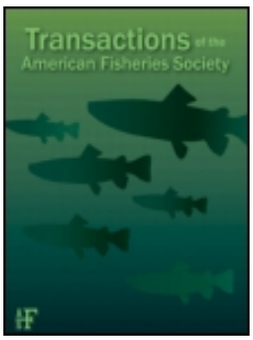

Transactions of the American Fisheries Society

ISSN: 0002-8487 (Print) 1548-8659 (Online) Journal homepage: https://www.tandfonline.com/loi/utaf20

Characterization and Inheritance of Six Microsatellite Loci in Lake Sturgeon

Eve McQuown , Graham A. E. Gall & Bernie May

To cite this article: Eve McQuown , Graham A. E. Gall & Bernie May (2002) Characterization and Inheritance of Six Microsatellite Loci in Lake Sturgeon, Transactions of the American Fisheries Society, 131:2, 299-307, DOI: 10.1577/1548-8659(2002)131<0299:CAIOSM>2.0.CO;2

To link to this article: https://doi.org/10.1577/1548-8659(2002)131<0299:CAIOSM>2.0.CO;2

Published online: 09 Jan 2011.

Transactions of the American Fisheries Society 131:299–307, 2002

NOTES

Characterization and Inheritance of Six Microsatellite Loci in Lake Sturgeon

EVE MCQUOWN,*[1] GRAHAM A. E. GALL, AND BERNIE MAY

Department of Animal Science, Meyer Hall, University of California, Davis, California 95616, USA

Abstract.—The lake sturgeon *Acipenser fulvescens* is a threatened species historically found in the Mississippi, St. Lawrence, and Hudson River drainage basins of North America. The complexity of the lake sturgeon genome necessitates confirmation of the Mendelian inheritance of molecular markers before its use in population-genetic studies. Six microsatellite loci were used to examine inheritance patterns in six lake sturgeon families. Three loci (*Spl120**, *Aox27**, and *Afu68b**) conformed to a simple, disomic model of Mendelian inheritance. Homeologous loci were observed for *Aox27** and *Afu68b*. Banding patterns, gametic segregation patterns, and evidence of double-reduction events in the progeny support a tetrasomic mode of inheritance at three loci (*Spl35**, *Spl101**, and *Spl106**). A model of inheritance was not tested for these presumptive duplicated loci due to the small number of observations of each phenotype. A number of aberrant progeny phenotypes were observed that could have resulted from double reduction, mutation, unreduced gametes, or null alleles. The results indicate that the lake sturgeon genome is evolving from tetrasomy to disomy. We caution that attention should be paid to the quality of artificial fertilization in the production of lake sturgeons for supplementing natural populations.

The lake sturgeon *Acipenser fulvescens* belongs to the family Acipenseridae and is one of five North American species of the genus *Acipenser*. The original distribution of this species included the Mississippi, St. Lawrence, and Hudson River drainage basins of North America (Guenette et al. 1993). Overfishing, loss of habitat through the destruction of spawning grounds and the construction of dams, and the reduction of water quality by pollution have all contributed to the overall decline of the lake sturgeon. The Convention on Trade in Endangered Species (CITES, Appendix II) listed it as a threatened species in 1998. The general goals of management agencies today are to restore the numbers of lake sturgeons to historical levels and to promote self-sustaining populations.

Genetic analysis of the lake sturgeon is limited to a few published studies on molecular variability. Guenette et al. (1993) used 14 restriction enzymes to examine the variation in the mitochondrial genome and found only three polymorphic mitochondrial restriction sites. Of the 40 restriction enzymes used by Ferguson et al. (1993) to search for variation in the mitochondrial DNA (mtDNA) molecule, only two showed polymorphism; these corresponded to two mtDNA haplotypes observed in a later study by Ferguson and Duckworth (1997). Most recently, Pyatskowit et al. (2001) studied the inheritance patterns of five microsatellite loci in 10 families of lake sturgeons and found evidence of both tetrasomy and disomy.

While mtDNA is valuable for determining the existence of evolutionary lineages, lack of variability has prevented the detection of genetic differences among lake sturgeon populations (Ferguson and Duckworth 1997). Since their discovery, microsatellites have been used frequently in studies of population structure (e.g., Gibbs et al. 1997; Wenburg et al. 1998; Broders et al. 1999). The value of these markers results from their high level of variability coupled with ease of analysis. Additionally, microsatellites do not require lethal sampling of the fish, which is valuable when working with a threatened or endangered species. The analysis of microsatellite data in population and evolutionary genetic studies is based on the assumptions of selective neutrality, codominance, and Mendelian inheritance. Validation of these assumptions is critical for lake sturgeon because of their potential polyploid character (Blacklidge and Bidwell 1993). The presence of null alleles, the level of duplication, and the actual mode of inheritance can be confirmed through inheritance studies of families of known parentage.

