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Genetic Comparison of Lake Sturgeon Populations: Differentiation Based on Allelic Frequencies at Seven Microsatellite Loci

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***ABSTRACT**. The lake sturgeon (*Acipenser fulvescens*) has recently become a high priority for restoration management because of the near extinction of the species from many areas of North America. The identification of the level of population differentiation that naturally exists among lake sturgeon populations will be useful in the development of management plans to conserve and restore diversity, and in the choice of donor populations to use for re-introduction. Genetic variation among and within 210 lake sturgeon collected from seven locations (St. Lawrence River, Des Prairies River (tributary to the St. Lawrence River), Mattagami River (Hudson Bay drainage), Menominee River (Lake Michigan drainage), Wolf River (Lake Michigan drainage), Niagara River, and Lake Erie) was examined based on allelic variation at seven microsatellite loci (four disomic and three putative tetrasomic). High levels of variability were detected at these loci. Analyses revealed an average of 8.6 alleles per locus (range 5 to 12 alleles per locus) and heterozygosity values at the four disomic loci ranging from 0.46 to 0.66. Multivariate factor analysis of Nei's genetic distance values produced three distinct population groups that were organized by geography: 1) Mattagami (northern Quebec), 2) Menominee/ Wolf (Lake Michigan—Wisconsin), and 3) St. Lawrence/ Des Prairies/ Niagara/ Erie (lower Great Lakes). Differences based on G-tests summed over all loci occurred between all possible paired comparisons of the collections (*$P < 0.01$*). These analyses indicated that lake sturgeon populations are differentiated within the Great Lakes basin. Managers of this species will need to identify individual populations in their jurisdictions and provide separate consideration for their conservation and rehabilitation.*

***INDEX WORDS:** Lake sturgeon, genetics, microsatellites, population structure, conservation.*

INTRODUCTION

Lake sturgeon, *Acipenser fulvescens*, had a native range in eastern North America that included the Great Lakes, and the Mississippi River and Hudson Bay drainages (Noakes *et al*. 1999). Lake sturgeon were listed as a threatened species by the Convention on International Trade in Endangered Species (CITES) in 1998. Factors leading to the decline in overall number of lake sturgeon include: overexploitation (Ferguson and Duckworth 1997) and habitat alteration, such as pollution and dam construction. These factors have lead to disrupted natural water flow regimes and barriers to annual migration cycles to and from spawning areas. Reestablishment of self-reproducing lake sturgeon populations is hindered by the low reproductive potential of this species. Sturgeons attain sexual matu-

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rity at ages ranging from 12 to 33 years of age (Scott and Crossman 1974) and spawn infrequently once mature. Natural recruitment is a slow process in the long-lived sturgeon under natural conditions, but is slowed further in altered waterways due to reduced reproductive success when mature adults are blocked from movement to spawning habitats. Water flow rates are especially critical for sturgeon (Ferguson and Duckworth 1997, Hay-Chmielewski and Whelen 1997). When flow rates are slow, mortality increases due to egg clumping which asphyxiates eggs and increases fungal infections. When rates are high, contamination by toxic substances can occur due to surface runoff and eggs can move to unsuitable habitats. If flow rates are highly variable, egg re-absorption can be high.

Until recently genetic studies of lake sturgeon have been limited to a few studies using mitochondrial DNA (mtDNA) variation. Low levels of mtDNA variability were reported in lake sturgeon by Guenette *et al*. (1993) who found only three polymorphic sites by using 14 restriction enzymes. Similarly, Ferguson *et al*. (1993) found two polymorphic sites previously described by Guenette *et al*. (1993) using 40 restriction enzymes. Ferguson and Duckworth (1997) reported low levels of variability within mtDNA among lake sturgeon populations. Collections from the Great Lakes/St. Lawrence and Mississippi drainages had a single haplotype expressed and sturgeon from Hudson/James Bay displayed this haplotype plus one additional haplotype. The authors used this evidence to support a two refugia hypothesis whereby postglacial lake sturgeon recolonized the contemporary distribution range by using two different routes of colonization. Mitochondrial DNA has not been sufficiently variable to detect population-level differences and thus help identify management units of lake sturgeon (Ferguson and Duckworth 1997). More variable genetic characters would provide a better means by which to compare sturgeon collections from different localities and to measure levels of population differentiation.

