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Development and standardization of disomic microsatellite markers for lake sturgeon genetic studies

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Summary

Lake sturgeon (*Acipenser fulvescens*) are of conservation concern in North America. To facilitate the recovery of this fish species, an understanding of their population genetic structure is necessary to develop and implement spatially and temporally appropriate management actions. Until recently, few genetic data using nuclear loci have been collected, primarily due to the paucity of suitable genetic markers because most microsatellite loci in lake sturgeon appeared to be tetrasomic. The authors identified nine microsatellite loci (from 254 examined) that were putative polymorphic disomic loci and tested their conformance to a disomic mode of inheritance using three lake sturgeon families. The objectives of the study were to: (i) confirm the disomic status of the nine loci through inheritance testing, and (ii) standardize the genetic markers among participating laboratories. At all nine loci, disomic inheritance were confirmed, and all nine loci segregated independently in the 26 of 36 loci pairs possible to test. One of the nine loci showed non-Mendelian segregation, possibly due to meiotic drive and/or selection. Three progeny had peak patterns inconsistent with disomy at one or more loci. The nine loci when combined with four microsatellite loci previously confirmed in other studies as disomic in lake sturgeon now yield a suite of 13 microsatellite markers. These 13 markers have been standardized among four other laboratories to facilitate building an inter-laboratory genetic database for lake sturgeon.

Introduction

Lake sturgeon (*Acipenser fulvescens*) historically ranged throughout the Great Lakes basin, Hudson Bay drainage, and Mississippi River of North America (Harkness and Dymond, 1961). Their numbers have been reduced by overfishing, dams and other migration impediments and diminished habitat quality and/or loss (Auer, 1999). Lake sturgeon are now listed as either endangered, threatened, or of special concern in most states within their historic range, as an Appendix II species under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and as a species of concern under the United States Endangered Species Act. As with many other sturgeon species, late sexual maturity and an intermittent spawning pattern (Harkness and Dymond, 1961) slow the ability of lake sturgeon to quickly rebound.

To facilitate the recovery of lake sturgeon, management efforts have focused on regulating or, in most cases, eliminating harvest (Welsh, 2004), providing fish passage over dams (Peake et al., 1997; Amaral et al., 2002), and restoring spawning habitat (Bruch, 1999). Reintroduction of lake sturgeon to locations where they have been extirpated, as well as stocking to increase the abundance of existing stocks, has been implemented to a limited extent (Schram et al., 1999; Runstrom et al., 2002). Approaches employed in conservation genetics can help to prioritize populations for active management, to identify suitable donor and recipient populations for stocking, and to guide interjurisdictional coordination by delineating appropriate management units.

Microsatellite loci are useful genetic markers for intraspecific population genetic studies because they are codominant, biparentally inherited, putatively neutral, and have a relatively high mutation rate (Goldstein and Schlotterer, 1999). Evidence of duplicated microsatellite loci has been observed in many sturgeon species (e.g. Jenneckens et al., 2001; Rodzen and May, 2005; Shao et al., 2005), as well as other fish species (e.g. David et al., 2003). The lake sturgeon genome appears to be tetraploid-derived and in the early stages of the diploidization process, with a mix of tetrasomic and disomic microsatellite loci (e.g. Pyatskowit et al., 2001; McQuown et al., 2002; Welsh et al., 2003). Duplicated loci can complicate population genetic analyses because of the difficulty in determining gene dosages and the assumption of diploidy is incorporated into many statistical tests.

In 1999, lake sturgeon geneticists and managers in the United States met to address priorities for the collection and analysis of genetic data that could be incorporated into management plans. Critical needs that were identified included the development of additional genetic markers and the standardization of those markers among the various laboratories (Lowie, 1999). To eliminate the inherent difficulties associated with tetrasomic loci, future lake sturgeon genetic marker development required identifying nuclear microsatellite loci that are disomic. Following selection of appropriate genetic markers, standardization of marker use and allelic designations was deemed critical for building a genetic database among the laboratories.

Building a North American genetic database of lake sturgeon is difficult because of differences among laboratories in analytic techniques and scoring procedures. Integration of genetic data collected at different laboratories will permit a more comprehensive understanding of lake sturgeon population structure. Many laboratories are using different genotyping platforms and apparent allele sizes can vary depending on electrophoretic conditions (Haberl and Tautz, 1999). Standardization of microsatellite loci also requires consistent allele scoring within and between laboratories. With the development of new microsatellite markers, the timing for such

standardization is optimal because few genetic data at nuclear loci have been previously collected.