In this paper, the inheritance of six microsatellite loci is described for six families of lake sturgeons.

* Corresponding author: emcquown@dfs.state.va.us

[1] Present address: Forensic Biology Section, Virginia Division of Forensic Science, 700 North 5th Street, Richmond, Virginia 23219, USA.

Received September 8, 2000; accepted August 30, 2001

The primary objective of the study was to develop informative markers that will aid in examining population structure in this threatened fish species.

Methods

Source of fish.—In May 1995, lake sturgeons were captured on the Des Prairies River, Montreal, Quebec. Details of the matings are described in Pyatskowit et al. (2001). The parental combinations of the six families (from two females and four males) used in this study are listed in Table 4 below. Lake sturgeon fry were stored either in lysis buffer or at −80°C. DNA extraction followed a hexadecyltrimethylammonium bromide (CTAB) method (Grewe et al. 1993) as modified by Pyatskowit et al. (2001).

Microsatellite development.—Due to the low number of published lake sturgeon microsatellite primers, a general screen was conducted to search for informative loci among primers designed for amplification in related species. A total of 113 primer pairs from shovelnose sturgeon *Scaphirhynchus platorynchus* (McQuown et al. 2000) were tested on five lake sturgeon individuals from the Mattagami River to evaluate polymorphism and the quality of amplification. Twenty-two of these primer pairs were chosen to be optimized and further tested on lake sturgeons. The remaining primers were discarded because they did not amplify, showed nonspecific products, had numerous bands (resulting in the inability to accurately score those individuals), or were monomorphic.

The 22 shovelnose sturgeon primer pairs were tested along with 16 from lake sturgeons (May et al. 1997) and 6 from Atlantic sturgeons *Acipenser oxyrinchus* (King et al. 2001) by amplifying DNA from eight lake sturgeon individuals (Mattagami River). Thirty-nine of the remaining primer pairs were rejected because of unclear product resolution on the gels, inability to score due to multiple bands, failure to amplify, or monomorphism. Loci *Spl35*, Spl101*, Spl106*, Spl120** (McQuown et al. 2000), and *Aox27** (King et al. 2001) were selected for further optimization and, ultimately, use in this study.

Microsatellite locus *Afu68** (May et al. 1997) weakly amplified a second banding pattern which, when gel-extracted and sequenced, proved to be the product of a separate locus. Sequencing of one of the gel-extracted bands by Davis Sequencing (Davis, California) revealed nine base pair differences in the flanking region that enabled the design of a new, locus-specific forward primer (Oligo, Molecular Biology Insights). The repeat motif, GATA, is identical for the two loci, but at least 15 extra repeats are found in the new locus. The primer pair consists of this new forward primer (F: AACAATATGCAACTCAGCATAA) and the reverse primer for *Afu68**. The locus was named *Afu68b** to identify this locus as being derived from amplification products of *Afu68**. Reaction conditions were optimized for this locus and led to a sixth locus used in this study. As the inheritance patterns of *Afu68** were examined by Pyatskowit et al. (2001), this locus was not included in this study.

Reaction conditions.—DNA from 50 progeny of each family was amplified and the phenotypes scored for each locus. Polymerase chain reaction (PCR) amplification consisted of a 20-μL reaction containing 50 ng DNA template, 0.22 mM of each deoxynucleotide triphosphate (dNTP), 0.4 μM of each primer (the 5′ end of the forward primer was fluorscein-labeled), 0.5 unit *Taq* DNA polymerase (GIBCO), and 1× GIBCO PCR buffer (200 mM tris-HCl, pH 8.4; 500 mM KCl). Magnesium chloride concentrations were 1.5 mM (*Afu68b*, Spl101**), 2.5 mM (*Spl120**), 3.0 mM (*Spl35**), or 3.125 mM (*Aox27*, Spl106**). Amplification was conducted in an MJ Research PTC-100 thermocycler using a touchdown protocol (Fishback et al. 1999) to eliminate artifact bands. Initial denaturing was at 95°C for 1 min followed by 15 cycles of 95°C for 30 s, 65°C for 1 min, and 72°C for 1 min. Upon completion of each cycle, the annealing temperature was decreased 1°C until 50°C was reached. At the end of these 15 cycles, an additional 15 cycles were run with 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 min. Following amplification, 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 the PCR product in a 1:1 ratio and then stored at 4°C.

