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Inheritance of microsatellite loci in the polyploid lake sturgeon (*Acipenser fulvescens*)

Jonathan D. Pyatskowit, Charles C. Krueger, Harold L. Kincaid, and Bernie May

Abstract: Inheritance in the expression of amplicons for four microsatellite primer pairs was determined using 10 families created from gametes of wild lake sturgeon (*Acipenser fulvescens*). Loci *Afu34* and *Afu68* expressed a maximum of two even-intensity bands per individual and had progeny genotype ratios that fit disomic inheritance ($P > 0.05$). Some variation exhibited at *Afu34* and *Afu68* was attributable to a null allele. Genotype expression at both loci also indicated that one female parent had transmitted unreduced gametes. Primer Afu39 amplified products that exhibited four gene doses, where genotype counts fit expected ratios for disomic inheritance ($P > 0.05$) indicating amplification of products from two disomic loci that share alleles. Meiotic drive was evident at the *Afu39* loci based on a test for random segregation ($P < 0.05$). Only the expression of *Afu19* gave evidence of tetrasomic inheritance based on a single progeny potentially produced by a double reduction gamete. No evidence for proposed octoploid inheritance was observed.

Key words: diploid, tetraploid, octoploid, meiotic drive.

Résumé : L'hérédité d'amplicons produits à l'aide d'amorces spécifiques de quatre microsatellites a été déterminée chez 10 familles issues de gamètes provenant d'esturgeons jaunes (*Acipenser fulvescens*) sauvages. Les locus *Afu34* et *Afu68* ont produit au maximum deux bandes d'intensité égale par individu et ont montré des ratios génotypiques compatibles avec une hérédité disomique ($P > 0{,}05$). Une certaine variation observée chez les locus *Afu34* et *Afu68* était attribuable à un allèle nul. L'expression génotypique pour ces deux locus indiquait qu'un parent femelle avait transmis des gamètes non réduits. Les amorces Afu39 ont amplifié des produits correspondant à quatre doses géniques et dont le décompte génotypique suggérait une hérédité disomique ($P > 0{,}05$). Les auteurs concluent qu'il s'agit d'amplicons provenant de deux locus disomiques qui ont des allèles en commun. Une transmission méiotique préférentielle a été observée pour le locus *Afu39*, lequel ne montrait pas une ségrégation aléatoire ($P < 0{,}05$). Seul le locus *Afu19* a montré une hérédité de type tétrasomique et cela basé sur un seul descendant potentiellement issu d'un gamète doublement réduit. Aucune évidence d'une hérédité octoploïde, proposée antérieurement, n'a été observée.

Mots clés : diploïde, tétraploïde, octoploïde, transmission méiotique préférentielle.

[Traduit par la Rédaction]

Introduction

Lake sturgeon (*Acipenser fulvescens*) were once abundant throughout the Great Lakes drainage but overharvesting, pollution, and the damming of rivers reduced sturgeon populations to low levels, sometimes to extinction. For example, the commercial harvest of sturgeon in Lake Erie was 2.25×10^6 kg in 1860 but by 1895 had declined by 80% (Scott and Crossman 1973). Most populations today exist at low levels. Recently, fishery management agencies (e.g., Wisconsin Department of Natural Resources) have undertaken programs to restore lake sturgeon populations. Often the descriptions of these programs recognize genetics as an important component of fishery management, but operationally fail to incorporate genetic provisions into the program (e.g., Menominee Indian Tribe of Wisconsin 1994).

The genetics of lake sturgeon is poorly understood because of their unique life history and sampling difficulties. First, the life history of lake sturgeon makes investigations, such as heritability studies, difficult to perform. Sexual maturation takes 10–30 years and subsequent spawning occurs at intermittent intervals of 1–6 years (Priegel and Wirth 1971). Second, sampling enough individuals as juveniles or adults for population genetics studies is difficult because of

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Table 1. Family designations, parental identification number, and parental genotypes used to examine the inheritance of allelic variation of microsatelite loci in lake sturgeon.

			Afu19		*Afu34*		*Afu39*		*Afu68*	
Family	Female	Male	F	M	F	M	F	M	F	M
K1	195	159	*1112*	*1112*	*2Ø*	*12*	*1113*	*1113*	*44*	*3Ø*
K2	195	193	*1112*	*1112*	*2Ø*	*12*	*1113*	*1122*	*44*	*4Ø*
K3	195	194	*1112*	*1111*	*2Ø*	*12*	*1113*	*1113*	*44*	*44*
K4	195	160	*1112*	*1112*	*2Ø*	*22*	*1113*	*1112*	*44*	*12*
K6	197	193	*1111*	*1112*	*12*	*12*	*1111*	*1122*	*23*	*4Ø*
K7	197	159	*1111*	*1112*	*12*	*12*	*1111*	*1113*	*23*	*3Ø*
K8	197	160	*1111*	*1112*	*12*	*22*	*1111*	*1112*	*23*	*12*
P1	84	85	*1111*	*1112*	*12*	*22*	*1111*	*1111*	*34*	*44*
P2	84	87	*1111*	*1222*	*12*	*12*	*1111*	*1111*	*34*	*11*
P3	84	88	*1111*	*1112*	*12*	*22*	*1111*	*1113*	*34*	*44*