We designed 254 primer pairs from four microsatellite-enriched libraries and screened those primer pairs on lake sturgeon individuals to identify markers that appeared to target polymorphic disomic loci (Welsh et al., 2003). The objectives of our study were to: (i) confirm the disomic status of selected loci through inheritance testing, and (ii) standardize the markers for disomic microsatellite loci among participating genetic laboratories.

Materials and methods

Sample collection

Crosses of adult lake sturgeon caught from the Sturgeon River, Michigan were made by the Michigan Department of Natural Resources. A single female was crossed with four different males to produce four families. Spawning sturgeon were caught in nets at the spawning site. Milt was collected by gently squeezing males and using a separate syringe for each male to collect the sperm. Eggs were collected by gently squeezing the female, and eggs were then placed in a one-gallon plastic tub. The eggs were divided into smaller containers, and a single male was used to fertilize a single subsample of eggs. The eggs were sent to the Wolf Lake Hatchery, Michigan for further development. The larval fish were killed, frozen, and transferred to ethanol. Fin clips from the parents were also collected, stored in ethanol, and then shipped to the Genomic Variation Laboratory, University of California at Davis.

Inheritance testing

From the 254 primer pairs designed during microsatellite marker development (Welsh et al., 2003), nine loci (AfuGs 9, 56, 63, 74, 112, 122, 160, 195, and 204) consistently amplified, had a sufficient number of alleles (>2), and had banding patterns on acrylamide gels (imaged with a Molecular Dynamics fluorimager) that appeared consistent with disomic inheritance. A banding pattern that is consistent with potential disomy has a maximum of two alleles (two bands) for each individual and, when two alleles are present, the bands have equal intensities.

Genomic DNA was extracted from a piece of the fin clip from each parent and from the tail of the larval fish from each of the four families, using the Sigma GenElute^{TM} Mammalian Genomic DNA Miniprep Kit and its suggested protocol. Using the polymerase chain reaction (PCR), 10 ng of extracted DNA were amplified at eight of the microsatellite loci (AfuG 9 excluded) in 10 μl reactions with: 20 mM Tris-HCl, pH 8.4; 50 mM KCl; 0.8 mM dNTPs; varying amounts of $MgCl_2$ (Table 1); 0.05 μM (FAM- or TET-labeled) or 0.1 μM (HEX-labeled) forward primer; 1 μM unlabeled reverse primer; and 0.4 units of *Taq* DNA polymerase. For locus AfuG 9, 10 μl PCR reactions consisted of: 50 mM Tris-HCl, pH 8.3; 10 mM KCl; 5 mM $(NH_4)_2SO_4$; 0.2 mM dNTPs; 2 mM $MgCl_2$; 0.2 μM FAM-labeled forward primer; 0.2 μM unlabeled reverse primer; and 2 U FastStart Taq DNA polymerase. MJ Research PTC100 thermocyclers were used and amplification parameters (excluding AfuGs 9 and 56) were: 95°C for 1 min 30 s; 30 cycles of 95°C for 1 min, 52–55°C (Table 1) for 45 s, 72°C for 2 min; a plus-A extension of 60°C for 45 min; ending with a 4°C hold. Further optimization of AfuG 9 due to inconsistent amplification resulted in the following amplification parameters for this locus: 95°C for 4 min; 40 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 45 s; 72°C for 7 min, ending with a 4°C hold. The following touchdown protocol was used for the amplification of AfuG 56 to eliminate the amplification of a secondary locus: 94°C for 1 min; 20 cycles of 92°C for 30 s and 70°C for 40 s with a 0.5°C decrease in the second step each cycle; 20 cycles of 92°C for 30 s and 60°C for 40 s with a 1-s increase in the second step each cycle; ending with a 4°C hold. Amplified samples were then diluted (Table 1), and 1 μl of diluted product was added to 4 μl of loading buffer (36% formamide, 12% loading dye, 1.4% ROX-labeled 400HD ladder); at this point, loci were pooled into three groups (Table 1). The amplified product was separated on a 5.5% acrylamide gel running at 2600 v for 6000 scans on a Bio-Rad BaseStation^{TM}. Gels were analyzed using the accompanying Cartographer® software.