PCR products were separated on 5% denaturing polyacrylamide gels (19:1 acrylamide:bis-acrylamide; 7 M urea; 0.5× tris−boric acid (borate)−EDTA [TBE] buffer). Gels were run at 35 W for 45 min (*Aox27*, Afu68b**), 1 h 20 min (*Spl120**), or 1 h 30 min (*Spl35*, Spl101*, Spl106**), and the labeled products were visualized on a Molecular Dynamics 595 Fluorimager. Each gel contained an allelic ladder to ensure consistent scoring of alleles. Each allele at a locus was randomly named with symbols (1–9, A) to simplify scoring and reporting in this manuscript. Alleles were sized using

TABLE 1.—Alleles at each locus expressed in terms of the number of base pairs (bp) and the characters used to represent them when scoring the gels.

Locus	Allele (bp)	Character
	Disomic loci	
*Spl120**	*278	3
	*262	2
	*258	6
	*254	1
*Aox27**	*138	3
	*134	2
	*130	1
	*126	5
*Afu68b**	*193	8
	*185	9
	*181	1
	*177	3
	*173	6
	*165	4
	Duplicated loci	
*Spl35**	*258	4
	*252	3
	*248	8
	*246	7
	*244	2
	*240	1
*Spl101**	*315	9
	*311	8
	*307	1
	*303	5
	*298	2
	*290	4
	*283	6
*Spl106**	*251	A
	*247	4
	*243	3
	*238	2
	*234	1
	*230	8
	*225	6
	*219	9

FragmeNT Analysis; the symbol of each allele and corresponding size appears in Table 1.

Data analysis.—Distinguishing a locus as provisionally disomic or duplicated was accomplished by examining the phenotypic banding patterns of the 13 Mattagami River lake sturgeon individuals during screening and optimization of the six microsatellite loci. A locus was provisionally considered disomic when individuals displayed single bands (indicating homozygosity) or two bands of even intensity (indicating heterozygosity) (Figure 1). Chi-square analyses were used to test for conformance of the presumptive disomic loci to a model of disomic Mendelian inheritance. Progeny with phenotypes at the disomic loci not predicted by the parental phenotypes were not included in the statistical analysis.

Other loci provisionally considered duplicated

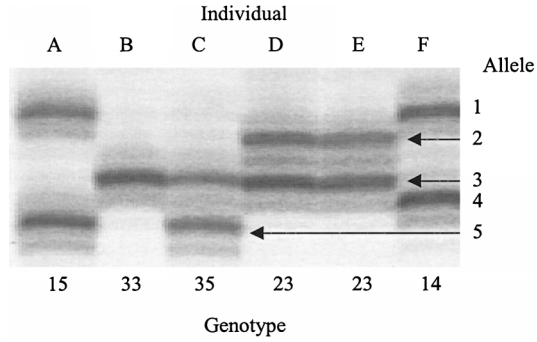

FIGURE 1.—Locus *Afu68b** showing even-intensity, two-banded patterns of heterozygous individuals at disomic loci. Individual B is homozygous at this locus.

expressed multiple-banded patterns, with some individuals showing asymmetrically stained two- or three-banded phenotypes (Figure 2; A, B, C, and E) suggestive of gene dosage or four-banded phenotypes with each band equally represented (Figure 2; G and H). Traditionally, gene dosage for allozymes has been scored assuming that band intensity corresponds to allele dosage (Marsden et al. 1987; Shaklee and Phelps 1992; Allendorf and Danzmann 1997). While some believe that dosage is not accurately reflected in PCR-based amplification products (Wagner et al. 1994), others have assigned dosage using microsatellite loci follow-

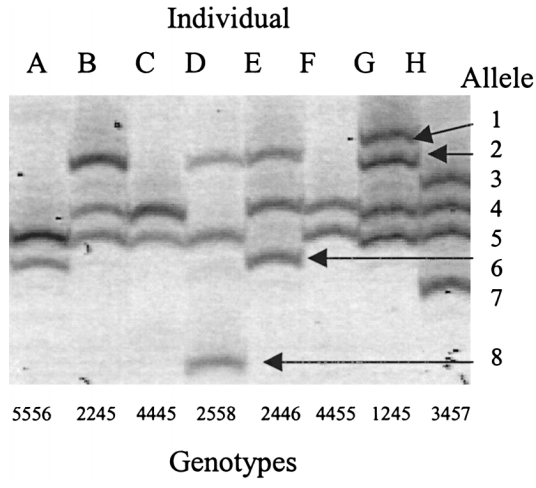

FIGURE 2.—Locus *Spl106** showing asymmetrical two-banded (A, C) and three-banded (B, D, E) phenotypes and four-banded, even-intensity phenotypes (G, H), as seen with duplicated loci. Individual F is an example of two bands of even intensity, which is considered representative of two allele doses at each band. The presumptive four-gene-dose genotypes are shown below the phenotypes.