Note: Ø is a null allele determined from the inheritance studies. The base pair size at *Afu19* of allele *1* = 133 (clone) and *2* = 130, at *Afu34 1* = 140 and *2* = 137 (clone), at *Afu39 1* = 133, *2* = 127, and *3* = 124 (clone), and at *Afu68 1* = 128, *2* = 124, *3* = 120 (clone), and *4* = 112. *Afu19* may be one tetrasomic locus (see Table 2). *Afu39* may represent two disomic loci that share alleles (see Table 5).

their low abundance and distribution over large geographic areas during most of their life history (Kempinger 1996). Only during the spawning season do numbers of adults assemble into spawning habitat and are vulnerable to sampling. In addition, nonlethal genetic techniques are required to study sturgeon because in most places the species is endangered or threatened. Thus, genetic techniques employed must not depend on samples from internal tissues (e.g., heart, liver) that require fish to be sacrificed. For example, white muscle biopsies from lake sturgeon in Wisconsin were used in the detection of six polymorphic allozyme loci (Bartley 1987).

Genetic studies of sturgeon are further complicated by the reported octoploid nature of the species (Blacklidge and Bidwell 1993). If sturgeon have an octoploid genome, interpretation of banding patterns on gels will be complicated and difficult without confirmatory inheritance studies. Such studies were required of allozyme loci in salmonids in order to understand their disomic inheritance with residual tetrasomy (e.g., May 1980).

Techniques recently developed to examine variation at microsatellite loci facilitate genetic studies of sturgeon in three ways. First, microsatellite loci can be examined with nonlethal tissue sampling. DNA for microsatellite studies can be extracted from fin, barbel, or scale tissue (Wirgin and Waldman 1994). Second, microsatellite loci sometimes provides greater discriminatory power than other genetic markers, especially when genetic variation exists at low levels in other genetic characters. Microsatellite loci have a much higher mutation rate and consequently often more alleles per locus than allozymes (e.g., Estoup et al. 1993; Grant et al. 1999) and thus permit more potential genetic differences at microsatellite loci to accumulate among populations. Genotypes inferred from gels can be compared to Hardy–Weinberg expectations or used to estimate allelic frequencies to be used in gene diversity analyses to describe population structure. However, confirmation of Mendelian inheritance of microsatellite variation is important, especially for polyploid-derived species such as sturgeon, to correctly translate allelic variation from banding patterns on gels (Allendorf et al. 1975).

This study describes the inheritance of allelic variation at microsatellite loci in lake sturgeon and tests the hypothesis of octoploid inheritance in this species. Segregation and mode of inheritance in single pair matings (families) are reported here for five loci previously described by May et al. (1997).

Materials and methods

Nomenclature

Eleven microsatellite primer pairs (May et al. 1997) were tested for amplification quality and genetic variability in DNA samples from the parents of the 10 lake sturgeon families. These primer pairs were originally named LS-## for Lake Sturgeon plus a sequence number (##, May et al. 1997). However, to conform to current nomenclature for microsatellite loci, we herein rename the primers to Afu## using the first letter of the genus and the first two letters of the species name combined with the same sequence number as used before. Primers are referred to in the text by using regular font (Afu#) whereas references to loci use italics (*Afu*#). Alleles are represented throughout the text as single digit numerals in italics for ease of discussion; their specific base-pair sizes are given in the heading for Table 1.

Sample collection

One mature female and three mature males were captured from the Menominee River in Wisconsin below the White Rapids dam using electrofishing and held in 1.8-m diameter tanks. A portion of fin was sampled from each fish with one half stored in 100% ethanol and the other half in lysis buffer (150 mM EDTA, 50 mM Tris pH 8.0, 2% *n*-lauroylsarcosine). Milt was collected from each male, using a syringe and stored on ice. Eggs were stripped from the female, divided into three lots, and each lot was fertilized with milt from a different male to create three different families. Fertilized eggs were incubated at the Wild Rose State Fish Hatchery (Wisconsin Department of Natural Resources) for several days before transport to rearing facilities at the Resource Ecology and Management Facility, Cornell University. All fry from these families (N = 14, 30, and 20) were sampled immediately after hatch because of chronic mortality caused by a fungal infection. Seven families were also created from two females and four males captured in the Des Prairies River at Montreal, Quebec. Seven families were created instead of eight, because one male was crossed with only one female (instead of two). One-hundred fry from each of the seven families were sampled. Fifty fry were stored at –80°C and 50 fry were stored in lysis buffer. Families created from the Menominee and Des Prairies River fish were established without prior knowledge of parental genotypes. Regardless of how the sam-

ples were stored (frozen, in alcohol, or in lysis buffer) ample DNA for analysis was extracted.