Provisional disomy was confirmed in the families by examining the peak patterns in the traces from the parents and offspring generated on a Bio-Rad BaseStation. A locus was provisionally disomic if a maximum of two alleles was observed in the majority of individuals and, when an individual was heterozygous, the two peaks had approximately equal heights. If an individual was trisomic or tetrasomic at a locus, it was assumed that relative peak heights would correspond to gene dosage. For example, if an individual is trisomic and has two copies of allele A and a single copy of allele B, the peak height of allele A would be nearly twice the peak height of allele B.

For those loci identified as provisionally disomic, chi-square tests were performed to test for the conformance of offspring genotype ratios to those expected under Mendelian segregation for disomic loci and for joint segregation of loci. Segregation in the females and males was tested independently. Because a single female was used for all matings, all her offspring were pooled to test for Mendelian segregation in the mother. Significance for both the test for Mendelian segregation and the test for joint segregation was assessed at an α-level of 0.05, after correcting for multiple comparisons using the sequential Bonferroni correction (Rice, 1989).

Standardization

Microsatellite markers that targeted polymorphic disomic loci were standardized among eight other genetic laboratories. Standardization kits were sent to participating laboratories containing instructions for optimized PCR conditions, one to three lake sturgeon DNA extracts and their corresponding genotypes (known samples), and three lake sturgeon DNA extracts without stated genotypes (unknown samples). The known samples represented the range of alleles observed thus far, and the number of known samples varied, depending on the number of alleles possible at that locus. Following genotyping of samples within the standardization kit, results were compared with those obtained at our laboratory and discrepancies were resolved through personal communications.

Results

During the analysis of offspring genotypes, errors in parental assignment became apparent as the impossibility of observed progeny genotypes resulting from the designated parents was revealed in one of the four families. For this one family, the correct parents were represented in the parental sample

Table 1
Summary of 13 standardized disomic microsatellite markers for lake sturgeon (*Acipenser fulvescens*) population genetic studies

	Sequences (5′–3′)	Annealing temperature (°C)	$MgCl_2$ concentration (mM)	Repeat motif	Number of alleles (n = 735)	Allele size range (bp)	Dilution	Multiplex group
AfuG 9	F: CATAATGTAAAGCAAAAGT R: ACCTGAAATGTATGTTATG	52	1.5	$(GATA)_{22}$	11	124–168	1 : 20	1
AfuG 56	F: ACTAAACCCAGCACAGAAAATCAG R: GAAGCCCATCCCACAGGTT	Touchdown	1.5	$(AAAC)_9$	4	258–274	1 : 2	1
AfuG 63	F: TCCTGGCTAGCGAACGAA R: CTTTTAAATGGGGGACAGACTAT	52	1.5	$(AAAC)_8$	5	127–147	1 : 8	4
AfuG 74	F: CTACAAAGACGGGTTACG R: AGCGACTGTCTGGTTTTC	52	1.5	$(AAAC)_6$	4	218–230	1 : 8	1
AfuG 112	F: TATTGTTCCTTTATGGTTATG R: TATTTCACTGTCTGTTGTATGTA	52	1.5	$(GATA)_{19}$	8	240–268	1 : 8	3
AfuG 122	F: AACACGACAACAAACTTATTCA R: TGTGTTTCTATGTCTGTCTGTCTA	52	1.0	$(GATA)_{13}$	11	147–187	1 : 8	4
AfuG 160	F: CCGCAGCATTAGGTCAAA R: CCCCAGTGGAAATAATAATGTA	52	1.75	$(AAAC)_8$	7	127–151	1 : 12	3
AfuG 195	F: ATTCCTCCAGCCGTATTATTA R: AAGCAGTTAGTTTATGTGGTTGTG	55	1.5	$(AAAC)_7$	3	161–173	1 : 4	2
AfuG 204	F: TGACCAGGCACCGTAACTTTG R: TAATGTCGCCGGCTCTGGTCTA	55	1.25	$(AAAC)_5$	2	141–145	1 : 8	4
Afu 68[1]	F: TTATTGCATGGTGTAGCTAAAC R: AGCCCAACACAGACAATATC	52	1.5	$(GATA)_{13}$	11	108–152	1 : 8	2
Afu 68b[1]	F: AACAATATGCAACTCAGCATAA R: same as Afu 68	52	1.5	$(GATA)_{28}$	12	153–197	1 : 4	2
Aox 27[1]	F: AATAACAATAACGGCAGAACCT R: TGTGTTGCTCAAGACAGTATGA	52	1.5	$(ATTT)_5(ATTC)(ATTT)_3$	3	130–138	1 : 8	3
Spl 120[1]	F: ATTCCATGAGCAACACCACA R: TGATGGTCTGATGAGATCGG	Touchdown	1.5	$(TATC)_{15}$	9	254–290	1 : 4	4