TABLE 2.—Chi-square test for Mendelian segregation for three presumptive disomic loci with observed progeny phenotypes and the number of progeny (parentheses). Individuals with phenotypes not predicted by the phenotypes of their parents are indicated by bold italics; they were not included in the analysis. Chi-square values with a significance level of 0.05 are shown with the degrees of freedom.

Locus	Family	Parent phenotypes (female/male)	Progeny phenotypes				χ^2
*Spl120**	K1	11/11	11 (50)				
	K2	11/11	11 (49)				
	K3	11/11	11 (50)				
	K4	11/23	13 (22)	12 (22)	***11*** (2)	***123*** (1)	0.02 (1)
	K6	26/11	12 (29)	16 (19)			0.93 (1)
	K7	26/11	16 (23)	12 (26)	***23*** (1)		0.08 (1)
*Aox27**	K1	11/13	11 (29)	13 (21)			0.981 (1)
	K2	11/23	13 (23)	12 (26)	***123*** (1)		0.08 (1)
	K3	11/13	13 (22)	11 (28)			0.50 (1)
	K4	11/15	11 (28)	15 (21)			0.74 (1)
	K6	11/23	12 (23)	13 (26)	***1223*** (1)		0.08 (1)
	K7	11/13	11 (25)	13 (23)			0.02 (1)
*Afu68b**	K1	48/13	14 (13)	38 (13)	34 (17)	18 (7)	4.08 (3)
	K2	48/46	48 (9)	44 (15)	68 (16)	46 (9)	3.49 (3)
	K3	48/49	44 (13)	49 (12)	89 (13)	48 (12)	0.08 (3)
	K4	48/16	68 (12)	18 (10)	46 (9)	14 (8)	0.90 (3)
			48 (2)	***1668*** (1)	***1688*** (1)	***1148*** (1)	
	K6	44/46	46 (22)	44 (26)			0.18 (1)
	K7	44/13	34 (23)	14 (19)	***44*** (2)	***46*** (1)	0.22 (1)

ing the assumptions used for scoring duplicated loci (May et al. 1997; Pyatskowit et al. 2001). Multiple reamplifications of several individuals gave consistent banding patterns, indicating that band intensity could be used to score dosage. However, no statistical model of inheritance for these duplicated loci was tested due to the small number of observations for each phenotypic class.

Results

Disomic Loci

Three loci were classified as disomic: *Spl120**, *Aox27**, and *Afu68b**. While most of the individuals examined at the disomic loci expressed a typical disomic banding pattern, some aberrant phenotypes were observed in the offspring (Table 2). These included three bands of even intensity; three bands with one band darker in intensity than the other two, even-intensity bands; bands found only in the female parent; and bands that were not found in either parent.

Evidence for homeologous loci was observed at *Aox27**. All individuals expressed a band of 126 base pairs. Phenotypes at this locus consisted of three bands (allele 126 and two other bands of even intensity) or two bands (allele 126 and one other band). When only band 126 and another band were expressed, band 126 was often darker in intensity than when observed in the presence of two other bands. These observations led us to conclude that

TABLE 3.—Observed progeny phenotypes that were unexpected based on the parental phenotypes. Numbers of progeny are given in parentheses.

Locus	Family	Parent phenotypes (female/male)	Progeny phenotypes				
*Spl106**	K2	1344/117A	344A (1)	1137A[a] (1)			
	K3	1344/1134	11344 (1)				
	K4	1344/124A	1334 (1)	1114 (1)			
	K6	1344/117A	13789 (2)	1478A (1)	14789 (1)	11467 (1)	1467A (2)
			14467 (1)				
*Spl101**	K1	1225/1118	1111 (1)	1226 (1)	1126 (1)	1256 (1)	
	K2	1225/1446	1112 (1)	124456 (1)			
	K3	1225/1256	1266 (2)	2566 (4)	2266 (1)	1566 (2)	5566 (1)
	K4	1225/1169	2256 (1)	1124 (1)	2566 (1)	156699 (1)	
	K6	1589/1446	1345 (1)	1445668 (1)	124 (1)	144668 (1)	
	K7	1589/1118	1155 (1)	1255 (1)	1145 (1)	1558 (1)	1458 (1)
*Spl35**	K3	33ØØ/2237	3777 (1)				
	K4	33ØØ/1247	3377 (1)				
	K7	223Ø/2233	238Ø (1)	1234 (1)			

[a] Any unexpected phenotype consisting of four bands with one band darker than the others or three bands with two bands darker than the other are shown here as having two copies of the allele(s) represented by the darker banding pattern. For example, a phenotype 137A with the 1 band being darker than the others is shown here as 1137A. Phenotypically, it is difficult to distinguish 1137A from 11137A.

there were two homeologous loci, *Aox27** and another locus that appeared monomorphic in this study and always exhibited two copies of allele 126.