Microsatellites are hypervariable, codominant nuclear DNA markers in which variation is partitioned in one to five base pair repeat motifs (Zajc *et al*. 1997). Microsatellites typically have a higher level of polymorphism than traditional nuclear loci, such as allozymes. In addition, DNA for microsatellite analyses can be easily extracted from tissues obtained by non-lethal sampling (fins, hair, feces), which is essential when working with threatened or endangered species. Pyatskowit *et al*. (2001) and McQuown *et al*. (2002) used microsatellite markers to confirm the Mendelian inheritance patterns of microsatellites in families of lake sturgeon and found a mixture of disomy and tetrasomy.

The development of management plans and implementation of actions to restore lake sturgeon within its native range can benefit from an understanding of the genetic diversity of the lake sturgeon populations. This information is helpful in focusing management on evolutionarily significant units (Moritz 1994), in choosing donor populations to use as sources of reintroduction, and in formulating restoration goals regarding population structure. In the past, information about lake sturgeon population structure has been unavailable to agencies during their development and implementation of management programs (Czeskleba *et al*. 1985, Schram *et al*. 1999, Thuemler 1985). As the ability to gather genetic information has improved over the past decade, several management agencies have incorporated genetic considerations into recovery and rehabilitation programs, including genetic stock assessment (Hay-Chmielewski and Whelan 1997).

In this study, the population structure of lake sturgeon from six locations in the Great Lakes drainage and one location in the Hudson Bay drainage was investigated based on allelic variation at seven microsatellite loci. Genetic variation observed at these loci was described within and among the seven collections.

MATERIALS AND METHODS

Collection Sites

Fin clips were obtained from lake sturgeon collected from seven locations between 1995 and 1999 (Fig. 1, Table 1). Fish from the Mattagami River (Hudson Bay drainage, Ontario) represented the northern range of lake sturgeon distribution and were the most geographically distant from the other six populations. The other six collections were from the southern geographic range in the Great Lakes basin and included fish from the St. Lawrence River (New York), Menominee River (Wisconsin), Des Prairies River (Quebec), Niagara River (New York), Wolf River (Wisconsin), and Lake Erie. Information has been published elsewhere concerning the life history of sturgeon in the Mattagami River (Noakes *et al*. 1999), Wolf River (Probst and Cooper 1955, Folz and Meyers 1985, Kempinger 1988), Menominee River (Thuemler 1985, 1997), the St. Lawerence River (Carlson 1995), and Des Prairies River (Fortin *et al*. 1993). The geographic

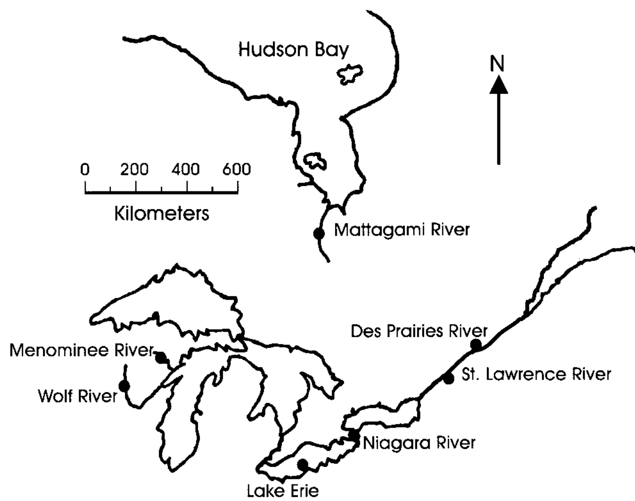

FIG. 1. Sample locations of seven lake sturgeon collections that were analyzed for allelic variation at seven microsatellite loci.

distribution of the collections did not cover the range of the species and was limited in part because of the difficulty in obtaining samples. Also, this project was viewed as a first step in investigating the population structure of lake sturgeon. If population differentiation was observed among these collections then a more geographically thorough study would be warranted.