Primer information, including sequences, annealing temperature, $MgCl_2$ concentration, repeat motif of the clone, number of alleles (based on unpublished data from 19 populations), allele size ranges, dilution amounts for PCR product prior to loading on gel, and multiplex group (PCR products combined prior to loading). Information in table complements reagent and thermocycler information described in Materials and Methods section.

[1]Afu 68 (May et al., 1997); Afu 68b (McQuown et al., 2002); Aox 27 (King et al., 2001); Spl 120 (McQuown et al., 2000).

collection; however, the parents of the offspring were misidentified. Using genotypic information, the correct father was identified. The two families with the same parents were combined. All progeny from the three families were combined for segregation tests in the female.

All nine loci were classified as provisionally disomic based on observed peak patterns. In the majority of individuals, the maximum number of observed alleles was 2, and peak heights were equivalent when individuals were heterozygous. However, three of the progeny from the same family (04374) had aberrant genotypes. One appeared trisomic at five of the nine loci, with peak heights corresponding to varying allele dosages. This individual was homozygous at three of the remaining loci and appeared to be disomic at the other remaining locus (AfuG 122). A second individual was trisomic at a single locus (AfuG 9), while apparently disomic at the remaining six informative loci. The third aberrant offspring appeared tetrasomic at three loci; the remaining loci were either uninformative [homozygous (three loci) or lack of amplification (one locus)], or apparently disomic (two loci). The latter two loci that appeared disomic may have two copies of each of the two alleles, consistent with tetrasomy.

All loci were tested for conformance to disomic ratios expected under Mendelian segregation. Based on these tests, one locus, AfuG 204, showed non-Mendelian segregation in female 00630 (Table 2). At this locus, the female was the only informative parent because all the fathers were homozygous. The female transmitted allele 141 more frequently than allele 145. Equal peak heights were observed in the female and all progeny showed peak heights corresponding to equal dosages for heterozygous individuals.

Segregation patterns at locus AfuG 122 indicated the possible presence of a null allele. The mother was originally genotyped as a homozygote (179/179), and amplification results could not be obtained for father 04399, despite consistent amplification at all other loci. When the mother was genotyped as a homozygote, there was a large number of mismatches for homozygous individuals in the families that could be tested. The presence of a null allele was therefore incorporated into the parental genotypes, with the mother being genotyped as a heterozygote and father 04399 being genotyped as a homozygote for the null allele. Offspring ratios then conformed to the expectations of Mendelian segregation. The presence of a null allele at locus AfuG 122 has also been confirmed in lake sturgeon population genetic studies, where 14 of 16 spawning populations had a significant heterozygote deficit (Welsh, 2006).

Most pairs of loci could be tested for independent segregation in at least one of the parents. Loci combinations that could not be tested (because either one parent was homozygous or both parents had the same genotype) include: (i) AfuG 56 with AfuGs 74, 160, 112, 63, and 204; and (ii) AfuG 195 with AfuGs 74, 160, 112, 63, and 204. No differences from expectations of independent segregation of loci were observed.

The inheritance analysis confirmed the identification of nine primer pairs that targeted disomic microsatellite loci. The nine microsatellite markers as well as four other microsatellite loci identified in previous studies (Pyatskowit et al., 2001; McQuown et al., 2002) were sent to participating laboratories for standardization (Table 1). Four laboratories completed standardization to date (see Acknowledgements). Correct scoring of unknowns ranged from 75% to 100%. Genotyping platforms used by those four laboratories and our own laboratory included the FMBIOII®, ABI Prism® 377 DNA Sequencer, ABI Prism® 3700 DNA Analyzer, and Bio-Rad BaseStation^{TM}.

Discussion

Lake sturgeon are actively undergoing diploidization, as observed by Pyatskowit et al. (2001), McQuown et al. (2002), and Welsh et al. (2003), presenting an unusual glimpse at the evolutionary pathway of gene duplication. The loci analyzed in this study were selected because they showed a banding pattern consistent with a disomic mode of inheritance, and we expected conformance with the ratios expected with Mendelian segregation. All of the parents and offspring (excluding three individuals) displayed peak patterns consistent with a disomic mode of inheritance at these loci.