Chi-square analysis found that all families at *Aox27** and *Afu68b** and families K4, K6, and K7 at *Spl120** conformed to a disomic, Mendelian model of inheritance (Table 2). At *Spl120**, families K1, K2, and K3 were from parents homozygous for a common allele, and all progeny had phenotypic banding patterns identical to those seen in the parents. In the test for independent assortment of loci, all three possible combinations in the males were tested and only that for male 160 at *Spl120** and *Afu68b** was significant ($n = 33$, $\chi^2 = 6.81$, $r = 0.29$, $P = 0.05$). We are reluctant to attach much significance to this finding with such a small sample size. It was not possible to test for joint segregation of the disomic loci in the female parents.

Duplicated Loci

Three loci were classified as duplicated: *Spl35**, *Spl101**, and *Spl106**. Most individuals examined at the duplicated loci had a characteristic tetrasomic banding pattern, that is, four bands of uniform intensity, three bands with one band being darker than the other two bands of equal intensity, two bands with one band darker than the other, or two bands of uniform intensity (Figure 2). Aberrant phenotypes observed in the offspring included more copies of an allele than was present in the parents, five bands of even intensity, and three bands of even intensity (Table 3).

Discussion

The high variability of all of these loci makes them useful in further lake sturgeon genetic studies, but confirmation of the pattern of inheritance is essential before use in population-genetic stud-

TABLE 4.—Counts of progeny phenotypes that could have arisen from double-reduction events or unreduced gametes in the parents. The number of events refers to the count of progeny phenotypes that could be explained by unreduced or double-reduction gametes being transmitted from that particular parent. The asterisk indicates one individual that could have arisen through a double-reduction event in either the male or female.

	Identification number		Number of events	
Family	Female	Male	Female	Male
Double reduction				
K1	195	159	1	0
K2	195	193	0	1
K3	195	194	1	12
K4*	195	160	1	2
K6	197	193	0	0
K7	197	159	2	0
Unreduced gametes				
K1	195	159	0	0
K2	195	193	0	1
K3	195	194	0	0
K4	195	160	1	3
K6	197	193	0	2
K7	197	159	0	0

ies. Three microsatellite loci fit a disomic model of inheritance (*Spl120**, *Aox27**, and *Afu68b**), and three loci appear to be segregating tetrasomically (*Spl35**, *Spl101**, and *Spl106**), suggesting the existence of duplicate loci.

At duplicate loci, the majority of individuals expressed banding patterns characteristic of and consistent with a tetrasomic locus. For example, the male parent of family K4 showed a phenotype at *Spl106** consisting of four bands of even intensity corresponding to genotype *124A.* All possible male gametes based on a tetrasomic mode of inheritance (*12*, *14*, *1A*, *24*, *2A*, and *4A*) were observed in the K4 progeny (data not shown). In addition, all possible gametes at *Spl35** and *Spl101** were found in the progeny of families K4 and K6, respectively, providing further support for tetrasomic inheritance. In addition, a large number of aberrant phenotypes could be explained only by the occurrence of double-reduction events in the parents.

Potential double-reduction events occurred in high frequency at the duplicated loci and were observed in all families except K6 (Table 4). This phenomenon is observable when an allele present at one dose in the parents is represented by two doses in the progeny (Marsden et al. 1987). For example, the K3 cross of a 1225 female and a 1256 male gave a progeny phenotype of 1266 at *Spl101**. This expression could be explained by assuming the female transmitted 12 to the progeny while the 66 came from the male as a double-reduction gamete.

Evidence of the transmission of unreduced gametes may suggest abnormal segregation patterns in the parents of these families, particularly among males (Table 4). One example was the occurrence of three even-intensity bands in two individuals at the disomic loci *Spl120** and *Aox27**, which could be explained by the transmission of an unreduced gamete from the male parents. Evidence for transmission of an unreduced gamete also was found at the duplicated locus *Spl101**, where one individual had a phenotype of four bands, with two bands darker in intensity.

