	0.058	0.107	0.056
Spl 120	*N*	79	100	77	101	48	43	29	18
	254	0.544	0.290	0.513	0.525	0.594	0.442	0.224	0.444
	257	0.139	0.015	0.071	0.025	0.167	0.140	0.086	–
	258	–	–	–	–	–	–	**0.345**	–
	262	–	0.115	0.032	0.040	0.031	0.105	0.069	0.056
	273	**0.101**	–	–	–	–	–	–	–
	277	0.006	0.015	0.260	0.282	0.156	0.221	0.138	0.250
	281	0.152	0.110	0.032	0.020	–	–	0.034	0.250
	285	–	0.435	0.091	0.099	0.042	0.081	0.103	–
	289	0.057	0.020	–	0.010	0.010	0.012	–	–
AfuG 63	*N*	79	100	80	100	46	43	27	18
	123	–	–	*0.006*	*0.010*	–	–	–	–
	127	0.177	–	0.262	0.340	0.283	0.314	0.185	0.194
	135	0.095	0.160	0.125	0.055	0.120	0.058	–	0.222
	139	0.721	0.710	0.494	0.520	0.413	0.291	0.500	0.500
	143	0.006	0.130	0.112	0.075	0.185	0.337	0.167	0.083
	147	–	–	–	–	–	–	**0.148**	–
AfuG 68b	*N*	79	100	80	100	47	43	30	18
	154	–	–	*0.006*	*0.030*	*0.021*	–	–	–
	158	–	–	0.012	0.010	0.043	0.256	0.333	0.194
	162	–	–	**0.012**	–	–	–	–	–
	166	–	0.300	0.012	0.025	0.021	0.023	0.017	–
	170	–	0.055	0.037	0.030	0.021	0.046	0.083	0.111

Appendix 1 continued

Locus	Size (bp)	WH (79)	ON (100)	LW (80)	MS (101)	MO (48)	PS (43)	SR (30)	EFC (18)
	174	0.089	–	0.106	0.175	0.181	0.151	0.183	0.167
	178	0.411	0.020	0.469	0.380	0.330	0.244	0.100	0.139
	182	0.272	0.350	0.137	0.170	0.160	0.070	0.100	0.028
	186	0.032	0.020	0.019	0.040	0.011	0.081	0.050	0.111
	190	–	0.255	0.131	0.110	0.149	0.023	–	0.250
	194	0.196	–	0.056	0.025	0.064	0.105	0.133	–
	198	–	–	–	**0.005**	–	–	–	–
AfuG 56	*N*	79	100	79	100	47	43	29	18
	258	–	**0.005**	–	–	–	–	–	–
	262	–	0.075	0.120	0.205	0.223	0.093	0.172	0.194
	266	0.937	0.520	0.867	0.790	0.777	0.907	0.810	0.639
	270	**0.051**	–	–	–	–	–	–	–
	274	0.013	0.400	0.013	0.005	–	–	0.017	0.167
AfuG 160	*N*	77	99	78	98	48	43	30	18
	130	–	–	–	0.010	0.021	0.046	–	–
	134	0.766	0.803	0.647	0.684	0.687	0.814	0.700	0.639
	146	0.214	0.096	0.340	0.306	0.292	0.139	0.300	0.361
	150	0.019	0.096	0.013	–	–	–	–	–
	154	–	**0.005**	–	–	–	–	–	–
AfuG 195	*N*	79	100	78	101	48	43	29	18
	161	0.532	0.530	0.603	0.713	0.667	0.709	0.362	0.472
	165	0.468	0.470	0.397	0.287	0.333	0.291	0.638	0.528
AfuG 9	*N*	79	100	79	98	46	43	29	18
	268	0.240	0.060	0.063	0.082	0.076	0.070	0.155	0.056
	272	–	0.090	0.006	–	–	–	–	–
	276	0.120	–	0.063	0.036	0.098	0.012	–	0.194
	280	0.202	–	0.006	0.005	0.011	–	–	–
	284	0.367	–	0.538	0.673	0.663	0.395	0.379	0.500
	288	0.007	0.220	0.051	0.116	0.054	0.325	0.259	0.028
	292	–	0.010	0.177	0.087	0.087	0.163	–	0.194
	296	–	0.435	0.089	0.051	–	0.023	0.190	0.028
	300	–	0.015	0.006	0.010	0.011	0.012	0.017	–
	304	–	*0.150*	–	–	–	–	–	–
	308	–	**0.005**	–	–	–	–	–	–
	312	–	**0.015**	–	–	–	–	–	–
AfuG 204	*N*	79	100	80	101	48	43	30	18
	141	0.905	0.995	0.537	0.614	0.500	0.709	0.850	0.944
	145	0.095	0.005	0.462	0.386	0.500	0.291	0.150	0.056
AfuG 74	*N*	79	100	80	101	48	43	30	18
	218	0.373	0.085	0.669	0.698	0.698	0.849	0.567	0.722
	222	–	0.250	0.025	0.015	0.010	0.093	0.250	–
	226	0.627	0.665	0.306	0.287	0.292	0.058	0.183	0.278