Microsatellite Analysis

Lake sturgeon collections were analyzed at four disomic (*Afu 68*, *Afu 68b*, *Aox 27*, and *Spl 120*) and three presumptive tetrasomic microsatellite loci (*Spl 35*, *Spl 101*, and *Spl 106*). McQuown *et al.* (2002) and Pyatskowit *et al.* (2001) provided a description of these loci in terms of their allelic variation and inheritance.

DNA was extracted from fin tissues using either the TNES-Urea method of White and Densmore (1992) or the CTAB method of Grewe *et al.* (1993) as modified by Pyatskowit *et al.* (2001). PCR reactions consisted of 50 ng DNA template, 0.22 mM of each dNTP, 0.4 μM of each primer, 0.5 Unit Taq DNA Polymerase (GIBCO, Invitrogen, Carlsbad, California) and 1X GIBCO PCR buffer. Water was added to bring the total reaction volume to 20 μL. Magnesium chloride concentrations were specific for each locus (Table 2). Amplification followed a touchdown PCR protocol (Fishback *et al.* 1999) and was conducted in a MJ Research PTC-100 thermocycler as follows: initial denaturing at 95°C for 1 min followed by 15 cycles of 95°C for 30 s; 65°C for 1 min; 72°C for 1 min. Upon completion of each cycle, annealing temperature was decreased 1°C until 50°C was reached. An additional 15 cycles were run which consisted of denaturation at 95°C for 1 min followed by annealing at 50°C for 1 min and a final extension at 72°C for 1 minute. Twenty µL of formamide loading dye (98% formamide, 0.09% bromophenol blue, 0.09% xylene cyanol FF, 0.01 mM ph 8.0 stock EDTA) was added to each individual reaction, and PCR products were stored at 4°C.

PCR products were separated on 5% polyacrylamide gels (19:1 acrylamide:bis-acrylamide; 7 M urea; 0.5 X TBE buffer). Gels were run at 35 W for 45 min (*Afu 68*, *Afu 68b*, *Aox 27*), 1 h 20 min (*Spl 120*), or 1 h 30 min (*Spl 35*, *Spl 101*, *Spl 106*) and visualized on a Molecular Dynamics 595 Fluorimager. The 5′-end of one primer of each pair was fluorscein-labelled which allowed for visualization without additional staining. Alleles were sized using FragmeNT Analysis, and each gel contained an allelic ladder to assist in consistent scoring of alleles.

Statistical Analysis

Conformance of allelic frequencies at disomic loci to Hardy-Weinberg expectations within samples was assessed by log-likelihood *G*-test (Levene 1949, Sokal and Rohlf 1981). Individuals with aberrant banding patterns (see Banding Patterns in Results) were not included in any analyses. Presumptive tetrasomic loci were not tested for conformance to Hardy-Weinberg expectations because 1) sample sizes per collection were too small for the expected number of genotype classes (15 classes when three alleles are present), 2) effective population sizes would also have to be incredibly large to randomize the possible gamete pool when large numbers of alleles are present, and 3) the type of segregation will effect the distribution (two disomic loci sharing the same alleles will not give the expected distribution if they have different allelic frequencies at the two loci). Genetic differences among samples were assessed with heterozygosity calculations, *G*-tests, and genetic distance coefficients (Nei 1972). To perform these calculations, variation at the duplicated loci was assigned to a single locus.

Allele counts by locus were compared statistically by contingency table analysis with *G*-test (Sokal and Rohlf 1981). The critical values used to

TABLE 1. Location, year of collection, number of individuals sampled (N), and lifestage of the fish (S = spawning adult, A = non-spawning adult, J = juvenile, and A? = individuals whose maturity level was not known, but appeared to be adults based on length, weight, and behavior) of seven lake sturgeon collections analyzed for variation at seven microsatellite loci. Fish sampled provides the number of fish within a collection sampled at different times, places, and life stage.