Duplicated loci have been used in population-genetic analyses (Krueger and May 1987; Estoup et al. 1998), paternity and kinship analyses (Bruford and Wayne 1993; Queller et al. 1993), and inbreeding and fitness studies (Buza et al. 2000), but inheritance of the loci should be examined to determine if the loci are segregating according to Mendelian expectations (Allendorf and Danzmann 1997). However, without making assumptions about inheritance patterns, duplicated microsatellite loci can be used in the examination of population structure. This application has been done by estimating allele frequencies by a direct count of the number of alleles at a particular locus in an individual (Krueger and May 1987; Perkins et al. 1995; Allendorf and Danzmann 1997). Additionally, Ronfort et al. (1998) have written a computer program to analyze population structure over several loci in autopolyploids that includes a calculation entailing the expected rate of double-reduction events.

Several individuals had phenotypes at the disomic and duplicated loci that could be explained by the occurrence of a mutation; these progeny phenotypes showed bands not present in the parents, bands found in only one parent, or banding patterns characteristic of a four-dose locus, but they did not fit the patterns expected based on parental phenotypes. Mutations occur at a high rate at microsatellite loci and are thought to arise through slippage in DNA replication in which nucleotide repeats are subject to a high rate of single-motif insertions and deletions (Metzgar et al. 2000). In essence, confirmation of a mutation requires sequencing the DNA of the unique allele(s) in the progeny and the parental alleles to confirm microsatellite motifs (Banks et al. 1999).

The phenotype of any individual that had an unexpected phenotype based on those of the par-

ents was compared with the expected phenotypes at all loci for all families to discriminate between mutational events and accidental mixing of individuals among families. The phenotypes of 3 out of 18 individuals were incompatible with the parental phenotypes yet were consistent with those expected from the parents of another family. It is possible that these three progeny were mistakenly mixed with the wrong family during spawning or rearing, but for the phenotypes of the remaining 15 individuals mutation remains the most likely explanation. The mutation rate was not calculated owing to the uncertainty as to whether a particular aberrant phenotype actually resulted from mutation. Nevertheless, calculation of the mutation rate may prove to be important in future studies, when the time of divergence between populations is of concern (Kimmel et al. 1996) or when restoration plans are being formulated and maintenance of the genetic variation of a population is elemental (Deng and Lynch 1996). However, the lake sturgeon may have an advantage with its tetraploid-derived genome, as it is thought that variation is lost at a slower rate in polyploid organisms than in diploid organisms (Moody et al. 1993).

At *Spl35**, the high frequency (22%) of phenotypes with three bands of uniform intensity can be explained by the presence of null alleles. Null alleles are nonamplifying alleles that can arise from point mutations in the primer annealing sites (Donini et al. 1998). Also, several individuals had two bands of even intensity at this locus, suggesting the presence of two doses of each allele or one dose of each allele and the presence of two null alleles. As an example, six progeny originally scored as 1144, 1177, or 2277 from the K4 cross of a female scored as 3333 and a male scored as 1247 were notable for not expressing the female band and having excessive gene dosage from the male parent. Based on the assumption that null alleles exist, the likely explanation for these six progeny is that they received two alleles from the male and two null alleles from the female. Under these assumptions the progeny phenotypes would be rescored as 14θθ, 17θθ, and 27θθ, and the female phenotype would be changed from 3333 to 33θθ.

If null alleles are not accounted for in population-genetic analyses, inaccurate interpretations could be made when estimating the levels of heterozygosity and inbreeding. Redesigning primers has been shown to reveal additional alleles that had not previously amplified (Callen et al. 1993; Ishibashi et al. 1996). In addition, a number of researchers have used various statistical measures to account for the potential presence of null alleles (Pemberton et al. 1995; Brookfield 1996; Gibbs et al. 1997).

Finally, the issue of abnormal transmission occurring in artificial crosses must be addressed. Pallid sturgeon *S. albus* fry from artificial crosses that did not exhibit normal swimming behavior at hatch were found to exhibit asymmetrical banding patterns or to have more than two alleles at disomic loci (B. May, unpublished data). In contrast, strictly disomic banding patterns have been observed consistently in natural pallid sturgeon populations. The lake sturgeon fry used for this study were sacrificed either 2 or 3 d posthatch with no concern about developmental status. All the parental phenotypes in this study exhibited normal banding patterns, yet abnormal patterns were found in the offspring following artificial spawning; it is possible that the high frequency of atypical banding patterns that we observed would not be found in wild populations. In fact, only 11 aberrant phenotypes were observed among seven loci used in a population-genetic study of 210 lake sturgeon individuals distributed among seven populations by McQuown et al. (unpublished data). This finding suggests that high frequencies of aberrant phenotypes are not indicative of the genetic nature of wild lake sturgeon populations, and the highly variable loci developed here should prove useful in delineating lake sturgeon population-genetic structure.