DNA extraction

DNA extraction used the CTAB (cetyltrimethylammonium bromide) procedure described by Grewe et al. (1993), but modified as follows: 30 μL of proteinase K (10 mg/mL stock) was used in each sample and samples were incubated overnight at 55°C before extraction. This procedure differed from Grewe et al. (1993) in that it used three times the amount of proteinase K (300 μg vs. 100 μg) and the samples were incubated longer (10–12 h vs. 30–60 min). The extended incubation step helped breakdown the tough fin tissue and generated greater DNA yields.

Polymerase chain reaction conditions

Fifty-microlitre reactions were run using a Perkin–Elmer 4800 Cycler. Each reaction mixture contained 1 μL of DNA (~20 ng/μL), 5 μL of Gibco 10× buffer (Gaithersburg, Md.), 1 μL each of forward and reverse locus specific primer (20 μM), and 0.2 μL of Gibco Taq polymerase (5 U/μL). Each mixture contained $MgCl_2$ (see May et al. 1997) and 2.0 μL (for Afu22, Afu23, Afu39, Afu57, Afu58, and Afu69) or 3.5 μL (Afu19, Afu34, Afu54, Afu62, and Afu68) of dNTPs (2.5 mM) to optimize the mixture for specific primers. Filtered, deionized water was added to each mixture to bring the total volume to 50 μL. In addition, a mineral oil overlay was added to each reaction mixture before placement into the thermocyler. An amplification consisted of three min at 94°C, and then 33 cycles of one min at 94°C, 30 s at 57°C, 30 s at 72°C, and then, 5 min at 72°C. Afu34 differed in that the optimal annealing temperature was 59°C rather than 57°C. DNA amplification was confirmed by resolving 10 μL of the PCR reaction on a 3.5% agarose gel (Nusieve GTG – SeaKem LE, 1:1) stained with ethidium bromide.

Gel conditions

Amplified DNA samples were resolved on a 4% metaphor gel at 460 V with the 0.5× TBE (Tris-borate EDTA) gel buffer circulated through a Neslab RTE 100 cooling unit which maintained the temperature at 17–18°C. A 10-μL aliquot of amplified DNA was concentrated to 5 μL in a Speed Vac. One microlitre of Sigma dye (St. Louis, Mo.) was added to each sample before the samples were loaded onto the gel. Electrophoresis of amplified DNA used a prerun step at 460 V for 5 min in 12–13°C uncirculated buffer. Voltage was then turned off, and the buffer temperature was increased to 17–18°C. Voltage and buffer circulation was then restored for 0.75–1.5 h (run time was determined by the clone size: 15 min for every 25 base pairs). Gels were stained with ethidium bromide and a Polaroid picture was taken to score individuals. Relative band intensity was determined by eye (e.g., 1112 vs. 1122). Asymmetry in PCR products was assumed to reflect gene dosage.

Fragment sizes were determined as follows. The forward primer (see May et al. 1997) for *Afu19*, *34*, *39*, and *68* was purchased with a 5′ fluorescein label, and representative genotypes were amplified and examined electrophoretically. Twenty-five microlitres of 98% formamide loading buffer was added to 25 μL of the amplified product. This mixture was denatured at 95°C for 2 min and then snap-cooled on ice for at least 5 min prior to loading. Eight microlitres of sample was loaded on a 5% polyacrylamide (19:1) gel with 7.5 M urea (20 cm × 32 cm × 0.4 mm). Gels were run at 40 W (50°C) between 1 and 1.5 h depending on the fragment sizes and imaged with a Molecular Dynamics 595 fluorimager. Bands were sized using Molecular Dynamics' FRAGMENNT ANALYSIS software and Bio-Rad fluorescent low-range fluoroscein ladder (170-3123, Hercules, Calif.).

PCR products exhibited the same sizes as determined by their original sequencing as reported by May et al. (1997) with the exception of the Afu39 clone. Reexamination of the original sequences for this clone showed the sequence in one direction counted 125 bp and 129 bp in the reverse direction. Both sequences had a region of unassignable bases. Based on the robustness of our sizing using the fluorimager (described above) for the three other clones, we conclude that the clone for Afu39 is 124 bp, not 129 bp as reported in May et al. (1997) nor 125 bp.

Table 2. Test for Mendelian segregation of chromosomes at *Afu19* in lake sturgeon.

	Parental genotypes		Progeny genotype counts			
Family	F	M	*1111*	*1112*	*1122*	χ^2 (1 d.f.)
K3	*111<u>2</u>*	*1111*	42	48		0.4
K6	*1111*	*111<u>2</u>*	35	56		4.84*
K7	*1111*	*111<u>2</u>*	49	50		0.01
K8	*1111*	*111<u>2</u>*	41	48	1†	0.55
P1	*1111*	*111<u>2</u>*	7	7		0
P2	*1111*	*1<u>2</u>22*		24	6	10.80**
P3	*1111*	*111<u>2</u>*	11	9		0.2

Note: Expected progeny genotypes are 1:1 ratio of homozygotes to asymmetrical heterozygotes. Underlined numbers are the alleles being tested.
†Denotes a double reduction event.
*Denotes a difference from expected ratios at the 0.05 level and ** at the 0.01 level.