The apparent triploidy observed in one individual of family 04374 could be due to retention of the second polar body during fertilization, or due to the transmission of an unreduced gamete from a parent. The triploidy may be a result from artificial mating. The presence of triploid individuals resulting from artificial crosses has also been observed in pallid sturgeon, *Scaphirhynchus platorynchus*, while pallid sturgeon individuals are consistently disomic in natural populations (McQuown et al., 2002). The individual that appears to be tetraploid may have resulted from a double reduction event, where a parent has one copy of an allele and the offspring has two copies (Marsden et al., 1987). The individual that was trisomic at a single locus could be due to a non-disjunction event (failure of a chromosome to separate correctly during meiosis).

An unexpected result was the excess transmission of a single allele at one locus, AfuG 204, by the female. The allele that is transmitted less frequently was not present in any of the fathers. Possible explanations for the differential transmission of one allele include the occurrence of meiotic drive and/or selection, either pre-zygotic or in the early stages of development. Meiotic drive occurs when an allele is preferentially transmitted during gamete formation, resulting in non-Mendelian segregation. Selection is also a possibility, as recent evidence suggests that microsatellite loci may not be strictly neutral (Fondon and Garner, 2004). Some microsatellites may be under selection, either directly or indirectly (through linkage), resulting in distorted segregation ratios. Excess transmission of a single allele at two microsatellite loci was also observed in artificial crosses of the sterlet sturgeon, *A. ruthenus* (Williot et al., 2005).

Although the lake sturgeon genome is actively returning to diploidy, the majority of their genome remains duplicated (Ludwig et al., 2001; Welsh et al., 2003; Fontana et al., 2004). This duplication can complicate population genetic studies because many of the analyses (e.g. testing for Hardy–Weinberg equilibrium and other tests reliant on allele frequency data) assume disomic segregation. Without inheritance testing, microsatellite loci may appear to be disomic, but actually may not meet the diploid assumption.

From a conservation genetics perspective, an understanding of the effect of polyploidy on genetic concerns resulting from small population sizes is important to effective management. Depending upon the degree of functional retention, duplicated loci could buffer an individual from fitness shortcomings resulting from inbreeding, by delaying the expression of deleterious recessive alleles (Allendorf and Waples, 1996). Duplicated *Sox* genes (transcription factors that affect development and sex determination) in *A. sturio* appear to still be

Table 2
Test for disomic segregation

Locus	Family	Female	Male	Offspring						N	Female (χ^2)	Male (χ^2)
AfuG 9	04374	140, 152	144, 144	140, 144	144, 152	140, 144, 152				46	0.68	NA
Observed				23	21	2						
Expected				23	23	0						
	04399		140, 144	140, 140	140, 152	140, 144	144, 152			51		2.37
Observed				11	20	7	13					
Expected				12.75	12.75	12.75	12.75					
	04400		140, 144	140, 140	140, 152	140, 144	144, 152			52		2.33
Observed				10	10	17	15					
Expected				13	13	13	13					
AfuG 56	04374	266, 266	266, 266	266, 266						67	NI	NI
Observed				67								
Expected				67								
	04399		266, 266	266, 266						47		NI
Observed				47								
Expected				47								
	04400		262, 266	266, 266	262, 266					46		0.09
Observed				22	24							
Expected				23	23							
AfuG 63	04374	127, 143	143, 143	127, 143	143, 143	127, 143, 143				87	4.55	NA
Observed				50	36	1						
Expected				43	43	0						
	04399		139, 139	127, 139	139, 143					60		NA
Observed				30	30							
Expected				30	30							
	04400		127, 127	127, 127	127, 143					65		NA
Observed				41	24							
Expected				32.5	32.5							
AfuG 74	04374	218, 226	218, 222	218, 218	218, 226	218, 222	222, 226	218, 222, 226, 226	218, 218, 226	65	0.04	1.29
Observed				18	18	12	15	1	1			
Expected				15.75	15.75	15.75	15.75	0	0			
	04399		218, 222	218, 218	218, 226	218, 222	222, 226			51		0.96
Observed				16	13	10	12					
Expected				12.75	12.75	12.75	12.75					
	04400		218, 226	218, 218	218, 226	226, 226				37	NA	NA
Observed				9	21	7						
Expected				9.25	18.5	9.25						
AfuG 112	04374	252, 256	244, 256	256, 256	252, 256	244, 256	244, 252	244, 252, 256	244, 244, 252, 256	38	3.77	4.12
Observed				13	11	6	6	1	1			
Expected				8.75	8.75	8.75	8.75	0	0			
	04399		256, 256	256, 256	252, 256					44		NA
Observed				27	17							
Expected				22	22							
	04400		256, 256	256, 256	252, 256					24		NA
Observed				16	8							
Expected				12	12							