Conclusions

Our findings revealed a single disomic locus (*Spl120**), pairs of disomic loci (*Afu68**/*Afu68b** and *Aox27**), and duplicate pairs of disomic loci behaving as tetrasomic loci (*Spl35**, *Spl101**, and *Spl106**). We propose the use of cytological studies to observe meiosis in both males and females to determine whether tetravalents or random bivalents are formed. Due to the uncertainty of the origin of abnormal phenotypes, cytogenetic analyses and further inheritance tests using controlled crosses of a large scale must be conducted with lake sturgeon before extensive population management activities are undertaken based on microsatellite genetic data. We further suggest that concern about the process of artificial fertilization used for lake sturgeon is appropriate and that there should be genetic examinations of fry for meiotic abnormalities. However, three disomic (*Spl120**, *Aox27**, and *Afu68b**) and three presumptive tetrasomic microsatellite loci (*Spl35**, *Spl101**, and

*Spl106**) have been developed and should prove useful in lake sturgeon population-genetic studies.

Acknowledgments

Jeff Rodzen, Charles Krueger, Natalia Belfiore, M. Fernanda Rodriguez, and three anonymous reviewers provided valuable suggestions and editing regarding this paper.

References

Allendorf, F. W., and R. G. Danzmann. 1997. Secondary tetrasomic segregation of *MDH-B* and preferential pairing of homeologues in rainbow trout. Genetics 145:1083–1092.

Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tschawytscha*). Journal of Heredity 90:281–288.

Blacklidge, K. H., and C. A. Bidwell. 1993. Three ploidy levels indicated by genome quantification in Acipenseriformes of North America. Journal of Heredity 84:427–430.

Broders, H. G., S. P. Mahoney, W. A. Montevecchi, and W. S. Davidson. 1999. Population genetic structure and the effect of founder events on the genetic variability of moose, *Alces alces*, in Canada. Molecular Ecology 8:1309–1315.

Brookfield, J. F. Y. 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. Molecular Ecology 5:453–455.

Bruford, M. W., and R. K. Wayne. 1993. Microsatellites and their application to population genetic studies. Current Opinion in Genetics and Development 3: 939–943.

Buza, L., A. Young, and P. Thrall. 2000. Genetic erosion, inbreeding, and reduced fitness in fragmented populations of the endangered tetraploid pea *Swainsona recta*. Biological Conservation 93:177–186.

Callen, D. F., A. D. Thompson, Y. Shen, H. Phillips, R. I. Richards, J. C. Mulley, and G. R. Sutherland. 1993. Incidence and origin of 'null' alleles in the $(AC)_n$ microsatellite markers. American Journal of Human Genetics 52:97–115.

Deng, H. W., and M. Lynch. 1996. Estimation of deleterious-mutation parameters in natural populations. Genetics 144:349–360.

Donini, P., P. Stephenson, G. J. Bryan, and R. M. D. Koebner. 1998. The potential of microsatellites for high throughput genetic diversity assessment in wheat and barley. Genetic Resources and Crop Evolution 45:415–421.

Estoup, A., F. Rousset, Y. Michalakis, J. M. Cornuet, M. Adriamanga, and R. Guyomard. 1998. Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). Molecular Ecology 7:339–353.

Ferguson, M. M., L. Bernatchez, M. Gatt, B. R. Konkle, S. Lee, M. L. Malott, and R. S. McKinley. 1993. Distribution of mitochondrial DNA variation in lake sturgeon (*Acipenser fulvescens*) from the Moose River basin, Ontario, Canada. Journal of Fish Biology 43(Supplement A):91–101.

Ferguson, M. M., and G. A. Duckworth. 1997. The status and distribution of lake sturgeon, *Acipenser fulvescens*, in the Canadian provinces of Manitoba, Ontario, and Quebec: a genetic perspective. Environmental Biology of Fishes 48:299–309.

Fishback, A. G., R. G. Danzmann, T. Sakamoto, and M. M. Ferguson. 1999. Optimization of semi-automated microsatellite multiplex polymerase chain reaction systems for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 172:247–254.

Gibbs, H. L., K. A. Prior, P. J. Weatherhead, and G. Johnson. 1997. Genetic structure of populations of the threatened eastern massasauga rattlesnake, *Sistrurus c. catenatus*: evidence from microsatellite DNA markers. Molecular Ecology 6:1123–1132.