Collection	Year	Fish Sampled	Total N	Life
St. Lawrence River				
Robert Moses Power Dam, Massena, New York	1995		35	S
Mattagami River				
Below a hydroelectric dam (lat. 50.04 N and long. 82.10 W)	1997		40	A
Menominee River				
Below the White Rapids Dam, Wisconsin	1995		21	S
Des Prairies River			15	
Below Quebec Hydroelectric Dam, between Montreal	1995	7		S
and Laval	1994	8		S
Niagara River			30	
Lower Niagara at Peggy's Eddy	1999	3		J
		2		S
		4		A?
	1998	6		J
Lower Niagara at Fort Niagara Drift	1999	4		J
Lower Niagara at Queenston Drift	1999	7		J
		3		A?
Upper Niagara at Tonawanda Island	1998	1		J
Wolf River				
Bamboo Bend, near Shiocton, Wisconsin	1995		30	S
Lake Erie			39	
Eastern Lake Erie	1998	1		A
	1996	2		A
		1		J
Site unknown, captured by commercial fishermen	1997	11		J
	1996	21		J
Middle Sister Island	1996	2		J
Unknown collection site	1996	1		J

reject the null hypothesis for the *G*-tests were increased (based on Sidak's multiplicative inequality) to account for the increase in type I error when multiple tests of the same hypothesis were made (Cooper 1968). Genetic distances calculated between collections were subjected to multivariate factor analysis and performed using Statistica for Windows version 5.1 (Statsoft Inc.) with factors extracted using a MINRES function. Allelic frequencies, heterozygosity, *G*-tests, and genetic distances were calculated using Genes in Populations 2.2 (designed by B. May and C. Krueger; written in C by W. Eng and E. Paul, available at http://animalscience.ucdavis.edu/extension/Gene.htm).

RESULTS

Genetic Variation within Collections

The average number of alleles found at each locus was 8.6 and ranged from five to 12 alleles (Table 2). The total number of alleles found in each population ranged from 34 (Mattagami River) to 55 (Lake Erie). Of the 60 alleles observed, only 10 occurred at frequencies of < 0.05 in all collections

TABLE 2. Number of alleles by locus found in collections, MgCl2 used for amplificationof DNA (mM), and sources of primers used to examine population genetic structure in seven lake sturgeon collections.

		Number of Alleles Observed								
Locus	Total Alleles Observed	St. Lawerence River	Mattagami River	Menominee River	Des Prairies River	Niagara River	Wolf River	Lake Erie	DNA amplification $MgCl_2$ (mM)	Primer sources
Afu 68	9	7	4	4	6	6	6	7	3.125	May *et al*. (1997)
Afu 68b	12	11	6	10	7	7	7	11	1.500	McQuown *et al*. (2002)
Aox 27	5	4	2	3	4	5	2	5	3.125	King *et al*. (2001)
Spl 35	10	8	5	6	7	9	4	9	3.000	McQuown *et al*. (2000)
Spl 101	8	8	6	6	8	8	5	7	1.500	McQuown *et al*. (2000)
Spl 106	10	9	5	7	6	8	8	9	3.125	McQuown *et al*. (2000)
Spl 120	6	6	6	6	6	6	6	6	2.500	McQuown *et al*. (2000)
Total	60	53	34	42	44	50	39	55		

(Table 3). *Aox 27* showed the least variability with the most common allele *130* ranging in frequency from 0.67 to 0.96 (Table 3).

Average heterozygosity over the four disomic loci was 0.57 and ranged from 0.46 in sturgeon from the Mattagami River to 0.66 in fish from the St. Lawrence River (Table 4). Average heterozygosities across all populations for the disomic loci were 0.49 (*Afu 68*), 0.76 (*Afu 68b*), 0.32 (*Aox 27*), and 0.70 (*Spl 120*). Deviations from Hardy-Weinberg equilibrium were detected in eight cases, four of which were for *Afu 68* (Table 4). All populations conformed to Hardy-Weinberg equilibrium at *Aox 27*.

Genetic Variation among Collections

Genetic distances calculated between each pair of collections ranged from 0.044 (between Lake Erie and Niagara River) to 0.33 (between Mattagami River and Niagara River; Table 5). In general, collections geographically close were genetically more similar to each other than those more distant (Fig. 1, Table 5). Based on genetic distances, the Mattagami River collection from the Hudson Bay drainage was the least similar genetically to the other six collections from the Great Lakes basin.

