Journal of Applied Ichthyology

J. Appl. Ichthyol. **27** (Suppl. 2) (2011), 24–33

ISSN 0175–8659

*Received: March 30, 2011*
*Accepted: July 25, 2011*
doi: 10.1111/j.1439-0426.2011.01873.x

# Neutral markers confirm the octoploid origin and reveal spontaneous autopolyploidy in white sturgeon, *Acipenser transmontanus*

By A. Drauch Schreier[1], D. Gille[2], B. Mahardja[1] and B. May[1]

[1]*Genomic Variation Laboratory, Department of Animal Science, University of California Davis, Davis, CA, USA;* [2]*Veterinary Genetics Laboratory, University of California Davis, Davis, CA, USA*

## Summary

The role of polyploidy in vertebrate genome evolution remains a fertile area of research and sturgeons (order Acipenseriformes) provide a unique model of genome duplication, with species possessing ~120, ~250 or ~360 chromosomes. Cytogenetic and molecular data have been used to support different hypotheses about the number of genome duplications in this polyploid series; however, few studies have examined inheritance in sturgeons, although evaluation of polysomic segregation ratios is crucial to inferring ancestral genome duplication level in a polyploid species. Here we examine the inheritance of eight microsatellite loci in fifteen white sturgeon (*Acipenser transmontanus*) families of known parentage to infer the level of genome duplication. Microsatellites were detected as four or eight copy loci. Numbers of alleles per locus, transmission frequencies of informative alleles, and gene copy numbers in parents reveal an ancient octoploid origin for white sturgeon. Comparison to the lake sturgeon genome suggests the ~250 chromosome state in sturgeon was achieved by multiple independent polyploid events. The discovery of spontaneous autopolyploids via microsatellite analysis and flow cytometry provides additional evidence of the plasticity of highly duplicated sturgeon genomes.

## Introduction

The ancient sturgeons and paddlefishes (order Acipenseriformes) provide a unique model of genome evolution in vertebrates, as their radiation contains multiple polyploidization events (Birstein et al., 1997). Acipenseriformes evolved from a common ancestor with a diploid chromosome number of 60 (Dingerkus and Howell, 1976; Birstein and Vasil'ev, 1987). Early investigations of sturgeon genome size and structure by karyotype or flow cytometry assorted extant species into three groups: those possessing ~120 (group A), ~250 (group B), or ~500 chromosomes (group C; Birstein and Vasil'ev, 1987; Birstein et al., 1993; Blacklidge and Bidwell, 1993). The arrangement of the ~120 chromosome American paddlefish, *Polyodon spathula*, karyotype into groups of tetrads (Dingerkus and Howell, 1976) led researchers to infer that all Acipenseriformes with ~120 chromosomes were similarly tetraploid derived, those possessing ~250 chromosomes were octoploid derived, and the sole species with ~500 chromosomes, *Acipenser mikadoi*, was hexadecaploid (16n) derived. Recent revisions to these classifications include the discovery of ~360 chromosomes in the shortnose sturgeon, *Acipenser brevirostrum*, (Kim et al., 2005) and only ~250 chromosomes in *Acipenser mikadoi* (Vishnyakova et al., 2008). Group C has been revised to include only the shortnose sturgeon with 360 chromosomes.

Some have proposed reconsideration of ploidy classifications for sturgeon. Fontana (1994) examined the nucleolar organizing regions (NORs) of four sturgeon species and concluded that the group A species had fully diploidized; therefore the group B species should be considered tetraploid, and *A. brevirostrum* hexaploid. Several subsequent papers have upheld these designations (Tagliavini et al., 1999; Fontana, 2002; Fontana et al., 2008). Ludwig et al. (2001) used the numbers of alleles per locus at six microsatellite loci in 20 sturgeon species to support the new categorizations. Others suggest these revisions are inappropriate. Birstein (2005) indicates that the high number of NORs in group A relative to teleost fishes does indeed reflect ancient tetraploidy. Also, Vasil'ev (2009) argues that the presence of duplicated genes and the karyotypic structure of the genome support an ancient tetraploid origin for the group A species, with subsequent diploidization and gene silencing. He further proposes two different scales for considering relationships between ploidy groups within the Acipenseriformes: the evolutionary scale of diploid (2n = 60), tetraploid (2n = 120), octoploid (2n = 250), and dodecaploid (2n = 360) as well as the recent scale of diploid (2n = 120), tetraploid (2n = 250) and hexaploid (2n = 360), reflecting the procession to diploidization in each lineage (Vasil'ev, 2009).

Regardless, designating group B and group C species as tetraploid and hexaploid based on extant levels of diploidization of the group A ploidy class may oversimplify and misrepresent the evolutionary history of Acipenseriformes. It is possible that group B and C species diverged from group A species before 120 chromosome genomes were fully diploidized and if this were the case, higher levels of polysomy might be expected in species with 250 and 360 chromosomes. No studies either inferring ploidy level or mechanisms of genome duplication cited above include gene segregation data in their analyses. However, these data are essential as only examination of segregation ratios in known families can provide evidence of polysomic inheritance and therefore the ancestral level of genome duplication in a species (Wright et al., 1983; Allendorf and Thorgaard, 1984; Marsden et al., 1987; David et al., 2003).

The white sturgeon (*Acipenser transmontanus*), a species with ~250 chromosomes (Hedrick et al., 1991; Fontana, 1994; Van Eenennaam et al., 1998a), is the largest North American freshwater fish, native to large river systems and estuaries along the west coast of the continent. Analysis of synaptone-

mal complex formation in white sturgeon spermatocytes suggested that although highly duplicated, the male white sturgeon genome may be fully diploidized as only bivalent pairing was observed (Van Eenennaam et al., 1998b). However, a previous study examining inheritance of microsatellite loci in white sturgeon found evidence of polysomy and genome duplication higher than tetraploidy (Rodzen and May, 2002). Here, In this study we follow the inheritance of eight additional microsatellite loci from known families of white sturgeon to examine levels of genome duplication. As genotype data suggest the presence of alternative ploidy states among the individuals examined, we employ flow cytometry to provide additional evidence of spontaneous autopolyploidy in a captive population of white sturgeon. Evidence of polysomic inheritance in loci present in ≥8 copies suggests that white sturgeon are ancient octoploids. The documentation of viable spontaneous autopolyploids in captive white sturgeon further suggests the plasticity of highly duplicated sturgeon genomes.

## Methods

### Sample collection

Fifteen full- and half-sibling white sturgeon families were created at Sterling Caviar LLC (Elverta, California) from crosses of five female (Y243, 065, Y3, Y192, 062) and three male (7219, 1178, 3d09) broodstock. Fin clips were collected from the eight broodstock and 48 one day post-hatch offspring from each cross were sacrificed for DNA extraction (N = 720). DNA was obtained from all samples using a standard DNA extraction kit (Promega, Fitchburg Center, WI, USA).

Blood samples were provided by farm personnel from 3 years old male (N = 3) and 7 years old female (N = 3) white sturgeon to examine alternative ploidy levels within the farm population with flow cytometry. Approximately 2.0 ml of blood was drawn from the caudal vein and mixed with 2.0 ml of heparin to prevent clotting. A single chicken blood sample was provided by UC Davis Hopkins Avian Facility personnel (Protocol # 15053) for use as a diploid control in flow cytometric ploidy analysis. All blood samples were stored on ice on their way to the lab for processing.

### Microsatellite genotyping

PCR was conducted with labeled primers for eight microsatellite loci developed for white, green (*A. medirostris)*, or Chinese (*A. sinensis)* sturgeon (Table 1). Amplification reactions consisted of 1.0 $\mu$l of 10× reaction buffer, 0.2 mM of each dNTP (Promega), 1.5–3.0 mM $MgCl_2$, 5.0 $\mu$M each of forward and reverse primers, 0.375 U Taq polymerase (Promega GoTaq) and $dH_20$ to a final volume of 10 $\mu$l. PCR was performed in either GeneAmp® 9700 PCR systems (Applied Biosystems, Pleasanton, CA, USA) or MJ Dyad™ DNA Engines (Biorad, Bio-Rad Laboratories, Clinical Diagnostics Group, Hercules, CA, USA; Table 1). PCR product was diluted with $dH_20$ and 1.0 $\mu$l of diluted product then was added to 9.0 $\mu$l of highly deionized formamide (Gel Company) and 0.1 $\mu$l of Applied Biosystems (ABI) Rox 400 HD size standard. Samples were denatured for 3 min at 95°C before loading on an ABI 3130xl Genetic Analyzer for fragment analysis. Size-calling of alleles was conducted in GENEMAPPER v4.0 software (Applied Biosystems, Pleasanton, CA, USA).