Grewe, P. M., C. C. Krueger, C. F. Aquadro, E. Bermingham, H. L. Kincaid, and B. May. 1993. Mitochondrial DNA variation among lake trout (*Salvelinus namaycush*) strains stocked into Lake Ontario. Canadian Journal of Fisheries and Aquatic Sciences 50:2397–2403.

Guenette, S., R. Fortin, and E. Rassart. 1993. Mitochondrial DNA variation in lake sturgeon (*Acipenser fulvescens*) from the St. Lawrence River and James Bay drainage basins in Quebec, Canada. Canadian Journal of Fisheries and Aquatic Sciences 50:660–666.

Ishibashi, Y., T. Saitoh, S. Abe, and M. C. Yoshida. 1996. Null microsatellite alleles due to nucleotide sequence variation in the grey-sided vole *Clethrionomys rufocanus*. Molecular Ecology 5:589–590.

Kimmel, M., R. Chakraborty, D. N. Stivers, and R. Deka. 1996. Dynamics of repeat polymorphisms under a forward-backward mutation model: within- and between- population variability at microsatellite loci. Genetics 143:549–555.

King, T. L., B. A. Lubinski, and A. P. Spidle. 2001. Microsatellite DNA variation in Atlantic sturgeon (*Acipenser oxyrinchus*) and cross-species amplification in the Acipenseridae. Conservation Genetics 2(2):103–119.

Krueger, C. C., and B. May. 1987. Genetic comparison of naturalized rainbow trout populations among Lake Superior tributaries: differentiation based on allozyme data. Transactions of the American Fisheries Society 116:795–806.

Marsden, J. E., S. J. Schwager, and B. May. 1987. Single-locus inheritance in the tetraploid tree frog *Hyla versicolor* with an analysis of expected progeny ratios in tetraploid organisms. Genetics 116:299–311.

May, B., C. C. Krueger, and H. L. Kincaid. 1997. Genetic variation at microsatellite loci in sturgeon: primer sequence homology in *Acipenser* and *Scaphirhynchus*. Canadian Journal of Fisheries and Aquatic Sciences 54:1542–1547.

McQuown, E. C., B. L. Sloss, R. J. Sheehan, J. Rodzen, G. J. Tranah, and B. May. 2000. Microsatellite analysis of genetic variation in sturgeon (Acipenseridae): new primer sequences for *Scaphirynchus* and

Acipenser. Transactions of the American Fisheries Society 129:1380–1388.

Metzgar, D., J. Bytof, and C. Wills. 2000. Selection against frameshift mutations limits microsatellite expansion in coding DNA. Genome Research 10: 72–80.

Moody, M. E., L. D. Mueller, and D. E. Soltis. 1993. Genetic variation and random drift in autotetraploid populations. Genetics 134:649–657.

Pemberton, J. M., J. Slate, D. R. Bancroft, and J. A. Barrett. 1995. Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. Molecular Ecology 4:249–252.

Perkins, D. L., J. D. Fitzsimmons, J. E. Marsden, C. C. Krueger, and B. May. 1995. Differences in reproduction among hatchery strains of lake trout at eight spawning areas in Lake Ontario: genetic evidence from mixed-stock analysis. Journal of Great Lakes Research 21(Supplement 1):364–374.

Pyatskowit, J. D., C. C. Krueger, H. L. Kincaid, and B. May. 2001. Inheritance of microsatellite loci in the polyploid-derivative lake sturgeon (*Acipenser fulvescens*). Genome 44:185–191.

Queller, D. C., J. E. Strassman, and C. R. Hughes. 1993. Microsatellites and kinship. Trends in Ecology and Evolution 8:66–75.

Ronfort, J., E. Jenczewski, T. Bataillon, and F. Rousset. 1998. Analysis of population structure in autotetraploid species. Genetics 150:921–930.

Shaklee, J. B., and S. R. Phelps. 1992. Chinook salmon NADP(+)-dependent cytosolic isocitrate dehydrogenase: electrophoretic and genetic dissection of a complex isozyme system and geographic patterns of variation. Biochemical Genetics 9/10:455–489.

Wagner, A., N. Blackstone, P. Cartwright, M. Dick, and B. Misof. 1994. Surveys of gene families using polymerase chain reaction: PCR selection and PCR drift. Systematic Biology 43:250–261.

Wenburg, J. K., P. Bentzen, and C. J. Foote. 1998. Microsatellite analysis of genetic population structure in an endangered salmonid: the coastal cutthroat trout (*Oncorhynchus clarki clarki*). Molecular Ecology 7:733–749.