Data analysis

Mode of inheritance for loci that exhibited four gene doses was determined with χ^2 tests of progeny genotype ratios (*1111*, *1112*, or *1122*) with ratios expected for tetrasomic inheritance (1:4:1) or disomic inheritance (1:2:1) from crosses that had one homozygous parent (*1111*) and one symmetrical heterozygous parent (*1122*) (Snedecor and Cochran 1989). In crosses where only one alternate allele was present in one of the parents, progeny genotypes were examined for the presence of a double reduction event that would indicated multivalent tetrasomic inheritance (Burnham 1962; Marsden et al. 1987*a*). For example, a cross between an asymmetrical heterozygous parent and a homozygous parent (*1112* × *1111*) would confirm tetrasomy if any progeny exhibited the (*1122*) genotype. In families that appeared to exhibit only two gene doses, the cross of heterozygous parents (*12* × *12*) was tested for goodness of fit (χ^2 test) to the disomic inheritance model 1:2:1. Meiotic drive, the unequal distribution of alleles into gametes, was tested with a χ^2 test (Marsden et al. 1987*a*; Snedecor and Cochran 1989).

Results

Five of 11 primers examined failed to amplify or were insufficiently resolved for analysis (Afu22, Afu23, Afu57, Afu58, and Afu69). No efforts were made to optimize these primers. One locus (*Afu62*) was homozygous for all parents, so inheritance tests could not be performed. *Afu62* was found to be polymorphic in a larger survey of individuals for population analysis (unpublished data). Another locus (*Afu54*) exhibited variation for which the amplicons from the same individual differed between PCR amplifications, resulting in its exclusion from the inheritance study. Inheritance of the variation observed with the four primers remaining are described below.

Afu19 exhibited two alleles with variation in band intensity of parent and progeny genotypes that indicated simultaneous expression of four gene doses (Table 2). Locus *Afu19* appeared to exhibit tetrasomy and was scored as a tetrasomic locus based on the presence of one progeny that appeared to

Table 3. Segregation of alleles at *Afu34* (interpreted as a single disomic microsatellite loci) in artificial crosses of lake sturgeon.

	Parental genotypes		Observed progeny genotypes					
Family	F	M	*11 or 1Ø*	*12*	*22 or 2Ø*	*112*	*122*	χ^2 *(d.f)*
K6	*12*	*12*	17	20	13			2.64 (2)
K7	*12*	*12*	13	21	14			0.79 (2)
K1	*2Ø*	*12*	12	12	22			0.09 (1)
K2	*2Ø*	*12*	12	10	20			0.10 (1)
K3	*2Ø*	*12*	11	11	22			0.00 (1)
K8	*12*	*22*	12	20				2.00 (1)
P1	*12*	*22*	3	8				2.27 (1)
P2	*12*	*12*	1	4	10	10	2	
P3	*12*	*22*	3	13				6.25 (1)*

Note: Underlined numbers represent alleles that are being tested for segregation. Unpredicted genotypes *112* and *122* (trisomic) in family P2 may have been the result of unreduced gametes at *Afu34*. As a result, χ^2 for family P2 is not defined because the expected value for this genotype is zero.

*Denotes a difference from expected ratios at the 0.05 level.

Fig. 1. *Afu39* gel showing the expression of three genotypes and three alleles. Note the asymmetry of genotypes *1113* and *1112* and the heteroduplex bands that migrate more slowly than the homoduplex or allelic bands.

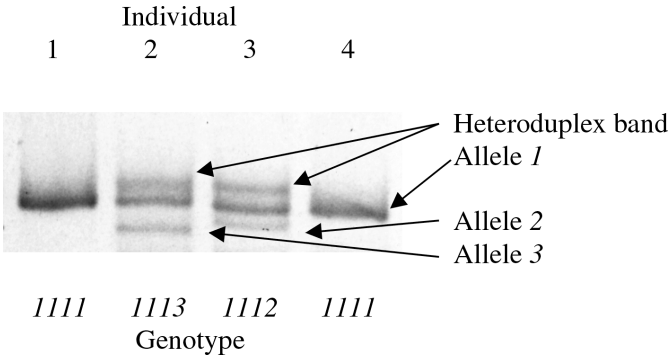


Table 4. Segregation of alleles at *Afu39* in artificial crosses of lake sturgeon.

	Parental genotypes		Gamete types that produced the progeny		
Family	F	M	*11*	*1X*	χ^2 (1 d.f.)
K2	*1113*	*1122*	23	4	6.69**
K4	*1113*	*1112*	60	34	7.19**
K4	*1113*	*1112*	65	16	13.79**
K7	*1111*	*1113*	29	21	1.28
K8	*1111*	*1112*	19	31	2.88
P3	*1111*	*1113*	4	6	0.4

Note: Underlined numbers identify the particular allele being tested for segregation. The "X" under gamete type denotes the presence of that allele in progeny. For example, in family K2 four progeny were produced from the *13* gamete in the female. Expected gamete types transmitted to the progeny from the parent is a 1:1 ratio of *11* to *1X* gametes.