Table 1
Conditions for microsatellite PCR in white sturgeon families

| Locus | $MgCl_2$ (mM) | Ta (°C) | Annealing time (s) | References |
|---|---|---|---|---|
| AciG 2 | 1.5 | 60 | 30 | Börk et al. (2008) |
| AciG 53 | 2.0 | 56 | 105 | Börk et al. (2008) |
| AciG 140 | 2.5 | 56 | 30 | Börk et al. (2008) |
| AciG 35 | 3.0 | 56 | 30 | Börk et al. (2008) |
| AciG 43 | 2.0 | 56 | 30 | Genbank: HM459582[a] |
| AciG 52 | 2.0 | 56 | 105 | Börk et al. (2008) |
| AciG 110 | 2.0 | 56 | 30 | Börk et al. (2008) |
| As015 | 2.5 | 56 | 30 | Zhu et al. (2005) |

[a]Forward primer sequence: TAATACAGCGGGGATGGAA; Reverse primer sequence: GCACAGTGAAAGCACGGTAA.

### Flow cytometry

Chicken blood collected for ploidy analysis by flow cytometry was mixed with equal parts of 2% dextran sulfate in a polystyrene tube and incubated at 37°C for 45 min. The resulting leukocyte layer was transferred to a new container and centrifuged at 123 × g for 5 min. The supernatant was aspirated and the chicken cell pellet was further processed in the same manner as white sturgeon samples. Approximately 1.0–2.0 ml of heparinized sturgeon blood was mixed with equal parts of 5.0 mM EDTA in phosphate buffered saline (PBS) and centrifuged at 123 × g for 5 min. The supernatant was aspirated and ACK buffer (1.55 M $NH_4Cl$ and 0.1 M $KHCO_3$) was applied to the white sturgeon blood cells and chicken leukocytes for 4 min to lyse any remaining erythrocytes. The lysis reaction was stopped by the addition of 3.0–4.0 ml of staining media (SM: PBS with 2.0 mM EDTA). Leukocytes were centrifuged at 123 × g for 5 min and pellets were treated with ACK buffer a second time. Leukocyte cell pellets were re-suspended in 500 $\mu$l of SM and counted by hemacytometer using Trypan blue and an inverted lens microscope. Aliquots of $1.0–2.0 \times 10^6$ cells were transferred to polystyrene tubes containing 1.0–2.0 ml of PBS and centrifuged at 123 × g for 5 min. Leukocytes were fixed in 200 $\mu$l of 70% ethanol for 10 min on ice and washed twice with 3.0–4.0 ml of SM. Fixed leukocytes were centrifuged at 341 × g for 5 min, re-suspended in 250 $\mu$l of RNase (100 $\mu g\ ml^{-1}$ in PBS), and incubated at room temperature for 30 min. The leukocytes were washed with SM and centrifuged at 341 × g for 5 min. The supernatant was aspirated and leukocytes re-suspended in 250–500 $\mu$l of propidium iodide (PI; 40 $\mu g\ ml^{-1}$ in PBS) for flow cytometry analysis.

### Microsatellite data analysis

We evaluated the level of genome duplication in white sturgeon in two ways. First, we examined the number of alleles / individual found at each locus in both the parents and progeny. We made comparisons between the average number of alleles / locus observed in families of dams exhibiting a higher ploidy level (Y192 and 065) and the nine remaining families with two sample *t*-tests, using the harmonic mean to account for differences in sample size (Sokal and Rohlf, 1981). Second, we examined the dosage of informative alleles in each parent. The high numbers of alleles detected per individual and size specific amplification bias in loci with allele size ranges spanning >100 base pairs made scoring allele dosage impossible. Therefore, we used the frequency that an informative allele (allele unique to one

parent) was transmitted to progeny in half-sibling families to determine parental allele dosage. We calculated 95% confidence intervals (CIs) around the frequency of allelic transmission and applying the rules of Mendelian inheritance, alleles with transmission frequency CIs overlapping 0.5 (50% of offspring inherited an allele) were considered to be present in a single copy in the parent, whereas CIs overlapping 0.83 suggested two copies of an allele. CIs with a lower bound $>0.83$ indicated more than two copies of an allele were present in the parent (Rodzen and May, 2002). In ambiguous cases, where the lower bounds of transmission frequency CIs were $>0.5$ but upper limits were $<0.83$, we conservatively classified the allele as present in a single dose.

We also evaluated white sturgeon genome structure by examining patterns of allelic transmission. We calculated the number of informative, single copy alleles that were transmitted by one parent to individual offspring. If a microsatellite system was present as a single tetrasomic locus, a parent could transmit a maximum of two alleles to an offspring. At a microsatellite system consisting of two tetrasomic loci or one octosomic locus, a single offspring could inherit up to four informative alleles from one parent. In this analysis, allelic transmission was examined on a per family basis as different allele combinations were informative in different crosses.

Finally, we looked for evidence of disomy in the white sturgeon genome by examining segregation frequencies of informative, single copy allelic pairs transmitted by parents. In these analyses, we assumed that double reduction was negligible in white sturgeon, a premise supported by the absence of multivalent pairing observed in male white sturgeon (Van Eenennaam et al., 1998b), although pairing is unknown in female white sturgeon. In the case where a primer pair is amplifying two or more disomic loci, certain alleles would never be co-transmitted to the same offspring, as alleles at the same disomic locus would segregate during meiosis. In contrast, two alleles originating from a single tetrasomic locus, two tetrasomic loci, or a single octosomic locus have a non-zero probability of co-transmission (1/6, 1/4, and 15/70, respectively). We used these Mendelian ratios to construct simple chi square tests to examine conformance of each locus to disomic, tetrasomic, and octosomic models of inheritance. Only pairs of informative, single copy alleles were included in this analysis. The presence of many microsatellite systems consisting of disomically inherited loci would suggest that white sturgeon are well progressed along the evolutionary path to diploidization.

#### Flow cytometry data analysis

Nuclear DNA content of the chicken and white sturgeon leukocytes was estimated by analyzing PI fluorescence with a BD FACScan flow cytometer (Becton Dickinson, Bedford, MA, USA) with a single argon laser with a 488 nm excitation beam and BD CELLQUEST analysis software (Becton Dickinson). We measured the fluorescence of 5000–30 000 stained leukocyte nuclei per sample. DNA content was estimated by comparing the fluorescence and genome size (2.5 pg per nucleus) of the diploid chicken standard to the resulting white sturgeon fluorescence by the equation DNA (pg) $= 2.5 \times (S/C)$, where S and C correspond to the mean fluorescence values of the white sturgeon and chicken leukocytes respectively (Rasch et al., 1970).

## Results

#### Microsatellite data

The numbers of alleles/individual/locus ranged from 1 to 11 (mean 2.06–7.65) across eight loci and 15 white sturgeon families (Table 2). Dams 065 and Y192 appeared to exhibit a higher level of genome duplication than other parents. At eight copy loci (see below), dam Y192 possessed from 6 to10 alleles/locus, while other white sturgeon parents possessed from 4 to 7 alleles/locus (Table 2.) Offspring in the families of Y192 and 065 had a significantly greater average number of alleles/locus at 8 of 8 and 5 of 8 loci, respectively (Table 2).

Segregation of informative alleles in offspring revealed that gene copy number ranged from 4 to 13 in parents, with the highest levels of duplication found in 065 and Y192, although dam 062 possessed at least 10 gene copies at AciG 35 (Table 3). Most other parents possessed eight or fewer gene copies at each locus, although sire 3d09 had nine copies at AciG 52 (Table 3). All loci were present in at least four copies (AciG 53, 140), with some loci in eight or more copies (AciG 2, 35, 43, 52, 110, As015; Table 3). At AciG 53 and 140, offspring exhibited 1–4 alleles/individual and offspring segregation data revealed that parents possessed 2–5 gene copies/locus. Only dams 065 and Y192 possessed $>4$ copies at these loci. The remaining loci were inferred to be in ≥8 gene copies, with individuals possessing from 2 to 9 alleles per locus, excluding 065 and Y192 and their families (Table 2). AciG 2 only exhibited 2–5 alleles per locus, but the presence of up to five alleles in many individuals suggests it too is duplicated in $>4$ copies. We infer