Multivariate factor analysis of genetic distances produced two axes that described 73.9% of the total variance and organized collections into three geographical groups (Fig. 2). Lake sturgeon from the Mattagami River in northern Ontario (Hudson Bay drainage) formed one group; fish from the Menominee and Wolf rivers in Wisconsin (upper Great Lakes drainage) formed a second group; and collections from Lake Erie and the Des Prairies, Niagara, and St. Lawrence rivers comprised the third group (lower Great Lakes-St. Lawerence River). While the upper and lower Great Lakes groups were more closely related to each other than the Hudson Bay group, significant differences existed among the three geographic groups based on G-test ($P < 0.05$). Differences occurred between all possible paired sample comparisons when G-tests were summed over all loci ($P < 0.01$; Table 5). Among all individual locus comparisons, only the Niagara River and Lake Erie collections showed no differences for the individual locus tests; although the test at *Spl 101* was nearly significant ($P < 0.06$).

Non-amplification

All individuals examined at *Aox 27* and *Spl 35* were successfully amplified. Out of 210 fish, four, five, two, three, and three individuals failed to amplify at *Afu 68*, *Afu 68b*, *Spl 120*, *Spl 106*, and *Spl 101*, respectively. Of these 17, eight individuals amplified successfully at the other six loci, and nine did not amplify at one or two other loci.

Banding Patterns

With the exception of seven fish at *Spl 120*, all individuals examined at the two-dose loci (*Afu 68*, *Afu 68b*, *Aox 27*, and *Spl 120*) displayed a characteristic disomic banding pattern, where heterozygous individuals showed two bands of equal intensity on the gels. Of the seven fish that were exceptions, six individuals expressed phenotypes of three even intensity bands, and one had three bands

TABLE 3. Allelic frequencies at seven microsatellite loci in seven collections of lake sturgeon. Allele refers to the size of an allele in base pairs (bp). The number of individuals in each population that were successfully amplified and included in the analysis is designated by n for each locus.

		Collection						
Locus	Allele (size bp)	St. Lawrence River	Mattagami River	Menominee River	Des Prairies River	Niagara River	Wolf River	Lake Erie
Afu 68								
n		35	39	21	15	30	30	36
	108	0.00	0.28	0.00	0.00	0.00	0.00	0.00
	112	0.59	0.47	0.62	0.53	0.37	0.40	0.42
	116	0.06	0.00	0.02	0.00	0.00	0.02	0.01
	120	0.13	0.00	0.05	0.20	0.05	0.12	0.06
	124	0.07	0.00	0.00	0.10	0.18	0.13	0.25
	128	0.13	0.08	0.31	0.10	0.28	0.32	0.14
	132	0.01	0.17	0.00	0.03	0.10	0.00	0.07
	140	0.00	0.00	0.00	0.03	0.02	0.00	0.06
	144	0.01	0.00	0.00	0.00	0.00	0.02	0.00
Afu 68b								
n		34	40	20	14	28	30	37
	157	0.00	0.01	0.05	0.00	0.02	0.00	0.03
	161	0.03	0.00	0.20	0.04	0.00	0.00	0.01
	165	0.06	0.06	0.02	0.18	0.00	0.08	0.04
	169	0.04	0.00	0.05	0.00	0.11	0.00	0.12
	173	0.25	0.06	0.27	0.25	0.30	0.13	0.26
	177	0.40	0.06	0.12	0.14	0.41	0.42	0.34
	181	0.09	0.48	0.10	0.21	0.00	0.08	0.01
	185	0.06	0.00	0.08	0.07	0.12	0.00	0.10
	189	0.03	0.32	0.03	0.00	0.02	0.22	0.04
	193	0.01	0.00	0.08	0.11	0.00	0.05	0.04
	197	0.02	0.00	0.00	0.00	0.02	0.02	0.00
	133	0.02	0.00	0.00	0.00	0.00	0.00	0.01
Aox 27								
n		35	40	21	15	30	30	39
	126	0.06	0.04	0.07	0.03	0.02	0.00	0.01
	130	0.71	0.96	0.90	0.73	0.73	0.95	0.67
	134	0.11	0.00	0.02	0.10	0.03	0.00	0.10
	138	0.11	0.00	0.00	0.13	0.20	0.05	0.21
	142	0.00	0.00	0.00	0.00	0.02	0.00	0.01
Spl 35								
n		35	40	21	15	28	30	39
	211	0.00	0.00	0.00	0.00	0.01	0.000	0.000
	240	0.24	0.05	0.32	0.02	0.21	0.30	0.09
	242	0.03	0.00	0.02	0.00	0.08	0.00	0.03
	244	0.29	0.70	0.30	0.40	0.34	0.49	0.51
	246	0.07	0.05	0.18	0.10	0.05	0.05	0.03
	248	0.04	0.06	0.02	0.02	0.04	0.00	0.01
	252	0.29	0.14	0.15	0.40	0.23	0.16	0.26
	258	0.01	0.00	0.00	0.05	0.02	0.00	0.04
	276	0.00	0.00	0.00	0.00	0.00	0.00	0.01
	280	0.02	0.00	0.00	0.02	0.02	0.00	0.03