*Denotes a difference from expected ratios at the 0.05 level and ** at the 0.01 level.

be the product of a double reduction gamete. This progeny (a *1122* offspring) was produced by a cross between a homozygous female and an asymmetric heterozygous male (*1111* × *1112*; family K8; Table 2). In family P2, the *22* gamete was underrepresented relative to the *12* gamete from the male parent ($P < 0.001$; Table 2), suggesting possible meiotic drive. A slower migrating heteroduplex band was formed in heterozygous individuals on *Afu19* gels.

Afu34 appeared to be a disomic locus with two alleles symmetrically expressed on gels as two gene doses (Table 3). χ^2 tests of progeny from two families (K6 and K7) created by crosses of heterozygotes (*12* × *12*) did not reject the disomic model. In a third family (P2) created by a cross of heterozygotes, many unbalanced heterozygous progeny (*112* and *122*) were observed. One explanation for these genotypes is that one parent, in this case the male, produced both reduced gametes (either *1* or *2* gametes) and unreduced gametes (*12* gametes). In families P1 and P3, an excess of heterozygote (*12*) progeny, 21 heterozygotes versus 6 homozygotes, were observed where a 1:1 ratio was expected (Table 3). The excess heterozygous class could have included both 12 and 122 offspring, although no assymmetry in expression was observed among heterozygotes. The same female parent was used in both families, as in family P2 which produced the trisomic individual described above (Table 1). In three other families, unexpected *Afu34* genotypes were scored among the progeny (K1–K3; Table 3). The apparent *22* female was crossed with *12* males, which should have produced only expected progeny genotypes of *12* and *22*. However, some individuals from these families were scored as *11* genotypes. Thus, the female could have produced these progeny only if she had a null allele and was actually a *2Ø* genotype. The null allele when combined with the *1* allele appears on the gel as a homozygous (*11*) individual.

Primer Afu39 revealed the expression of three alleles, and variation in band intensity of phenotypes was indicative of the simultaneous expression of four gene doses (Fig. 1; Table 4). In three out of six tests for normal segregation of alleles, the *2* and *3* alleles were underrepresented in the progeny, and this provided evidence of meiotic drive ($P < 0.001$; Table 4). Families K2 and K6 were used to test between the tetrasomic and disomic inheritance models (Table 5). The tetrasomic model was rejected ($P < 0.01$) and the disomic model was accepted ($P > 0.05$) for both families. No evidence of double reduction gametes (tetrasomy) occurred in the progeny from crosses of homozygotes and asymmetrical heterozygotes (Table 4). Thus, this primer ap-

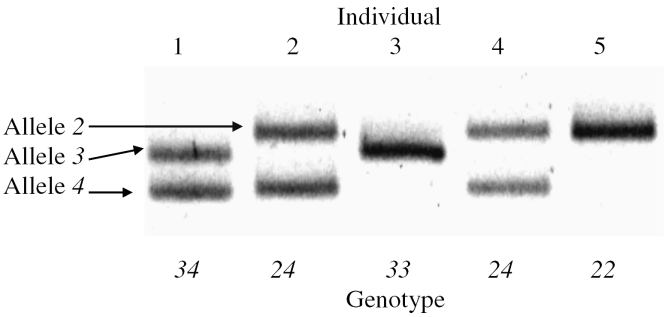
Table 5. Observed and expected segregation of a single tetrasomic microsatellite locus or two disomic microsatellite loci for *Afu39* in artificial crosses of lake sturgeon.

	Parental genotypes		Observed progeny genotypes†			
Family	F	M	*1111*	1112	1122	χ^2 (2 d.f.)
K2	*1113*	1122	23	37	13	
			[12.2]	[48.6]	[12.2]	12.38**
			{18.25}	{36.5}	{18.25}	2.76
K6	*1111*	1122	24	50	23	
			[16.2]	[64.6]	[16.2]	9.91**
			{24.25}	{48.5}	{24.25}	0.11

†[Expected tetrasomic 1:4:1]{expected disomic 1:2:1}.

**Denotes $P < 0.01$.

Fig. 2. *Afu68* gel showing four genotypes and three alleles. Note the absence of the asymmetrical genotypes that would indicate four gene doses.



peared to reveal the expression of two disomic loci (*Afu39*) that share similar alleles.

Afu68 was interpreted as a disomic locus with four alleles based on phenotypes expressed as two gene doses, consistent with disomy (Fig. 2; Table 6). In family P2, the cross of a *34* female with a *11* male yielded a number of progeny that expressed three alleles (*134* genotype, see Fig. 3). These progeny were unexpected based on the disomic inheritance model (Table 6). The *34* female may have transmitted both reduced (either a *3* or *4* allele) and unreduced (*34*) gametes to produce the three progeny genotypes observed (*13*, *14*, and *134*). Trisomic progeny were also produced in the same family at *Afu34* (family P2, Table 3). Families P1 and P3 had the same female parent as P2 (Table 1); however, no discrepancy in segregation was noted. A null allele was detected at *Afu68* based on the presence of unpredicted homozygote progeny such as *1Ø* in family K1 (Table 7). The unpredicted homozygotes can be explained by scoring the males in these crosses with a null allele. A disomic locus, whose amplicons were larger, was observed inconsistently and was presumed to be the homeolog of the disomic *Afu68* we studied.