Table 2
Numbers of microsatellite alleles observed in parents and offspring at each locus

| | Alleles | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | Y243 | $F_1$ ($\bar{X}$) | 065 | $F_1$ ($\bar{X}$) | Y3 | $F_1$ ($\bar{X}$) | Y192 | $F_1$ ($\bar{X}$) | 062 | $F_1$ ($\bar{X}$) | 7219 | $F_1$ ($\bar{X}$) | 1178 | $F_1$ ($\bar{X}$) | 3d09 | $F_1$ ($\bar{X}$) |
| AciG 2 | 3 | 1–3 (2.49) | 3 | 1–3 (3.10*) | 2 | 1–3 (2.32) | 2 | 1–3 (2.59*) | 3 | 1–4 (2.56) | 3 | 1–4 (2.64) | 3 | 1–4 (2.61) | 2 | 1–3 (2.55) |
| AciG 53 | 3 | 1–3 (2.41) | 2 | 1–2 (1.96) | 2 | 1–2 (1.96) | 4 | 2–4 (3.24*) | 2 | 1–2 (1.92) | 2 | 1–4 (2.25) | 2 | 1–4 (2.32) | 2 | 1–4 (2.32) |
| AciG 140 | 3 | 1–4 (2.67) | 3 | 1–4 (3.28*) | 2 | 1–4 (2.43) | 3 | 2–5 (3.14*) | 3 | 1–4 (2.62) | 2 | 1–4 (2.54) | 4 | 2–5[b] (3.04) | 3 | 2–5[b] (2.90) |
| AciG 35 | 6 | 4–8 (6.14) | 6 | 4–10 (7.65*) | 6 | 3–8 (6.08) | 6 | 5–9 (7.37*) | 5 | 5–8 (6.82) | 7 | 4–9 (6.69) | 6 | 3–10 (6.70) | 7 | 5–10 (7.04) |
| AciG 43 | 6 | 2–7 (4.31) | 5 | 2–9 (5.67*) | 6 | 2–7 (4.37) | 9 | 4–9 (6.22*) | 4 | 3–7 (4.69) | 5 | 2–8 (5.17) | 5 | 2–9[b] (5.36) | 3 | 2–8 (4.62) |
| AciG 52 | 6 | 4–8 (5.58) | 7 | 4–10 (6.98*) | 5 | 3–8 (5.60) | 10 | 5–11 (7.41*) | 7 | 4–9[a] (6.02) | 6 | 4–10[b] (6.68) | 4 | 4–9[b] (5.79) | 5 | 4–11[b] (6.48) |
| AciG 110 | 6 | 3–7 (5.35) | 4 | 3–6 (4.75) | 5 | 3–7 (4.78) | 8 | 4–9 (6.51*) | 4 | 3–6 (4.87) | 6 | 2–8 (5.22) | 5 | 3–9[b] (5.43) | 4 | 3–9[b] (4.75) |
| As015 | 4 | 2–5 (3.84) | 5 | 2–7 (4.94*) | 4 | 2–6 (4.57) | 9 | 4–10 (7.12*) | 4 | 3–6 (4.67) | 4 | 3–9[b] (4.97) | 5 | 3–9[b] (5.18) | 4 | 2–10[b] (4.92) |

Four copy loci are AciG 53 and 140. Eight copy loci are AciG 2, 35, 43, 52, 110, and As015.
[a]One offspring from 062x7219 and 2 offspring from family 062x3d09 had 9 alleles at AciG 52.
[b]Offspring with unusually high allele numbers are from families with 065 and Y192 as dams.
*Significantly greater mean number of alleles/individual relative to 9 other families from dams Y243, Y3, and 062 ($P < 0.001$).

Table 3
Minimum estimates of gene copy number at microsatellite loci in white sturgeon parents. Only informative alleles (alleles unique to one parent in a cross; $A_I$), are used in this analysis. N is the number of offspring evaluated. Dams are Y243, 065, Y3, Y192, 062; sires are 1178, 7219, 3d09

| Locus | Parent | Genotype | Minimum copy No. | $A_I$ | Allele count / N | Transmission frequency ± 95% CI | Est. dosage |
|---|---|---|---|---|---|---|---|
| AciG 2 | Y243 | *283* / 287 / 295 | 3 | *295* | 27 / 48 | 0.56 ± 0.14 | 1 |
| | 065 | *271* / 283 / 287 | 4 | *271* | 117 / 143 | 0.82 ± 0.06 | 2 |
| | Y3 | *283* / 295 | 2 | *295* | 25 / 48 | 0.52 ± 0.14 | 1 |
| | Y192 | *283* / 295 | 4 | *295* | 48 / 48 | 1.00 | > 2 |
| | 062 | *283* / 287 / 299 | 3 | *299* | 67 / 144 | 0.47 ± 0.08 | 1 |
| | 1178 | *283* / 287 / 295 | 3 | *287* | 38 / 95 | 0.40 ± 0.10 | 1 |
| | | | | *295* | 39 / 95 | 0.41 ± 0.10 | 1 |
| | 7219 | *283* / 287 / 295 | 3 | *287* | 59 / 96 | 0.61 ± 0.10 | 1 |
| | | | | *295* | 38 / 96 | 0.40 ± 0.10 | 1 |
| | 3d09 | *283* / 287 | 3 | *287* | 84 / 96 | 0.88 ± 0.07 | 2 |
| AciG 53 | Y243 | *214* / 218 / 222 | 3 | *222* | 72 / 144 | 0.50 ± 0.08 | 1 |
| | Y192 | *214* / 218 / 222 / 242 | 5 | *222* | 119 / 144 | 0.83 ± 0.06 | 2 |
| | | | | *242* | 74 / 144 | 0.51 ± 0.08 | 1 |
| AciG 140 | Y243 | *164* / 172 / 180 | 3 | *164* | 57 / 96 | 0.59 ± 0.10 | 1 |
| | 065 | *158* / 164 / 180 | 5 | *158* | 30 / 84 | 0.63 ± 0.14 | 1 |
| | | | | *164* | 91 / 96 | 0.95 ± 0.04 | > 2 |
| | Y3 | *164* / 172 | 2 | *164* | 46 / 96 | 0.48 ± 0.10 | 1 |
| | Y192 | *164* / 168 / 172 | 4 | *164* | 79 / 96 | 0.82 ± 0.08 | 2 |
| | | | | *168* | 66 / 144 | 0.46 ± 0.08 | 1 |
| | 062 | *164* / 172 / 180 | 3 | *164* | 56 / 96 | 0.58 ± 0.10 | 1 |
| | 1178 | *158* / 164 / 172 / 180 | 4 | *158* | 91 / 191 | 0.48 ± 0.07 | 1 |
| | | | | *172* | 29 / 47 | 0.62 ± 0.14 | 1 |
| | | | | *180* | 48 / 95 | 0.51 ± 0.10 | 1 |
| | 7219 | *172* / 180 | 4 | *172* | 47 / 48 | 0.98 ± 0.04 | > 2 |
| | | | | *180* | 52 / 96 | 0.54 ± 0.10 | 1 |
| | 3d09 | *158* / 172 / 180 | 4 | *158* | 92 / 192 | 0.48 ± 0.07 | 1 |
| | | | | *172* | 37 / 48 | 0.77 ± 0.12 | 2 |
| | | | | *180* | 48 / 96 | 0.50 ± 0.10 | 1 |
| AciG 35 | Y243 | *250* / 254 / 258 / 266 / 274 / 286 | 8 | *250* | 96 / 96 | | > 2 |
| | | | | *254* | 17 / 48 | 0.35 ± .0.13 | 1 |
| | | | | *266* | 70 / 96 | 0.73 ± 0.09 | 1 |
| | | | | *274* | 45 / 96 | 0.47 ± 0.10 | 1 |
| | 065 | *238* / 242 / 250 / 254 / 282 / 286 | 13 | *238* | 127 / 142 | 0.89 ± 0.05 | > 2 |
| | | | | *242* | 119 / 142 | 0.84 ± 0.06 | 2 |
| | | | | *250* | 47 / 47 | 1.00 | > 2 |
| | | | | *254* | 40 / 47 | 0.85 ± 0.10 | 2 |
| | | | | *282* | 75 / 95 | 0.79 ± 0.08 | 2 |
| | Y3 | *238* / 250 / 258 / 274 / 286 / 306 | 7 | *238* | 121 / 143 | 0.85 ± 0.06 | 2 |
| | | | | *250* | 30 / 48 | 0.63 ± 0.14 | 1 |
| | | | | *274* | 67 / 96 | 0.70 ± 0.09 | 1 |
| | Y192 | *238* / 250 / 254 / 266 / 270 / 282 | 11 | *238* | 71 / 144 | 0.49 ± 0.08 | 1 |
| | | | | *250* | 48 / 48 | 1.00 | > 2 |
| | | | | *254* | 24 / 48 | 0.50 ± 0.14 | 1 |
| | | | | *266* | 81 / 96 | 0.84 ± 0.07 | 2 |
| | | | | *270* | 96 / 96 | 1.00 | > 2 |
| | | | | *282* | 48 / 96 | 0.50 ± 0.10 | 1 |
| | 062 | *238* / 250 / 254 / 274 / 278 | 10 | *238* | 125 / 144 | 0.87 ± 0.05 | 2 |
| | | | | *250* | 48 / 48 | 1.00 | > 2 |
| | | | | *254* | 48 / 48 | 1.00 | > 2 |
| | | | | *274* | 47 / 96 | 0.49 ± 0.10 | 1 |
| | | | | *278* | 31 / 96 | 0.65 ± 0.13 | 1 |
| | 1178 | *258* / 266 / 274 / 282 / 286 / 306 | 7 | *258* | 126 / 143 | 0.88 ± 0.05 | 2 |
| | | | | *266* | 63 / 142 | 0.44 ± 0.08 | 1 |
| | | | | *274* | 47 / 95 | 0.49 ± 0.10 | 1 |
| | | | | *282* | 61 / 143 | 0.43 ± 0.08 | 1 |
| | | | | *286* | 53 / 96 | 0.55 ± 0.10 | 1 |
| | | | | *306* | 101 / 191 | 0.53 ± 0.07 | 1 |
| | 7219 | *250* / 254 / 258 / 262 / 278 / 286 / 306 | 8 | *254* | 24 / 48 | 0.50 ± 0.14 | 1 |
| | | | | *258* | 72 / 144 | 0.50 ± 0.08 | 1 |
| | | | | *262* | 112 / 240 | 0.47 ± 0.06 | 1 |
| | | | | *278* | 89 / 192 | 0.46 ± 0.07 | 1 |
| | | | | *286* | 83 / 96 | 0.86 ± 0.07 | 2 |
| | | | | *306* | 119 / 192 | 0.62 ± 0.07 | 1 |
| | 3d09 | *254* / 258 / 262 / 270 / 278 / 286 / 306 | 7 | *254* | 32 / 48 | 0.67 ± 0.13 | 1 |
| | | | | *258* | 63 / 143 | 0.44 ± 0.08 | 1 |
| | | | | *262* | 138 / 239 | 0.58 ± 0.06 | 1 |
| | | | | *270* | 102 / 191 | 0.53 ± 0.07 | 1 |
| | | | | *278* | 98 / 191 | 0.51 ± 0.07 | 1 |
| | | | | *306* | 93 / 191 | 0.49 ± 0.07 | 1 |
| | | | | *286* | 46 / 96 | 0.48 ± 0.10 | 1 |