(continued)

TABLE 3. Continued.

		Collection						
Allele Locus	St. Lawrence (size bp)	Mattagami River	Menominee River	Des Prairies River	Niagara River	Wolf River	Lake River	Erie
Spl 101								
n		34	40	20	15	29	30	38
	283	0.01	0.00	0.00	0.07	0.04	0.00	0.01
	290	0.02	0.08	0.01	0.03	0.06	0.00	0.07
	294	0.02	0.01	0.15	0.03	0.14	0.07	0.09
	298	0.14	0.06	0.21	0.15	0.26	0.12	0.11
	303	0.37	0.46	0.37	0.22	0.26	0.42	0.35
	307	0.34	0.35	0.20	0.38	0.18	0.28	0.32
	311	0.07	0.04	0.05	0.08	0.05	0.10	0.06
	315	0.03	0.00	0.00	0.03	0.01	0.00	0.00
Spl 106								
n		35	40	21	15	29	29	35
	212	0.05	0.00	0.00	0.02	0.07	0.03	0.04
	219	0.03	0.00	0.00	0.00	0.00	0.02	0.01
	225	0.00	0.01	0.04	0.00	0.01	0.00	0.03
	230	0.01	0.00	0.00	0.00	0.03	0.06	0.03
	234	0.34	0.46	0.18	0.38	0.17	0.15	0.31
	238	0.20	0.26	0.37	0.13	0.26	0.24	0.28
	243	0.10	0.27	0.31	0.10	0.09	0.28	0.09
	247	0.21	0.01	0.06	0.22	0.28	0.13	0.19
	251	0.05	0.00	0.04	0.15	0.08	0.08	0.01
	257	0.01	0.00	0.01	0.00	0.01	0.01	0.00
Spl 120								
n		30	39	18	15	29	30	38
	254	0.38	0.21	0.47	0.50	0.38	0.52	0.50
	258	0.08	0.01	0.14	0.17	0.31	0.07	0.17
	262	0.18	0.13	0.28	0.17	0.07	0.10	0.05
	274	0.20	0.04	0.06	0.03	0.10	0.15	0.13
	278	0.13	0.22	0.03	0.10	0.03	0.07	0.01
	282	0.02	0.40	0.03	0.03	0.10	0.10	0.13

with one band darker than the other two. These banding patterns may be a result of the presence of a homeologous locus that is detected under certain PCR conditions.

All individuals examined at *Spl 35* showed a characteristic tetrasomic banding pattern, where heterozygotes showed one of three phenotypes: four bands of even intensity, three bands with one band darker than the other two bands, or two bands of even intensity. All phenotypes expressed at *Spl 101* and *Spl 106* showed typical tetrasomic banding with the exception of one individual at *Spl 101* who produced a phenotype of three bands of even intensity and three fish at *Spl 106* that expressed five even-intensity bands.

DISCUSSION

Population Differentiation

Based on genetic variation at seven microsatellite loci, the seven collections formed three distinct clusters that corresponded to geographic location: 1) Hudson Bay drainage (northern Ontario), 2) upper Great Lakes (Wisconsin), and 3) lower Great Lakes-St. Lawrence River. In addition, heterogeneity existed within each geographic group providing evidence for the existence of seven genetically differentiated populations. These results stand in stark contrast to previous studies using mtDNA. Low levels of variation were detected and little differentiation among collections was detected over a comparable geographic range (Guenette *et al*. 1993,

TABLE 4. Observed and expected heterozygosities at four disomic loci for seven collections of lake sturgeon. Average observed heterozygosities for each collection are in the bottom row and for each locus in the last column. * indicates that locus did not conform to Hardy-Weinberg expectations at P < 0.05.