WH = White River, ON = Lake of the Woods, LW = Lake Winnebago, MS = Mississippi River, MO = Missouri River, PS = Peshtigo River, SR = Sturgeon River, EFC = Chippewa River (East Fork). Sample sizes are in parentheses. *N* indicates the number of individuals genotyped for each locus. Private alleles are in bold. Alleles in italics are shared between source and reintroduced population pairs only

Appendix 2 Mitochondrial control region haplotype frequencies for 414 lake sturgeon across eight populations

Haplotype	WH (79)	ON (100)	LW (80)	MS (101)	MO (48)	PS (43)	SR (30)	EFC (18)	Total (499)
N_S	58	88	66	81	37	41	26	17	414
A	–	0.943	0.197	0.308	0.297	0.415	0.461	0.470	0.408
B	–	–	–	–	–	**0.097**	–	–	**0.010**
C	–	–	**0.015**	–	–	–	–	–	**0.002**
D	–	–	0.167	–	–	0.024	–	–	0.029
E	–	–	–	–	–	–	**0.077**	–	**0.005**
F	–	–	–	*0.025*	*0.054*	–	–	–	0.010
G	–	0.011	0.439	0.296	0.297	0.073	–	0.529	0.186
H	–	**0.015**	–	–	–	–	–	–	**0.002**
I	–	**0.015**	–	–	–	–	–	–	**0.002**
J	–	–	0.030	0.012	–	0.050	–	–	0.012
K	0.672	0.045	–	–	–	–	–	–	0.104
L	–	–	–	**0.012**	–	–	–	–	**0.002**
M	**0.034**	–	–	–	–	–	–	–	**0.005**
N	–	–	–	**0.049**	–	–	–	–	**0.010**
O	**0.259**	–	–	–	–	–	–	–	**0.036**
P	**0.017**	–	–	–	–	–	–	–	**0.002**
Q	–	–	–	–	–	**0.050**	–	–	**0.005**
R	–	–	0.076	0.160	0.054	0.024	–	–	0.051
S	0.017	–	0.030	0.136	0.297	–	–	–	0.060
T	–	–	**0.015**	–	–	–	–	–	**0.002**
U	–	–	–	–	–	0.219	0.461	–	0.051
V	–	–	–	–	–	**0.049**	–	–	**0.005**

WH = White River, ON = Lake of the Woods, LW = Lake Winnebago, MS = Mississippi River, MO = Missouri River, PS = Peshtigo River, SR = Sturgeon River, EFC = Chippewa River (East Fork). Sample sizes are in parentheses. N_S is the total number of sequences obtained for each population. Private haplotypes are in bold. Haplotypes in italics are shared between source and reintroduced population pairs only

References

Arrendal J, Walker CW, Sundqvist A, Hellborg L, Vilà C (2004) Genetic evaluation of an otter translocation program. Conserv Genet 5:79–88

Bandelt H, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16(1): 37–48

Bartron ML, Swank DR, Rutherford ES, Scribner KT (2004) Methodological bias in estimates of strain composition and straying of hatchery—produced steelhead in Lake Michigan tributaries. N Am J Fish Manage 24(4):1288–1299

Becker GC (1983) Fishes of Wisconsin. University of Wisconsin Press, Madison

Brown JR, Beckenbach AT, Smith MJ (1993) Intraspecific DNA sequence variation of the mitochondrial control region of white sturgeon (*Acipenser transmontanous*). Mol Biol Evol 10(2):326–341

Bruch RM, Binkowski FP (2002) Spawning behavior of lake sturgeon (*Acipenser fulvescens*). J Appl Ich 18:570–579

Carlson DM, Pflieger WL (1981) Abundance and life history of the lake, pallid, and shovelnose sturgeons in Missouri. Missouri Department of Conservation, Endangered Species Project SE-1-6 Final Report, Columbia