The mode of inheritance for *Afu62* could not be determined as parents for all the crosses were homozygous for the common allele. However, three alleles were found in an analysis of fish from several populations (unpublished data).

Table 6. Segregation of alleles at *Afu68* (interpreted as a single disomic microsatellite locus) in artificial crosses of lake sturgeon.

	Parental genotypes		Gamete type that produced progeny		
Family	F	M	*A*	*X*	χ^2 (d.f)
K1	*44*	*3Ø*	27	22	0.51 (1)
K4	*44*	*12*	25	23	0.08 (1)
K6	*23*	*4Ø*	48	46	0.04 (1)
K6	*23*	*4Ø*	58	36	5.15 (1)*
K7	*23*	*3Ø*	23	25	0.08 (1)
K8	*23*	*12*	45	33	1.85 (1)
K8	*23*	*12*	39	39	0.00 (1)
P1	*34*	*44*	9	5	1.14 (1)
P2	*34*	*11*	4	4	0.00 (1)
P3	*34*	*44*	10	10	0.00 (1)

Note: Underlined numbers identify the particular allele being tested for segregation. The "*X*" under gamete type denotes the presence of that allele in progeny. The "*A*" denotes the gamete bearing the alternate allele. Expected gamete types transmitted from the parent to the progeny is a 1:1 ratio of *A* to *X* gametes. See Table 7 for complete listing of observed genotypes for families K1, K6, and K7 with null alleles. Family P2 had 21 additional progeny of the unpredicted *134* genotype (trisomic). These progeny may have been the result of unreduced gametes produced by the P2 female (see P2 trisomic progeny at *Afu34* in Table 3).

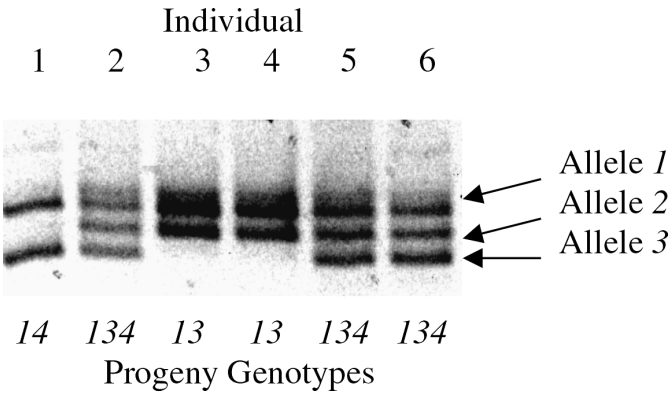
*Denotes a difference from expected ratios at the 0.05 level.

The simultaneous expression of four gene doses was similar to that found at *Afu19* and *Afu39* suggesting either a tetrasomic locus or two disomic loci.

Discussion

Based on these results, the lake sturgeon currently has a genome similar to salmonid fishes where some tetrasomic loci have become two disomic loci and others retain a tetrasomic state (Wright et al. 1983; Allendorf and Thorgaard 1984). Two (Afu34 and 68) of the four primers used in this study revealed the expression of two gene doses, while the other two primers (Afu19 and 39) showed four gene doses. Disomic inheritance occurred at *Afu34*, *Afu39*, and *Afu68* based on gene dose expression and progeny genotype ratios. Tests for disomic versus tetrasomic inheritance could not be performed for *Afu19*, because no families contained a parent that was a *1122* genotype (balanced heterozygote). At *Afu19*, the *1122* progeny produced by a double reduction gamete provided evidence of tetrasomy (see Mar-

Fig. 3. *Afu68* gel showing the unexpected *134* genotype found in progeny from the mating of a *34* female and an *11* male.



sden et al. 1987*a*) but this was a single case out of over 200 meioses involving the *2* allele. Octoploid inheritance as previously suggested based on a study of cell DNA content and chromosome counts of lake sturgeon (Birstein and Vasiliev 1987; Blacklidge and Bidwell 1993; Birstein et al. 1997) was not supported by the results of this study. While a second disomic locus was occasionally seen on Afu68 gels following some PCRs, no additional amplicons were seen at any of the loci exhibiting four gene doses. Additional inheritance data for five more loci with more alleles per locus supports this view that the lake sturgeon genome is a tetraploid derivative and not octoploid (unpublished data). Use of either disomic or tetrasomic inheritance models simplifies the interpretation of variation at microsatellite loci over more complicated polyploid models. These models are well known in other species such as the tetraploid treefrog (*Hyla versicolor*; Marsden et al. 1987*a*), lake trout (*Salvelinus namaycush*; Marsden et al. 1987*b*), and Pacific salmon (*Oncorhynchus* sp.; Allendorf et al. 1975).