Table 3
(Continued)

| Locus | Parent | Genotype | Minimum copy No. | $A_I$ | Allele count / N | Transmission frequency ± 95% CI | Est. dosage |
|---|---|---|---|---|---|---|---|
| AciG 43 | Y243 | *301* / 305 / 309 / 347 / 351 / 363 | 7 | *301* | 28 / 95 | 0.29 ± 0.09[a] | 1 |
| | | | | *305* | 21 / 96 | 0.22 ± 0.08[a] | 1 |
| | | | | *347* | 21 / 48 | 0.44 ± 0.14 | 1 |
| | | | | *351* | 73 / 95 | 0.77 ± 0.08 | 2 |
| | | | | *363* | 55 / 96 | 0.57 ± 0.10 | 1 |
| | 065 | *293* / 297 / 305 / 347 / 351 | 10 | *293* | 117 / 143 | 0.82 ± 0.06 | 2 |
| | | | | *297* | 72 / 95 | 0.76 ± 0.09 | 2 |
| | | | | *305* | 82 / 95 | 0.86 ± 0.07 | 2 |
| | | | | *347* | 44 / 47 | 0.94 ± 0.07 | > 2 |
| | | | | *351* | 66 / 96 | 0.69 ± 0.09 | 1 |
| | Y3 | *297* / 301 / 309 / 313 / 351 / 354 | 6 | *297* | 26 / 95 | 0.27 ± 0.09[a] | 1 |
| | | | | *301* | 32 / 96 | 0.33 ± 0.09[a] | 1 |
| | | | | *313* | 26 / 48 | 0.54 ± 0.14 | 1 |
| | | | | *351* | 53 / 96 | 0.55 ± 0.10 | 1 |
| | | | | *354* | 78 / 143 | 0.55 ± 0.08 | 1 |
| | Y192 | *289* / 293 / 301 / 305 / 309 / 313 / 317 / 351 / 363 | 11 | *289* | 80 / 144 | 0.56 ± 0.08 | 1 |
| | | | | *293* | 77 / 144 | 0.53 ± 0.08 | 1 |
| | | | | *301* | 41 / 96 | 0.43 ± 0.10 | 1 |
| | | | | *305* | 88 / 96 | 0.92 ± 0.05 | > 2 |
| | | | | *313* | 21 / 48 | 0.48 ± 0.14 | 1 |
| | | | | *317* | 40 / 144 | 0.28 ± 0.07[a] | 1 |
| | | | | *351* | 42 / 96 | 0.44 ± 0.10 | 1 |
| | | | | *363* | 52 / 96 | 0.54 ± 0.10 | 1 |
| | 062 | *293* / 297 / 305 / 347 | 8 | *293* | 133 / 144 | 0.92 ± 0.04 | > 2 |
| | | | | *297* | 28 / 48 | 0.58 ± 0.14 | 1 |
| | | | | *305* | 67 / 96 | 0.70 ± 0.09 | 1 |
| | | | | *347* | 38 / 48 | 0.79 ± 0.11 | 2 |
| | 1178 | *301* / 309 / 313 / 351 / 359 | 6 | *301* | 53 / 95 | 0.56 ± 0.10 | 1 |
| | | | | *309* | 72 / 95 | 0.76 ± 0.09 | 2 |
| | | | | *313* | 56 / 143 | 0.39 ± 0.08[a] | 1 |
| | | | | *351* | 18 / 48 | 0.38 ± 0.14[a] | 1 |
| | | | | *359* | 107 / 238 | 0.45 ± 0.06 | 1 |
| | 7219 | *297* / 305 / 309 / 347 / 363 | 5 | *297* | 79 / 143 | 0.55 ± 0.08 | 1 |
| | | | | *305* | 25 / 48 | 0.52 ± 0.14 | 1 |
| | | | | *309* | 70 / 96 | 0.73 ± 0.09 | 1 |
| | | | | *347* | 49 / 96 | 0.51 ± 0.10 | 1 |
| | | | | *363* | 73 / 144 | 0.51 ± 0.08 | 1 |
| | 3d09 | *309* / 313 / 347 | 4 | *309* | 82 / 96 | 0.85 ± 0.07 | 2 |
| | | | | *313* | 49 / 144 | 0.34 ± 0.08[a] | 1 |
| | | | | *347* | 48 / 96 | 0.50 ± 0.10 | 1 |
| AciG 52 | Y243 | *178* / 182 / 186 / 194 / 198 / 228 | 8 | *178* | 37 / 48 | 0.77 ± 0.12 | 2 |
| | | | | *186* | 46 / 94 | 0.49 ± 0.10 | 1 |
| | | | | *198* | 114 / 142 | 0.80 ± 0.06 | 2 |
| | | | | *228* | 51 / 94 | 0.54 ± 0.10 | 1 |
| | 065 | *178* / 182 / 184 / 190 / 192 / 194 / 198 | 11 | *178* | 34 / 47 | 0.72 ± 0.13 | 2 |
| | | | | *184* | 92 / 143 | 0.64 ± 0.08 | 1 |
| | | | | *190* | 138 / 143 | 0.97 ± 0.03 | > 2 |
| | | | | *192* | 87 / 143 | 0.61 ± 0.08 | 1 |
| | | | | *198* | 114 / 143 | 0.80 ± 0.06 | 2 |
| | Y3 | *172* / 182 / 186 / 198 / 210 | 6 | *172* | 63 / 143 | 0.44 ± 0.08 | 1 |
| | | | | *186* | 74 / 96 | 0.77 ± 0.08 | 2 |
| | | | | *198* | 72 / 143 | 0.50 ± 0.08 | 1 |
| | Y192 | *178* / 182 / 190 / 194 / 198 / 202 / 206 / 210 / 224 / 242 | 11 | *178* | 34 / 48 | 0.71 ± 0.13 | 2 |
| | | | | *190* | 99 / 143 | 0.69 ± 0.07 | 1 |
| | | | | *198* | 61 / 143 | 0.43 ± 0.08 | 1 |
| | | | | *202* | 79 / 143 | 0.55 ± 0.08 | 1 |
| | | | | *206* | 75 / 143 | 0.52 ± 0.08 | 1 |
| | | | | *224* | 82 / 143 | 0.57 ± 0.08 | 1 |
| | | | | *242* | 72 / 143 | 0.50 ± 0.08 | 1 |
| | 062 | *178* / 182 / 184 / 186 / 190 / 192 / 194 | 7 | *178* | 22 / 37 | 0.59 ± 0.16 | 1 |
| | | | | *184* | 64 / 123 | 0.52 ± 0.09 | 1 |
| | | | | *186* | 60 / 86 | 0.70 ± 0.10 | 1 |
| | | | | *190* | 62 / 123 | 0.50 ± 0.09 | 1 |
| | | | | *192* | 66 / 123 | 0.54 ± 0.09 | 1 |
| | 1178 | *182* / 186 / 194 / 210 | 6 | *186* | 59 / 95 | 0.62 ± 0.10 | 1 |
| | | | | *194* | 44 / 48 | 0.92 ± 0.08 | > 2 |
| | | | | *210* | 59 / 132 | 0.45 ± 0.08 | 1 |
| | 7219 | *178* / 182 / 188 / 194 / 210 / 228 | 8 | *178* | 21 / 48 | 0.44 ± 0.14 | 1 |
| | | | | *188* | 187 / 240 | 0.78 ± 0.05 | 2 |
| | | | | *194* | 41 / 48 | 0.85 ± 0.10 | 2 |
| | | | | *210* | 77 / 144 | 0.53 ± 0.08 | 1 |