Table 2
Continued

Locus	Family	Female	Male	Offspring				N	Female (χ^2)	Male (χ^2)
AfuG 122	04374	179, X	167, 167	167, 179	167, X			58	5.56	NA
Observed				31	27					
Expected				29	29					
	04399		X, X	179, X	X, X			63		NA
Observed				39	24					
Expected				31.5	31.5					
	04400		167, 171	167, 179	171, 179	167, X	171, X	41		1.20
Observed				16	10	8	7			
Expected				10.25	10.25	10.25	10.25			
AfuG 195	04374	165, 165	165, 165	165, 165				87	NI	NI
Observed				87						
Expected				87						
	04399		161, 161	161, 165				44		NI
Observed				44						
Expected				44						
	04400		161, 165	165, 165	161, 165			78		0.20
Observed				41	37					
Expected				39	39					
AfuG 160	04374	135, 147	147, 147	147, 147	135, 147	135, 147, 147	135, 147, 147, 147	92	0.36	NA
Observed				42	48	1	1			
Expected				45	45	0	0			
	04399		135, 135	135, 135	135, 147			56		NA
Observed				28	28					
Expected				28	28					
	04400		147, 147	147, 147	135, 147			81		NA
Observed				39	42					
Expected				40.5	40.5					
AfuG 204	**04374**	**141, 145**	**141, 141**	**141, 141**	**141, 145**			**80**	**12.57, P = 0.0003**	NA
Observed				**43**	**37**					
Expected				**40**	**40**					
	04399		**141, 141**	**141, 141**	**141, 145**			**44**		NA
Observed				**29**	**15**					
Expected				**22**	**22**					
	04400		**141, 141**	**141, 141**	**141, 145**			**67**		NA
Observed				**48**	**19**					
Expected				**33.5**	**33.5**					

For each of nine loci, segregation was tested for three families: 04374, 04399, and 04400. The same female was used for all families, with each family having a different male as father. Observed and expected numbers of offspring genotypes are listed, along with number of offspring tested for each family (N). An 'X' in the genotype indicates a null allele. χ^2-value for segregation of female gametes calculated by combining all families; χ^2-value for segregation of male gametes tested within each individual family. Individuals with uninformative genotypes denoted by 'NA'. Significance tested at an α-level of 0.05 (d.f. = 1), after correcting for multiple comparisons using a sequential Bonferroni correction (Rice, 1989). Families and loci significantly different from Mendelian segregation expectations noted in bold.

under selection, despite the fact that the duplication event occurred a long time ago, indicating that these duplicated loci likely remain functional (Hett and Ludwig, 2005). Duplicated loci and their subsequent divergence can create greater allelic diversity through mutation. However, polyploids may be more vulnerable to outbreeding depression (Allendorf and Waples, 1996). If secondary segregation occurs (Allendorf and Danzmann, 1997), a second recombination event may increase the chances for the disruption of coadapted gene complexes. Thus, knowing lake sturgeon are polyploid derivatives helps assess potential fitness consequences of management decisions that may increase levels of inbreeding or outbreeding.

We identified nine polymorphic disomic microsatellite loci that, when combined with four loci previously identified as disomic in lake sturgeon (Pyatskowit et al., 2001; McQuown et al., 2002), provide 13 disomic loci useful in the study of lake sturgeon population genetics and the recovery of the species. This study provides a model for the development of genetic markers among laboratories. The fishery managers involved in lake sturgeon conservation had the foresight to understand the need for genetic standardization across laboratories, and researchers cooperatively attained that goal. Individual laboratories can accumulate substantial data, but the genetic data may mean little on a geographic scale larger than those of individual projects when standardization among laboratories has not occurred. Hopefully, the implementation of the model described here will increase standardization among laboratories and make genetic markers a more powerful tool for the conservation of species.

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