The unreduced gametes for the female of family P2 may have been accidentally caused by the artificial process used to make this cross (Tables 3 and 6). For example, the retention of the second polar body causing trisomy could have been induced by an unknown exposure to temperature extremes or a physical shock in egg handling. On the other hand, Blacklidge and Bidwell (1993) found one of eight lake sturgeon and 1 of 15 Gulf sturgeon (*A. oxyrhynchus desotoi*) to be triploids based on DNA content, implying a nonreduction gamete in one of the parents. It is of interest that nonreduction in our study only happened in one of the three crosses made with female 84 (Table 1). Perhaps nondisjunction occurs fairly frequently in this species.

Heterozygous individuals at loci (*Afu19*, *Afu34*, and *Afu39*) exhibited a "heteroduplex" band that aided in their genotype classification. These bands are believed to be formed from the complementary strands of the two different sequences found in a heterozygote. Because these new heteroduplex bands are pairings of sequences that differ in the number of repeat motifs, they do not pair exactly and tend to drag in the gel (migrate more slowly). The heteroduplex bands appear as artifacts migrating slower than the homoduplex bands, even though their molecular weight would suggest they should migrate between the two primary amplicons. Usually the two types of heteroduplex bands comigrate although in some cases both are observed.

Table 7. Lake sturgeon families that had evidence of a null allele segregating at *Afu68*.

	Parental genotypes		Observed progeny genotypes					
Family	F	M	*2Ø*	*23*	*24*	*33 or 3Ø*	*34*	*4Ø*
K1	*44*	*3Ø*					27	22
K6	*23*	*4Ø*	23		25	13	33	
K7	*23*	*3Ø*	10	13		25		

Reading dosage in phenotypes derived from more than two gene doses as done in our study has been argued to be impractical (see Spruell et al. 1999). While amplification of low-concentration template DNA can lead to alteration in the resultant amplicon ratios from the original template ratios, we rarely observed differential amplification for normal template concentrations when the gels are imaged with fluorimagers or photo documentation. However, capillary gels were observed to not necessarily display the correct ratios as observed with whole-gel imaging (e.g., ABI 310s). A photo of the gene dosage pattern interpretable from our gels may be seen in Fig. 2 from May et al. (1997) that shows all five tetrasomic banding types from a cross of two parents with the same two bands of equal intensity in each (double heterozygotes) for Afu19 in Atlantic sturgeon (*A. oxyrhynchus oxyrhynchus*). Repeated amplifications of the same individuals always lead to the same ratios of bands in the amplicons. Current work with the same lake sturgeon families from the present study has uncovered additional four dose loci with more alleles per locus with many examples of the additional characteristic patterns for four dose loci, phenotypes with three bands with one of the three twice as dark as the other two and phenotypes with four bands of equal intensity (unpublished data). In other sturgeon species such as white sturgeon (*A. transmontanus*), phenotypes involving more than four gene doses with the presence of null alleles are problematic in interpretation (unpublished data).

The presence of null alleles was detected in individuals at *Afu34* and *Afu68* (Tables 3 and 7). Null alleles are often undetected during population studies; however, inheritance studies can readily confirm their presence. The presence of null alleles tends to inflate estimates of allelic frequencies in population studies and are problematic where parentage is to be inferred, as in behavioral studies. Null alleles (presumptively due to changes in the primer site) can sometimes be resolved by redesigning the primers (O'Reilly and Wright 1995).

Development and testing of additional microsatellite loci in sturgeon will be beneficial for future genetic studies, especially for investigations of population structure (see McQuown et al. 2000). Although development of species-specific primers is expensive, microsatellite primers often cross-amplify in related species; thus, new primers would likely be widely applicable in many studies once developed (May et al. 1997; Morris et al. 1996). The inheritance of allelic variation at loci as revealed by new primers should be tested. For example, without this study, the four gene doses

observed at *Afu39* could have been presumed to exhibit tetrasomic as opposed to disomic inheritance.

This research showed that allelic variation at microsatellite loci can be interpreted using disomic and tetrasomic inheritance models instead of a proposed, more complicated, octosomic model (Blacklidge and Bidwell 1993). DNA content and chromosome number studies (reviewed in Birstein et al. 1998) coupled with our microsatellite studies (herein and unpublished data) place the tetrasomic derivative lake sturgeon intermediate between the disomic genomes for pallid (*S. albus*) and shovelnose (*S. platyrhynchus*) sturgeon and the octosomic derivative genome for white (*A. transmontanus*) and green (*A. medirostris*) sturgeon. Confirmation of the mode of inheritance at these loci validates their future use in genetic population studies of lake sturgeon. The ability to examine these loci with tissue obtained via nonlethal sampling greatly enhances the usefulness of these loci in the study of threatened or endangered sturgeon species.

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References

Allendorf, F.W., and Thorgaard, G.H. 1984. Tetraploidy and the evolution of salmonid fishes. *In* Fish Physiology. Vol. 8. *Edited by* W.S. Hoar, D.J. Randall, and J.R. Brett. Academic Press, New York. pp. 407–454.