Table 3
(Continued)

| Locus | Parent | Genotype | Minimum copy No. | $A_I$ | Allele count / N | Transmission frequency ± 95% CI | Est. dosage |
|---|---|---|---|---|---|---|---|
| | | | | *228* | 91 / 192 | 0.47 ± 0.07 | 1 |
| | 3d09 | *178* / 182 / 188 / 194 / 210 | 9 | *178* | 44 / 48 | 0.92 ± 0.08 | > 2 |
| | | | | *188* | 183 / 227 | 0.81 ± 0.05 | 2 |
| | | | | *194* | 41 / 48 | 0.85 ± 0.10 | 2 |
| | | | | *210* | 59 / 132 | 0.45 ± 0.08 | 1 |
| AciG 110 | Y243 | *262* / 291 / 299 / 303 / 307 / 327 | 6 | *291* | 71 / 144 | 0.49 ± 0.08 | 1 |
| | | | | *299* | 43 / 96 | 0.45 ± 0.10 | 1 |
| | | | | *307* | 53 / 96 | 0.55 ± 0.10 | 1 |
| | | | | *327* | 70 / 144 | 0.49 ± 0.08 | 1 |
| | | | | *327* | 70 / 144 | 0.49 ± 0.08 | 1 |
| | 065 | *262* / 292 / 299 / 303 | 6 | *299* | 138 / 143 | 0.97 ± 0.03 | > 2 |
| | | | | *303* | 29 / 48 | 0.60 ± 0.14 | 1 |
| | Y3 | *262* / 292 / 303 / 323 / 335 | 6 | *303* | 43 / 48 | 0.90 ± 0.09 | 2 |
| | | | | *323* | 40 / 95 | 0.42 ± 0.10 | 1 |
| | | | | *335* | 69 / 143 | 0.48 ± 0.08 | 1 |
| | Y192 | *262* / 266 / 291 / 292 / 299 / 301 / 303 / 347 | 11 | *266* | 42 / 48 | 0.88 ± 0.09 | 2 |
| | | | | *291* | 77 / 144 | 0.53 ± 0.08 | 1 |
| | | | | *299* | 90 / 96 | 0.94 ± 0.05 | > 2 |
| | | | | *301* | 75 / 144 | 0.52 ± 0.08 | 1 |
| | | | | *303* | 23 / 48 | 0.48 ± 0.14 | 1 |
| | | | | *347* | 70 / 144 | 0.49 ± 0.08 | 1 |
| | 062 | *262* / 295 / 296 / 299 | 5 | *295* | 61 / 96 | 0.64 ± 0.10 | 1 |
| | | | | *296* | 63 / 144 | 0.44 ± 0.08 | 1 |
| | | | | *299* | 74 / 96 | 0.77 ± 0.08 | 2 |
| | 1178 | *262* / 266 / 292 / 295 / 303 | 7 | *266* | 155 / 191 | 0.81 ± 0.05 | 2 |
| | | | | *292* | 75 / 96 | 0.78 ± 0.08 | 2 |
| | | | | *295* | 93 / 191 | 0.49 ± 0.07 | 1 |
| | | | | *303* | 27 / 48 | 0.56 ± 0.14 | 1 |
| | 7219 | *262* / 266 / 292 / 299 / 303 / 323 | 6 | *266* | 88 / 192 | 0.46 ± 0.07 | 1 |
| | | | | *292* | 52 / 96 | 0.54 ± 0.10 | 1 |
| | | | | *299* | 24 / 48 | 0.50 ± 0.14 | 1 |
| | | | | *303* | 25 / 48 | 0.52 ± 0.14 | 1 |
| | | | | *323* | 96 / 192 | 0.50 ± 0.07 | 1 |
| | 3d09 | *262* / 292 / 307 / 319 | 5 | *292* | 82 / 96 | 0.85 ± 0.07 | 2 |
| | | | | *307* | 97 / 192 | 0.51 ± 0.07 | 1 |
| | | | | *319* | 114 / 240 | 0.48 ± 0.06 | 1 |
| As015 | Y243 | *189* / 193 / 197 / 209 | 4 | *209* | 44 / 96 | 0.46 ± 0.10 | 1 |
| | 065 | *189* / 193 / 197 / 213 / 221 | 6 | *213* | 112 / 143 | 0.78 ± 0.07 | 2 |
| | | | | *221* | 94 / 143 | 0.66 ± 0.08 | 1 |
| | Y3 | *191* / 193 / 197 / 209 | 5 | *191* | 64 / 143 | 0.45 ± 0.08 | 1 |
| | | | | *209* | 79 / 96 | 0.82 ± 0.08 | 2 |
| | Y192 | *185* / 191 / 193 / 197 / 209 / 213 / 221 / 225 / 233 | 11 | *185* | 52 / 95 | 0.55 ± 0.10 | 1 |
| | | | | *191* | 60 / 143 | 0.42 ± 0.08 | 1 |
| | | | | *209* | 68 / 95 | 0.72 ± 0.09 | 1 |
| | | | | *213* | 127 / 143 | 0.89 ± 0.05 | > 2 |
| | | | | *221* | 62 / 143 | 0.43 ± 0.08 | 1 |
| | | | | *225* | 69 / 143 | 0.48 ± 0.08 | 1 |
| | | | | *233* | 72 / 143 | 0.50 ± 0.08 | 1 |
| | 062 | *189* / 193 / 209 / 213 | 5 | *209* | 51 / 96 | 0.53 ± 0.10 | 1 |
| | | | | *213* | 113 / 144 | 0.78 ± 0.07 | 2 |
| | 1178 | *185* / 189 / 193 / 197 / 209 | 7 | *185* | 103 / 190 | 0.54 ± 0.07 | 1 |
| | | | | *189* | 75 / 95 | 0.79 ± 0.08 | 2 |
| | | | | *197* | 40 / 48 | 0.83 ± 0.10 | 2 |
| | | | | *209* | 23 / 47 | 0.49 ± 0.14 | 1 |
| | 7219 | *189* / 193 / 197 / 217 | 7 | *189* | 75 / 95 | 0.79 ± 0.08 | 2 |
| | | | | *197* | 48 / 48 | 1.00 | > 2 |
| | | | | *217* | 135 / 240 | 0.56 ± 0.06 | 1 |
| | 3d09 | *189* / 193 / 197 / 217 | 6 | *189* | 73 / 96 | 0.76 ± 0.08 | 2 |
| | | | | *197* | 38 / 48 | 0.79 ± 0.11 | 2 |
| | | | | *217* | 121 / 239 | 0.51 ± 0.06 | 1 |

AciG 35 to be present in more than eight copies, as three parents (Y192, 065, 062) possessed > 8 copies of locus AciG 35, including one parent (065) with > 12 copies (Table 3). Three of the progeny of 062 possessed nine alleles at AciG 52, although we can only infer that 062 has a minimum of 7 or 8 gene copies at this locus, as two of her alleles are uninformative (Tables 2 and 3).

The number of single copy informative alleles transmitted to individual offspring ranged from 0 to 4 in 065, 0–6 in Y192, and 0–4 in the remaining parents. Informative alleles present in > 1 copy were not considered so these intervals represent minimum estimates. This analysis was not possible in AciG 2 or AciG 53 due to a paucity of informative alleles, but at four copy locus AciG 140, parents transmitted up to two single

copy alleles to individual offspring. At As015, a locus also limited by few informative alleles, this analysis was only possible with families of Y192, and she transmitted 0–6 alleles to individual offspring.

The co-segregation frequency of informative single copy alleles within families provided some evidence of disomic inheritance in two loci. In AciG 43, alleles *305* and *347* from dam Y243 did not segregate together in family Y243x1178, although allele *305* was found at a relatively low frequency in this family (20% of offspring). In family Y192x7219, alleles *313* and *317* did not segregate together but allele *317* was present in only 10% of offspring. Several AciG 43 alleles in multiple families were observed at lower frequencies than expected, suggestive of meiotic drive or some other non-Mendelian process (Table 3). Alleles *254* and *262* at AciG 35 from sire 7219 did not co-segregate in family Y3x7219 and these alleles were found in 50% and 48% of offspring, respectively. This allelic pair was informative only in one other family Y3x3d09, where *254* and *262* co-segregated in nine offspring. For the majority of allele pairs across the eight loci, co-segregation patterns conformed to models consistent with a single tetrasomic locus (7.1%), two tetrasomic loci (11%), multiple polysomic models (60%), or no model (21%) of inheritance.

#### Flow cytometry data

We estimated the DNA content of three female and three male white sturgeon relative to a diploid chicken control using flow cytometry. Figure 1 is a representative plot that combines flow cytometry histogram data from two females (Fe 1 and Fe 2) with markedly different PI fluorescences as well as the chicken control. Genome size ranged from 8.51 pg per cell to 11.39 pg per cell in white sturgeon, indicating that the presence of multiple ploidy classes (Table 4).