Carvalho GR (1993) Evolutionary aspects of fish distribution: genetic variability and adaptation. J Fish Biol 43(Suppl A): 53–73

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary process in conservation biology. Trends Ecol Evol 15(7):290–295

DeHaan PW, Libants SV, Elliott RF, Scribner KT (2006) Genetic population structure of remnant lake sturgeon populations in the Upper Great Lakes Basin. Trans Am Fish Soc 135:1478–1492

Drauch AM (2006) Genetic considerations of lake sturgeon reintroductions in the Midwestern United States. MS thesis. Purdue University, Lafayette

Drauch AM, Rhodes OE Jr (2007) Genetic evaluation of the lake sturgeon reintroduction program in the Mississippi and Missouri Rivers. N Am J Fish Manage 27(2):434–442

Einum S, Fleming IA (2001) Implications of stocking: ecological interactions between wild and released salmonids. Nordic J Freshw Res 75:56–70

Felsenstein J (1989) PHYLIP: phylogeny inference package (version 3.2). Cladistics 5:164–166

Fitzsimmons NN, Buskirk SW, Smith MH (1997) Genetic changes in reintroduced Rocky Mountain bighorn sheep populations. J Wild Manage 61(3):863–872

Fluxus Technology, Ltd (2004) Network vers. 4.2.1. Available: http://www.fluxus-engineering.com/sharenet.htm. Cited 21 May 2007

Fritzner NG, Hansen MM, Madsen SS, Kristiansen K (2001) Use of microsatellite markers for identification of indigenous brown trout in a geographical region heavily influenced by stocked domestic trout. J Fish Biol 58:1197–1210

Glaubitz JC (2004) CONVERT: a user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Mol Ecol Notes 4:309–310

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available at http://www2.unil.ch/popgen/softwares/fstat.htm. Cited 5 August 2006

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48:361–372

Harkness WJK, Dymond JR (1961) The Lake Sturgeon. Ontario Department of Lands and Forests, Fish and Wildlife Branch, Maple

Hughes KA, Sawby R (2004) Genetic variability and life-history evolution. In: Ferrière R, Dieckmann U, Couvet D (eds) Evolutionary conservation biology. Cambridge University Press, Cambridge

Hurlbert SH (1971) The nonconcept of species diversity: a critique and alternative parameters. Ecology 52(4):577–586

IUCN (1996) IUCN/SSC guidelines for reintroductions. 41st meeting of the IUCN Council, Gland, Switzerland, May 1995. Available at http://www.iucnsscrsg.org/images/IUCNPosition.pdf. Cited 5 August 2006

Jordan DS (1878) Manual of the vertebrates of the northern United States, including the district east of the Mississippi River, and north of North Carolina and Tennessee, exclusive of marine species. Jansen and McClurg, Chicago

Kalinowski ST (2005) HP-Rare 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol Ecol Notes 5:187–189

Kimura M (1980) A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Kincaid, H (1993) Breeding plan to preserve the genetic variability of the Kootenai River white sturgeon. U.S. Fish and Wildlife Service report (Project 93-27) to Bonneville Power Administration, Portland, Oregon

King TL, Lubinski BA, and Spidle AP (2001) Microsatellite DNA variation in Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) and cross-species amplification in the Acipenseridae. Conserv Genet 2:103–119

Lachner EA (1956) The changing fish fauna of the upper Ohio basin. In: Tryon CA, Shapiro MA (eds) Man and the waters of the upper Ohio basin. Special publication 5, Pymatuning Laboratory of Field Biology. University of Pittsburgh, Pittsburgh

Lacy RC (1997) Importance of genetic variation to the viability of mammalian populations. J Mamm 78(2):320–335

Latch EK, Rhodes OE Jr (2005) The effects of gene flow and population isolation on the genetic structure of reintroduced wild turkey populations: Are genetic signatures of source populations retained? Conserv Genet 6:981–997

Leberg PL (2002) Estimating allelic richness: effects of sample size and bottlenecks. Mol Ecol 11:2445–2449

LI-COR, Inc. (1997) LI-COR 4200 Series genetic analysis manual. LI-COR, Inc., Lincoln, Nebraska

McQuown EC, Sloss BL, Sheehan RJ, Rodzen J, Tranah GJ, May B (2000) Microsatellite analysis of genetic variation in sturgeon: new primer sequences for *Scaphirhynchus* and *Acipenser*. Trans Am Fish Soc 129:1380–1388

McQuown E, Gall GAE, May B (2002) Characterization and inheritance of six microsatellite loci in lake sturgeon. Trans Am Fish Soc 131:299–307