Allendorf, F.W., Utter, F.M., and May, B.P. 1975. Gene duplication within the family Salmonidae: II. Detection and determination of the genetic control of duplicate loci through inheritance studies and the examination of populations. *In* Isozymes IV. Genetics and Evolution. *Edited by* C.L. Markert. Academic Press, New York. pp. 415–432.

Bartley, D.M. 1987. The genetic structure of chinook and coho salmon populations in California, with a note on the genetic variability in sturgeon (Acipenseridae). Ph.D. thesis, University of California, Davis and San Diego State University, Davis, Calif.

Birstein, V.J., and Vasiliev, V.P. 1987. Tetraploid–octoploid relationships and karyological evolution in the order Acipenseriformes (Pisces); Karyotypes, nucleoli, and nucleolus-organizer regions in four acipenserid species. Genetica, **72**: 3–12.

Birstein, V.J., Hanner, R., and DeSalle, R. 1997. Phylogeny of the Acipenseriformes: Cytogenetic and molecular approaches. *In* Sturgeon Biodiversity and Conservation. *Edited by* V.J. Birstein, W.E. Bemis, and J.R. Waldman. Kluwer Academic Publishers, Dordrecht, The Netherlands.

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

Burnham, C.R. 1962. Discussions in cytogenetics. Burgess Publishing, Minneapolis, Minn.

Estoup, A., Presa, P., Krieg, F., Vaiman, D., and Guyomard, R. 1993. $(CT)_n$ and $(GT)_n$ microsatellites: A new class of genetic markers for *Salmo trutta* L. (brown trout). Heredity, **71**: 488–496.

Grant, W.S., Garcia-Marin, J.L., and Utter, F.M. 1999. Defining population boundaries for fishery management. *In* Genetics in Sustainable Fisheries Management. *Edited by* S. Mustafa. Fishing News Books, Blackwell Science, Oxford. pp. 27–72.

Grewe, P.M., Krueger, C.C., Aquadro, C.F., Bermingham, E., Kincaid, H.L., and May, B. 1993. Mitochondrial DNA variation among lake trout (*Salvelinus namaycush*) strains stocked into Lake Ontario. Can. J. Fish. Aquat. Sci. **50**: 2397–2403.

Kempinger, J.J. 1996. Habitat, growth, and food of young lake sturgeons in the Lake Winnebago system, Wisconsin. N. Am. J. Fish. Manag. **16**: 102–114.

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

Marsden, J.E., Krueger, C.C., Kincaid, H.L., and May, B. 1987*b*. Inheritance of duplicated fumarase and phosphoglucomutase loci in lake trout (*Salvelinus namaycush*). Heredity, **58**: 365–372.

May, B. 1980. The salmonid genome: Evolutionary restructuring following a tetraploid event. Ph.D. thesis, Pennsylvania State University, State College, Pa.

May, B., Krueger, C.C., and Kincaid, H.L. 1997. Genetic variation at microsatellite loci in sturgeon: Primer sequence homology in *Acipenser* and *Scaphirhyncus*. Can. J. Fish. Aquat. Sci. **54**: 1542–1547.

McQuown, E.C., Sloss, B.L., Sheehan, R.J., Rodzen, J., Tranah, G., and May, B. 2000. Microsatellite analysis of genetic variation in sturgeon: new primer sequences for *Scaphirynchus* and *Acipenser*. Trans. Am. Fish. Soc. **129**: 1380–1388.

Menominee Indian Tribe of Wisconsin. 1994. Menominee Reservation Lake Sturgeon Management Plan. U.S. Fish and Wildlife Service, LaCrosse, Wis.

Morris, D.B., Richard, K.R., and Wright, J.M. 1996. Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic studies of salmonids. Can. J. Fish. Aquat. Sci. **53**: 120–126.

O'Reilly, P., and Wright, J.M. 1995. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. J. Fish. Biol. **47**(Suppl. A): 29–55.

Priegel, G.R., and Wirth, T.L. 1971. The Lake Sturgeon: Its Life History, Ecology, and Management. Wisconsin Department of Natural Resources Publication, Madison, Wis. pp. 240–270.

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Fish. Res. Board Can. Bull. 184.

Snedecor, G.W., and Cochran, W.G. 1989. Statistical methods. Iowa State University Press, Ames, Iowa.

Spruell, P., Pilgrim, K.L., Greene, B.A., Habicht, C., Knudsen, K.L., Lindner, K.R., Olsen, J.B., Sage, G.K., Seeb, J.E., and Allendorf, F.W. 1999. Inheritance of nuclear DNA markers in gynogenetic haploid pink salmon. J. Hered. **90**: 289–296.

Wirgin, I.I., and Waldman, J.R. 1994. What DNA can do for you. Fisheries (Bethesda), **19**(7): 16–27.

Wright, J.E., Johnson, K., Hollister, A., and May, B. 1983. Meiotic models to explain classical linkage, pseudolinkage and chromosome pairing in tetraploid derivative salmonid genomes. *In* Isozymes. Curr. Top. Biol. Med. Res. **10**: 239–260.