## Discussion

These results support previous work with microsatellite markers suggesting that the white sturgeon genome is highly duplicated (Rodzen and May, 2002). None of the markers examined here appear to be strictly disomic, which refutes the claims of others that white sturgeon are fully diploidized (Van Eenennaam et al., 1998b; Fontana, 2002) and is consistent with Rodzen and May (2002), who found only one sex-specific disomic microsatellite marker in white sturgeon. At this locus, Atr 113, two males exhibited disomic inheritance while two females appeared tetrasomic. Ludwig et al. (2001) report one microsatellite locus presumed to be disomic in white sturgeon, but this conclusion was based only on the number of alleles / locus and no inheritance data were included. Also the samples examined in Ludwig et al. (2001) originated from an endangered white sturgeon population found by others to have very low genetic diversity (ADS; unpubl. data). Polysomic inheritance in white sturgeon characterized here and in Rodzen and May (2002) suggests that although bivalent pairing of chromosomes is occurring at least in males, not enough genetic differences have accumulated between chromosome copies to result in consistent preferential pairing of homologs (Stift et al., 2008). Lacking any information on meiotic chromosome behavior in female white sturgeon, we assume for the species a model of random bivalent pairing among homologous chromosomes (Rodzen and May, 2002; Stift et al., 2008).

Table 4
Mean propidium iodide (PI) fluorescence and estimated DNA content relative to the chicken control

| Sample | Mean PI fluorescence | Estimated DNA content (pg) | Putative ploidy |
|---|---|---|---|
| Chicken | 79.84 | 2.50 | 2n |
| Female 1 (Fe 1) | 293.9 | 9.20 | 8n |
| Female 2 (Fe 2) | 363.9 | 11.4 | 10n |
| Female 3 (Fe 3) | 333.4 | 10.4 | 10n |
| Male 1 (M1) | 279.8 | 8.76 | 8n |
| Male 2 (M2) | 271.9 | 8.51 | 8n |
| Male 3 (M3) | 282.7 | 8.85 | 8n |

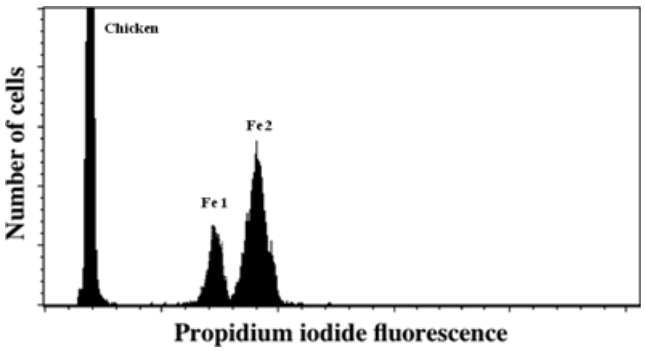


Fig. 1. Representative flow cytometry histogram of two female white sturgeon (Fe 1 and Fe 2) and chicken control. Fe 1 (8n) and Fe 2 (10n) represent two of three ploidy states we detected in white sturgeon with neutral markers. Using the chicken genome as a diploid standard, we calculate that Fe 2 has a genome size intermediate to that previously described for 8n white sturgeon and 12n shortnose sturgeon (Blacklidge and Bidwell, 1993). Fe 2 appears to be decaploid (10n), the same ploidy state detected in the offspring of dodecaploid (12n) dams Y192 and 065

Pooling data from this study and Rodzen and May (2002), we find 10 loci in $>4$ copies in the white sturgeon genome, suggesting that the species is octoploid derived and the classification of white sturgeon as tetraploid is invalid. One possible explanation for the presence of both four copy and eight copy loci in white sturgeon is the duplication of individual gene loci (Ludwig et al., 2001). AciG 35 appears to be an example of individual locus duplication, as it is found in a minimum of ten copies in an 8n individual (062) and in at least 13 copies in an individual with a more highly duplicated genome (065). However, if white sturgeon were evolutionary tetraploids that had experienced some tandem gene duplications, one would expect to observe an abundance of four copy loci with few loci exhibiting higher levels of duplication. Examining the 17 microsatellites characterized thus far in white sturgeon, we find there are actually more loci in $>4$ copies (10) than there are four copy loci (7), suggesting that localized gene duplication is an unlikely explanation for the presence of the eight copy loci (Rodzen and May, 2002; this study).

We might expect to observe four copy and eight copy loci in a genome shaped by an allopolyploid event. In an 8n allopolyploid, one might expect to find microsatellites systems consisting of two tetrasomic loci. One of two tetrasomic loci in a pair might become fixed for a null allele and may therefore be undetectable by PCR, producing microsatellites detected in only four copies such as AciG 140 and AciG 53. Given that modern sturgeon species are known not only to hybridize both

in the wild and in culture (Burtsev, 1972; Pirogovskiĭ et al., 1986; Sokolov and Vasil'ev, 1986a; Birstein et al., 1997; Ludwig et al., 2009) but also produce viable offspring (Burtsev, 1972; Sokolov and Vasil'ev, 1986b,c; Arefjev, 1989), allopolyploid steps in the radiation of the Acipenseriformes seems plausible. Vasil'ev (1999) and Fontana et al. (2008) provide models of Acipenseriform evolution containing allopolyploid steps.

The alternative scenario of genome duplication in white sturgeon is autopolyploidy. In an ancient 8n autopolyploid, microsatellites originally present in eight copies as octosomic loci would gradually decay into pairs of disomic loci. A more recently derived 8n autopolyploid would possess loci predominantly inherited in octosomic ratios. Co-segregating allelic pairs at four copy locus AciG 140 exhibit behavior consistent with transmission from a single tetrasomic locus while co-segregating allelic pairs at loci with ≥8 copies conform to patterns expected for alleles originating from either a single tetrasomic locus, two tetrasomic loci, or conform to multiple models of inheritance. Conformance to multiple models may be due to the similarity in segregation ratios expected for various inheritance models (e.g. 0.25 for two tetrasomic loci vs 0.21 for a single octosomic locus) or to an inheritance pattern intermediate to tetrasomy and octosomy. Additional examination of the white sturgeon genome with more markers and larger sample sizes is required to determine the mode of polyploidization in *A. transmontanus*.

Another group B species for which microsatellite inheritance data are available is the lake sturgeon. Although lake sturgeon and white sturgeon exhibit nearly identical genome sizes, lake sturgeon microsatellites were shown previously to be inherited as single tetrasomic loci, pairs of disomic loci, or single disomic loci (Pyatskowit et al., 2001; McQuown et al., 2002; Welsh and May, 2006). It is uncertain if the two locus types in lake sturgeon reflect segmental allopolyploidy (Stebbins, 1947; Johnson et al., 1987; Wolfe, 2001) or simply greater progression towards diploidization in another ancient octoploid. Differences in genome structure suggest that lake sturgeon and white sturgeon may be the result of different polyploidization events, which is supported by the most recent sturgeon phylogenies (Peng et al., 2007; Krieger et al., 2008). This reinforces the idea that it is impossible to make generalizations about the evolution of an entire ploidy class based only on data from a single species in that group.

The dams Y192 and 065 provide another example of the plasticity of sturgeon genomes, as these individuals possess a greater number of gene copies than other parents examined in this study. At loci present in eight copies (AciG 2, 43, 52, As015), Y192 exhibits a minimum of 10–11 copies per locus and 065 possesses a minimum of 6–11 copies per locus. Only one other parent exhibited >8 copies at one of these loci. Although sire 3d09 possesses a minimum of nine copies at AciG 52, he exhibits ≤8 or ≤4 copies at other eight and four copy loci, respectively, suggesting these data reflects either a novel individual duplication or a PCR error. Only two of 134 offspring from crosses of 3d09 with 8n females possess nine alleles, which is fewer than would be expected if 3d09 regularly were transmitting more than four alleles to his progeny.

The offspring of Y192 and 065 have significantly more alleles / locus than offspring from other crosses, confirming that higher copy number in these dams is not due to sample contamination. We detect fewer numbers of alleles / locus and gene copies in 065 and her offspring than in Y192 and her offspring, as 065 possesses a greater number of copies of just a few common alleles, many of which are uninformative.