		Collection							
Locus		St. Lawrence River	Mattagami River	Menominee River	Des Prairies River	Niagara River	Wolf River	Lake Erie	Average
Afu 68	*o*	0.57	0.39*	0.48	0.47*	0.43*	0.67	0.42*	0.49
	e	(0.62)	(0.66)	(0.52)	(0.65)	(0.74)	(0.71)	(0.73)	
Afu 68b	*o*	0.79	0.63	0.80*	0.86	0.68*	0.73	0.78	0.76
	e	(0.76)	(0.66)	(0.84)	(0.83)	(0.71)	(0.75)	(0.79)	
Aox 27	*o*	0.49	0.08	0.19	0.47	0.43	0.10	0.51	0.32
	e	(0.46)	(0.07)	(0.18)	(0.43)	(0.42)	(0.10)	(0.50)	
Spl 120	*o*	0.77*	0.74	0.72	0.67*	0.66	0.67	0.66	0.70
	e	(0.75)	(0.73)	(0.68)	(0.68)	(0.73)	(0.68)	(0.68)	
Average		0.66	0.46	0.55	0.62	0.55	0.54	0.59	

Ferguson *et al.* 1993, Ferguson and Duckworth 1997).

The genetic differentiation of the lake sturgeon from the Mattagami River from the Hudson Bay drainage may reflect different colonization origins from Great Lakes populations. The Mattagami River population was not only the least similar genetically to any other population in this study, but also showed the lowest overall variability. This population had the lowest total number of alleles and the lowest within-population heterozygosity (Tables 2, 4). Additionally, allele *108* at *Afu 68* occurred at a frequency of 0.28 in this collection and was found only in this population and not in any of the Great Lakes basin populations sampled (Table 3). Ferguson *et al.* (1993) suggested based on mtDNA variation that lake sturgeon in the northern part of their range may have originated from different refugia than lake sturgeon from the St. Lawrence River watershed. The high differentiation of the Mattagami River population (northern range) from the six Great Lakes basin populations (southern range) provides additional support for their hypothesis.

The results of this study represent a first step in understanding the population structure of lake sturgeon. This study was limited in the number of collections, number of individuals per collection (~30 fish), and in geographic scope. However, despite these limitations substantial genetic differentiation was detected. Sampling additional lake sturgeon populations may reveal the existence of a hierarchical population structure with population differentiation within rivers, among tributaries within drainages, and among two or more drainage basins. To detect such a population structure, samples must be collected from spawning adults. Samples from non-spawning adults may reflect mixtures of migrating populations. In this study, only fin tissues collected from the Menominee, Wolf, Des Prairies, and St. Lawrence rivers were from spawning adults, with individuals from the Mattagami River, Niagara River, or Lake Erie being either juveniles or non-spawning adults. Juvenile collections may result in increased capture of related fish that can affect estimates of genetic diversity and population divergence by increasing the observed genetic differentiation between collections (Wenburg *et al.* 1998) and reducing variation within collections. The inclusion of more loci than the seven used in this study would also increase the robustness of genetic analyses in future studies (Tarr *et al.* 1998, Wenburg *et al.* 1998). Currently, additional disomic microsatellite loci are being developed for future population studies.

Null Alleles

At some loci (except *Aox 27* and *Spl 35*), the DNA from two to five individuals per locus did not amplify after multiple attempts with altered PCR conditions. While DNA degradation or PCR inhibi-

TABLE 5. Pairwise comparisons of seven collections of lake sturgeon. Genetic distances (Nei 1972) are above the diagonal and G values (degrees of freedom) are below the diagonal (all G values exceeded a = 0.01).