McQuown E, Krueger CC, Kincaid HL, Gall GAE, May B (2003) Genetic comparison of lake sturgeon populations: differentiation based on allelic frequencies at seven microsatellite loci. J Great Lakes Res 29(1):3–13

Meffe GK (1995) Genetic and ecological guidelines for species reintroduction programs: application to Great Lakes fishes. J Great Lakes Res 21(Suppl. 1):3–9

Mock KE, Theimer TC, Rhodes OE Jr, Greenberg DL, Keim P (2002) Genetic variation across the historical range of the wild turkey (*Meleagris gallopavo*). Mol Ecol 11:643–657

Mock KE, Latch EK, Rhodes OE (2004) Assessing losses of genetic diversity due to translocation: long-term case histories in Merriam's turkey (*Meleagris gallopavo merriami*). Conserv Genet 5:631–345

Nei M (1972) Genetic distance between populations. Am Nat 106:283–292

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Nei M, Tajima F (1981) DNA polymorphism detectable by restriction endonucleases. Genetics 97:145–163

Nickelsen T (2003) The influence of hatchery coho salmon (Oncorhynchus kisutch) on the productivity of wild coho salmon populations in Oregon coastal basins. Can J Fish Aquat Sci 60:1050–1056

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation based exploration of accuracy and power. Mol Ecol 13:55–65

Pearson WD, Krumholz LA (1984) Distribution and status of Ohio River fishes. ORNL/Sub/79-7831/1. Oak Ridge National Laboratory, Oak Ridge

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudoin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. J Hered 95(6):536–539

Posada D, Crandall KA (2001) Intraspecific gene genealogies: trees grafting into networks. Trends Ecol Evol 16(1):37–45

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. Proc Natl Acad Sci USA 94:9197–9201

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86(3):248–249

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497

Runstrom A, Bruch RM, Reiter D, Cox D (2002) Lake sturgeon (*Acipenser fulvescens*) on the Menominee Indian Reservation: an effort toward co-management and population restoration. J Appl Ich 18:481–485

Sambrook J, Russell D (2001) Molecular cloning: a laboratory manual, 3rd edn, vol 1. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Schneider S, Roessli D, Excoffier L (2000) Arlequin: a software for population genetics data analysis, Version 3.1. Genetic and Biometry Laboratory, University of Geneva, Switzerland

Schram ST, Lindgren J, Evrard LM (1999) Reintroduction of lake sturgeon in the St. Louis River, Western Lake Superior. N Am J Fish Manage 19:815–823

Scott WB, Crossman EJ (1973) Freshwater Fishes of Canada. Fish Res Bd Can, Ontario-Bulletin 184

Scribner KT (2002) Development of a management plan for lake sturgeon within the Great Lakes basin based on population genetic structure: mitochondrial DNA development and analysis. GLFC Research Completion Report, Great Lakes Fishery Commission, Ann Arbor

Stephen CL, deVos JC Jr, Lee TE Jr, Bickham JW, Heffelfinger JR, Rhodes OE Jr (2005a) Population genetic analysis of Sonoran pronghorn (*Antilocapra americana sonoriensis*). J Mamm 86:782–792

Stephen CL, Whittaker DG, Gillis G, Cox LL, Rhodes OE Jr (2005b) Genetic consequences of reintroductions: an example from pronghorn antelope in Oregon. J Wild Manage 69:1463–1474

Stockwell CA, Leberg PL (2002) Ecological genetics and the translocation of native fishes: emerging experimental approaches. W N Am Nat 62(1):32–38

Swofford DL (2000) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 1.0b10. Sinauer Associates, Sunderland

Tajima F (1983) Evolutionary relationship of DNA sequences in finite populations. Genetics 105:437–460

Trautman MB (1981) The fishes of Ohio. Ohio State University Press, Columbus

Vasemägi A, Gross R, Paaver T, Koljonen M-L, Nilsson J (2005) Extensive immigration from compensatory hatchery releases into wild Atlantic salmon population in the Baltic Sea: spatio-temporal analysis over 18 years. Heredity 95(1):76–83

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Mol Ecol 15:1419–1439

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38(6):1358–1370

Welsh AB, Blumberg M, May B (2003) Identification of microsatellite loci in lake sturgeon, *Acipenser fulvescens*, and their variability in green sturgeon, *Acipenser medirostris*. Mol Ecol Notes 3(1):47–55

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice Hall, Upper Saddle River