The cause of the unusual gene copy number in these females is uncertain, although one possibility is that they descended from the fusion of an unreduced gamete with a normal gamete. Pyatskowit et al. (2001) and McQuown et al. (2002) discovered hatchery-reared lake sturgeon that were the products of unreduced gametes donated by one parent in artificial crosses, although the cause of abnormal meiosis was unclear. Three wild adult white sturgeon sampled in the Columbia and Sacramento Rivers exhibited unusually high numbers of alleles / locus across loci, suggesting that the phenomenon of unusual gene copy number is not exclusive to captive rearing (ADS, unpubl. data). Karyotypic and cytological studies have revealed differences in chromosome number and DNA content between wild and captive bred white sturgeon as well as wild sturgeon originating from different populations (Hedrick et al., 1991; Blacklidge and Bidwell, 1993; Fontana, 1994; Van Eenennaam et al., 1998b; Zhou et al., 2011). Blacklidge and Bidwell (1993) report individual lake sturgeon and Gulf sturgeon (*Acipenser oxyrinchus desotoi*) with genome sizes that are 3n to the 2n genome size measured in the species. It was suggested that interspecific hybridization may have caused the observed increase in genome size (Blacklidge and Bidwell, 1993), but as Y192 and 065 are the progeny of controlled crosses in captivity, hybridization can be ruled out. Zhou et al. (2011) conducted flow cytometry analysis on nine sturgeon species and discovered several examples of intraspecific variation in genome size, particularly amongst captive bred individuals. One of twelve adult white sturgeon examined by these authors possessed a genome size 1.5 times larger than the typical white sturgeon genome (Zhou et al., 2011).

Assuming white sturgeon are octoploid derived and Y192 and 065 are the products of unreduced gametes, they would be dodecaploid (12n). The transmission of six (AciG 43, AciG 52, AciG 110) and seven (As015) informative alleles to each offspring by Y192 and up to five informative alleles (AciG 52) by 065 is consistent with dodecaploidy. If Y192 and 065 are dodecaploids, their crosses with 8n males would produce decaploid (10n) offspring. Indeed, the progeny of Y192 and 065 exhibited up to ten alleles at eight copy loci and 11 alleles at AciG 35. Although we were unable to perform flow cytometric analysis on the offspring of Y192 and 065, we discovered two individuals (Fe 2 and Fe 3) in a sample of six white sturgeon from the caviar farm that possessed unusually large genome sizes (Table 4). Previous investigation of genome size in sturgeon showed the putative 12n shortnose sturgeon (*Acipenser brevirostrum*) had an average DNA content of 13.075 ± 0.051 pg per cell while the 8n white sturgeon had a mean genome size of 9.463 ± 0.043 (Blacklidge and Bidwell, 1993). Genome sizes of 10.44 pg per cell (Fe 3) and 11.39 pg per cell (Fe 2) seem to represent an intermediate 10n genome size between the previously observed 8n and 12n genome sizes in the Acipenseriformes. The parentage of Fe 2 and Fe 3 is unknown, but they originate from the same year class and may be sisters or half-sisters. The other female, Fe 1, originates from the same year class as Fe 2 and Fe 3 but may not share common parents. The males sampled (M1–M3) represent a different year class from the females and similarly may have different parentage. Unfortunately, we were unable to obtain blood samples Y192 and 065 to confirm their dodecaploidy. Farm records indicate thatY192 is still living and she will be sampled for flow cytometric analysis at the next available opportunity.

Alternative ploidy states within extant species have been documented in the wild for other fish species and several amphibians (Bogart, 1980; Legatt and Iwama, 2003). Diploid and octoploid populations of the frog *Ceratophyrs ornata* have been discovered and both diploid and tetraploid populations of *Odontophrynus* spp. exist (Bogart, 1980). Legatt and Iwama (2003) suggest that spontaneous polyploidy may be relatively common in lower fishes. One example is the dojo loach, *Misgurnus anguillicaudatus*, which can be found in diploid, triploid, and rare tetraploid forms in the wild (Arai et al., 1993). Spontaneous polyploids often are morphologically indistinguishable from individuals of 'normal' ploidy (e.g. this study) and the prevalence of this phenomenon in fishes may be underappreciated (Legatt and Iwama, 2003). Autopolyploids may be intentionally produced in aquaculture to improve growth rate and carcass quality of food fishes and invertebrates (Piferrer et al., 2009). However, the induction of autopolyploidy was not a management goal for the farmed population we describe.

The fate of spontaneous autopolyploids may be determined by a number of factors, including their fertility, the presence pre- or post-zygotic isolating mechanisms between spontaneous autopolyploids and 'normal' individuals, and their competitive advantage or disadvantage to the 'normal' population. We can only partially address the first two factors. The presence of 10n individuals in the farm population suggests that 12n females are fertile and can produce viable offspring when backcrossed to 8n males. This is not unexpected in a species where bivalent chromosome pairing at meiosis may be the norm. The 10n form is clearly viable, although nothing is known about its fertility. Backcrosses of a decaploid to either an octoploid or dodecaploid would produce aneuploid 9n or 11n offspring, respectively. Even if these individuals were viable, their unbalanced chromosome number (9n or 11n) may lead to unusual pairings at meiosis and the production of aneuploid gametes. Certain triploid salamanders, however, have been observed to bypass the constraints of aneuploidy by producing unreduced triploid gametes through pre-meiotic endomitotic replication (Bogart, 1980). Further research is required to determine the viability and fertility of 9n and 11n aneuploid forms.

The discovery of spontaneous autopolyploidy in white sturgeon here and in Zhou et al. (2011) calls into question the notion that genome duplication in lower vertebrates must necessarily occur through hybridization. Future research examining meiotic processes in sturgeon of both sexes may help us to better understand the potential for autopolyploidy in this lineage. Likewise, studies examining the reproductive potential of naturally produced hybrids, particularly the fertility of progeny from interploid crosses, will greatly increase our understanding of the role of hybridization in sturgeon speciation.

Our findings have implications for sturgeon conservation as well. Several conservation aquaculture programs are operating to preserve endangered sturgeon species such as the pallid sturgeon (*Scaphirhynchus albus*; Oldenburg et al. 2011), Kootenai River white sturgeon, an endangered distinct population segment (Ireland et al., 2002), and Adriatic sturgeon (*Acipenser naccarii*; Congiu et al., 2011). As similar artificial spawning techniques are utilized across sturgeon aquaculture programs, it is possible that the unintentional production of spontaneous autopolyploids is occurring in multiple facilities. Screening of additional sturgeon production facilities is necessary to ascertain the prevalence of spontaneous autopolyploidy in captive bred sturgeon. This need is most pressing in conservation focused programs, as some alternative ploidy states may exhibit lowered fertility. Introducing individuals with low fertility into wild populations would be counterproductive to conservation aquaculture goals. Experiments to identify environmental and genetic factors that may lead to spontaneous autopolyploidy in captive bred sturgeon are required so artificial spawning techniques can be modified, if necessary, to prevent its occurrence.

**Acknowledgements**

Funding for this work came from various sources, including the Bay-Delta Sport Fishing Enhancement Stamp Program under contract PO680104 by the Wildlife Forensics Laboratory, Division of Law Enforcement, California Department of Fish & Game, Rancho Cordova, CA 95670 and the Kootenai Tribe of Idaho, Bonners Ferry, ID 83805. ADS was supported by Hart Cole Goss and Block grant fellowships awarded by the UC Davis Department of Animal Science and Ecology Graduate Group, respectively. The authors would like to thank Peter Struffenegger, Eric Phillips, and Bobby Renschler at Sterling Caviar for providing blood and tissue samples and rearing eggs to hatching for this study. Thanks also to Jackie Pisenti at the UC Davis Hopkins Avian Facility for providing chicken blood for our diploid flow cytometry standard and Bob Kaufman for providing crucial information about preservation of sturgeon blood. We also appreciate the assistance of Jessica Petersen, Serge Doroshov, Arne Ludwig, and three anonymous reviewers who provided comments on earlier versions of this manuscript.

**References**

Allendorf, F. W.; Thorgaard, G. H., 1984: Tetraploidy and the evolution of salmonid fishes. In: Evolutionary genetics of fishes. B. J. Turner (Ed.). Plenum Press, New York, NY, pp. 1–53.

Arai, K.; Matsubara, K.; Suzuki, R., 1993: Production of polyploids and viable gynogens using spontaneously occurring tetraploid loach, *Misgurnus anguillicaudatus*. Aquaculture **117,** 227–235.

Arefjev, V. A., 1989: Karyotype variability in successive generations after hybridization between the great sturgeon *Huso huso* (L.) and the sterlet, *Acipenser ruthenus* L. J. Fish Biol. **35,** 819–828.

Birstein, V.J., 2005: Phylogeny and evolution of Acipenseriformes: new molecular and genetic data create new puzzles. In: Evolutionary biology: history and theory, vol **3**. Y.M. Gall and E. I. Kolchinsky (Eds). St. Petersburg**,** Russia, pp. 231–269.

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

Birstein, V. J.; Poletaev, A.; Goncharov, B. F., 1993: DNA content in Eurasian sturgeon species determined by flow cytometry. Cytometry **14,** 377–383.

Birstein, V. J.; Hanner, R.; DeSalle, R., 1997: Phylogeny of the Acipenseriformes: cytogenetic and molecular approaches. Env. Biol. Fish. **48,** 127–155.

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

Bogart, J. P., 1980: Polyploidy in evolution of amphibians and reptiles. In: Polyploidy: biological relevance. W. H. Lewis (Ed.). Plenum Press, New York, NY, pp. 341–378.