Collection	St. Lawrence	Mattagami	Menominee	Des Prairies	Niagara	Wolf	Lake Erie
St. Lawrence	*	0.232	0.089	0.052	0.073	0.073	0.052
Mattagami	414 (54)	*	0.231	0.204	0.329	0.178	0.221
Menominee	148 (54)	293 (47)	*	0.127	0.116	0.076	0.137
Des Prairies	81 (54)	274 (48)	153 (50)	*	0.122	0.130	0.064
Niagara	131 (55)	504 (52)	170 (52)	136 (52)	*	0.084	0.044
Wolf	145 (54)	332 (50)	138 (51)	163 (51)	188 (53)	*	0.078
Lake Erie	127 (57)	426 (54)	192 (54)	106 (55)	89 (55)	186 (55)	*

tion is a potential explanation, null alleles in a homozygous state can cause the failure of DNA to amplify. Null alleles are non-amplifying alleles that result from a nucleotide deletion or substitution at the priming site (Ishibashi *et al*. 1996). Null alleles can be confirmed with inheritance studies or DNA sequencing of the region where null alleles are found to determine if a mutation has occurred in the primer-binding site (Gibbs *et al*. 1997). Pyatskowit *et al*. (2001) identified null alleles at *Afu 68* based on inheritance studies. If null alleles are present, they may result in inflated homozygosity estimates because heterozygotes will be incorrectly scored as homozygotes. Thus, the heterozygote deficiency detected in four populations at *Afu 68* could be due to null alleles.

Segregation Patterns

In this study, with the exception of a few individuals at *Spl 120*, all individuals examined at the disomic loci (*Afu 68, Afu 68b*, *Aox 27*, and *Spl 120*) displayed the expected disomic banding pattern (McQuown *et al*. 2002, Pyatskowit *et al*. 2001). While McQuown *et al*. (2002) could not test a statistical model of inheritance for the presumptive tetrasomic loci (*Spl 35*, *Spl 101*, and *Spl 106*), the majority of individuals expressed banding patterns expected from a tetrasomic locus and showed gametic segregation ratios consistent with tetrasomy. With the exception of one individual at *Spl 101* and three fish at *Spl 106*, all individuals had a banding pattern characteristic of a four-dose tetrasomic locus. While unexpected banding patterns in wild sturgeon are occasionally observed (presumably indicative of unusual ploidy), a far greater number in progeny from artificial crosses are observed (McQuown *et al*. 2002; May unpublished data).

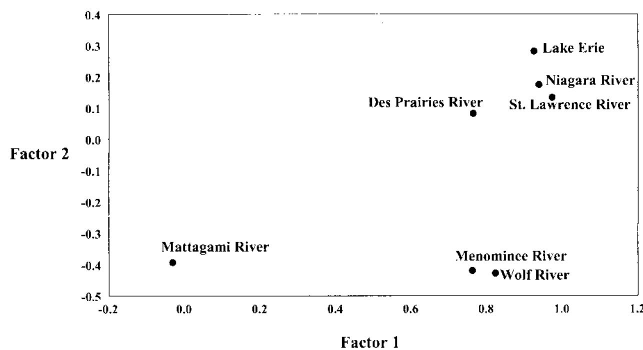

FIG. 2. Results of multivariate factor analysis of Nei's (1972) genetic distances calculated between lake sturgeon collections. Two factors explained 74% of the total variance.

Management Implications

A crucial goal in lake sturgeon conservation is to preserve genetic diversity over the range of the species. Species fitness and survival are maintained when extant genetic variability is not lost (Meffe and Carroll 1997). Genetic diversity is fundamentally organized within and among populations; thus, populations become the primary biological unit of focus for conservation management. When genetically differentiated populations are identified or suspected to exist, they need to be provided protection from stressors such as habitat degradation and overfishing. This study provides preliminary evidence for the existence of differentiated populations and should provide motivation within management agencies to conduct more extensive investigations into the population structure of lake sturgeon within their jurisdictions. If management programs include the stocking of lake sturgeon into lakes and streams, the source of donor individuals must be

carefully evaluated if the genetic integrity of unique populations is to be protected. For example, the stocking of sturgeon from the Wolf River in Wisconsin into the waters of the St. Lawrence River would jeopardize the genetic differences between these two populations. Donor individuals should be genetically similar to the existing remnant lake sturgeon populations in the waters to be stocked and great care should be given to using the large numbers of contributing parents. The conservation of the lake sturgeon populations will be accomplished when management strategies for lake sturgeon are organized around local population structure and include the careful consideration of stocking programs and address problems such as habitat degradation and overfishing.

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