Börk, K. B.; Drauch, A. M.; Israel, J. A.; Pedroia, J. K.; Rodzen, J.; May, B. P., 2008: Development of new microsatellite DNA primers for green and white sturgeon. Cons. Genet. **9,** 973–979.

Burtsev, I. A., 1972: Progeny of the intergeneric hybrid of beluga and sterlet. In: Genetics, hybridization, and selection of fish. B. I. Cherfas (Ed.). Academy of Sciences, Jerusalem, Israel, pp. 211–220.

Congiu, L.; Pujolar, J. M.; Forlani, A.; Cenadelli, S.; Dupanloup, I.; Barbisan, F.; Galli, A.; Fontana, F., 2011: Managing polyploidy in ex situ conservation genetics: the case of the critically endangered Adriatic sturgeon (*Acipenser naccarii*). PLoS ONE **6,** e18249.

David, L.; Blum, S.; Feldman, M.W.; Lavi, U.; Hillel, J., 2003: Recent duplication of the common carp (*Cyprinus carpio* L.) genome as revealed by analyses of microsatellite loci. Mol. Biol. Evol. **20,** 1425–1434.

Dingerkus, G.; Howell, W. M., 1976: Karyotypic analysis and evidence of tetraploidy in the North American paddlefish, *Polyodon spathula*. Science **194,** 842–844.

Fontana, F., 1994: Chromosomal nucleolar organizer regions in four sturgeon species as markers of karyotype evolution in Acipenseriformes. Genome **37,** 888–892.

Fontana, F., 2002: A cytogenetic approach to the study of taxonomy and evolution in sturgeons. J. Appl. Ichthyol. **18,** 226–233.

Fontana, F.; Congiu, L.; Mudrak, V. A.; Quattro, J. M.; Smith, T. I. J.; Ware, K.; Doroshov, S. I., 2008: Evidence of hexaploid karyotype in shortnose sturgeon. Genome **51,** 113–119.

Hedrick, R. P.; McDowell, T. S.; Rosemarck, R.; Aronstein, D.; Lannan, C. N., 1991: Two cell lines from white sturgeon. Trans. Am. Fish. Soc. **120,** 528–534.

Ireland, S. C.; Anders, P. J.; Siple, J. T., 2002: Conservation aquaculture: an adaptive approach to prevent extinction of an endangered white sturgeon population. In: Biology, management, and protection of North American sturgeon. W. Van Winkle, P. Anders, D. H. Secor and D. Dixon (Eds). Proc 28th Am Fish Soc Symp. American Fisheries Society, Bethesda, MD, pp. 211–222.

Johnson, K. R.; Wright, J. E., Jr; May, B., 1987: Linkage relationships reflecting ancestral tetraploidy in salmonid fish. Genetics **116,** 579–591.

Kim, D. S.; Nam, Y. K.; Noh, J. K.; Park, C. H.; Chapman, F. A., 2005: Karyotype of North American shortnose sturgeon *Acipenser brevirostrum* with the highest chromosome number in the Acipenseriformes. Ichthyol. Res. **52,** 94–97.

Krieger, J.; Hett, A. K.; Fuerst, P. A.; Artyukhin, E.; Ludwig, A., 2008: The molecular phylogeny of the order Acipenseriformes revisited. J. Appl. Ichthyol. **24**(Suppl. 1)**,** 36–45.

Legatt, R. A.; Iwama, G. K., 2003: Occurrence of polyploidy in fishes. Rev. Fish. Biol. Fish. **13,** 237–246.

Ludwig, A.; Belfiore, N. M.; Pitra, C.; Svirsky, V.; Jenneckens, I., 2001: Genome duplication events and functional reduction of ploidy levels in sturgeon (Acipenser, Huso, and Scaphirhynchus). Genetics **158,** 1203–1215.

Ludwig, A.; Lippold, S.; Debus, L.; Reinartz, R., 2009: First evidence of hybridization between endangered starlets (*Acipenser ruthenus*) and exotic Siberian sturgeons (*Acipenser baerii*) in the Danube River. Biol. Invasions **11,** 753–760.

Marsden, J. E.; Schwager, S. J.; May, B., 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.

McQuown, E.; Gall, G. A. E.; May, B., 2002: Characterization and inheritance of six microsatellite loci in lake sturgeon. Trans. Am. Fish. Soc. **131,** 299–307.

Oldenburg, E. W.; Guy, C. S.; Cureton, E. S.; Webb, M. A. H.; Gardner, W. M., 2011: Effects of acclimation on poststocking dispersal and physiological condition of age-1 pallid sturgeon. J. Appl. Ichthyol. **27,** 436–443.

Peng, Z.; Ludwig, A.; Wang, D.; Diogo, R.; Wei, Q.; He, S., 2007: Age and biogeography of major clades in sturgeons and paddlefishes (Pisces: Acipenseriformes). Mol. Phylo. Evol. **42,** 854–862.

Piferrer, R.; Beaumont, A.; Falguiere, J. C.; Flajšhans, M.; Haffray, P.; Colombo, L., 2009: Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture **293,** 125–156.

Pirogovskiĭ, M. I.; Sokolo, L. I.; Vasil'ev, V. P., 1986. *Huso huso* (Linnaeus, 1758). In: The freshwater fishes of europe, Vol **1,** Part II. J. Holčík (Ed.). AULA-Verlag, Wiesbaden, Germany, pp. 156–200.

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

Rasch, E. M.; Prehn, L. M.; Rasch, R. W., 1970: Cytogenetic studies of *Poeilia* (Pisces) II. Triploidy and DNA levels in naturally occurring populations associated with the gynogenetic teleost, Poecilia (Girard). Chromosoma **31,** 18–40.

Rodzen, J. A.; May, B., 2002: Inheritance of microsatellite loci in white sturgeon (*Acipenser transmontanus*). Genome **45,** 1064–1076.

Sokal, R. R.; Rohlf, F. J., 1981: Biometry. Freeman, New York, NY.

Sokolov, L. I.; Vasil'ev, V. P., 1986a: *Acipenser nudiventris* Lovetsky, 1828. In: The freshwater fishes of europe, Vol **1,** Part II. J. Holčík (Ed.). AULA-Verlag, Wiesbaden, Germany, pp. 207–226.

Sokolov, L. I.; Vasil'ev, V. P., 1986b: *Acipenser ruthenus* Linnaeus, 1758. In: The freshwater fishes of europe, Vol **1,** Part II. J. Holčík (Ed.). AULA-Verlag, Wiesbaden, Germany, pp. 228–262.

Sokolov, L. I.; Vasil'ev, V. P., 1986c: *Acipenser baeri* Brandt, 1869. In: The freshwater fishes of europe, Vol **1,** Part II. J. Holčík (Ed.). AULA-Verlag, Wiesbaden, Germany, pp. 262–284.

Stebbins, G.L., Jr, 1947: Types of polyploids: their classification and significance. Adv. Genet. **1,** 403–429.

Stift, M.; Berenos, C.; Kuperus, P.; van Tienderen, P. H., 2008: Segregation models for disomic, tetrasomic, and intermediate inheritance in tetraploids: a general procedure applied to Rorippa (yellow cress) microsatellite data. Genetics **179,** 2113–2123.

Tagliavini, J.; Williot, P.; Congiu, L.; Chicca, M.; Lanfredi, M.; Rossi, R.; Fontana, F., 1999: Molecular and cytogenetic analysis of the karyotype of the European Atlantic sturgeon, *Acipenser sturio*. Heredity **83,** 520–525.

Van Eenennaam, A. L.; Murray, J. D.; Medrano, J. F., 1998a: Mitotic analysis of the North American white sturgeon, *Acipenser transmontanus* Richardson (Pisces, Acipenseridae), a fish with a very high chromosome number. Genome **41,** 266–271.

Van Eenennaam, A. L.; Murray, J. D.; Medrano, J. F., 1998b: Synaptonemal complex analysis in spermatocytes of white sturgeon, *Acipenser transmontanus* Richardson (Pisces, Acipenseridae), a fish with a very high chromosome number. Genome **41,** 51–61.

Vasil'ev, V. P., 1999: Polyploidization by reticular speciation in Acipenseriform evolution: a working hypothesis. J. Appl. Ichthyol. **15,** 29–31.

Vasil'ev, V. P., 2009: Mechanisms of polyploid evolution in fish: polyploidy in sturgeons. In: Biology, conservation, and sustainable development of sturgeons. R. Carmona, A. Domezain, M. García-Gallego, J. A. Hernando (Eds). Springer-Verlag, Berlin, Germany, pp. 97–117.

Vishnyakova, K. S.; Mugue, N. S.; Zelenina, D. A.; Mikodina, E. V.; Kovaleva, O. A.; Madan, G. V.; Yegorov, Y. E., 2008: Cell culture and karyotype of sakhalin sturgeon *Acipenser mikadoi*. Biologicheskie Membrany **25,** 420–433. (In Russian).

Welsh, A.; May, B, 2006: Development and standardization of disomic microsatellite markers for lake sturgeon genetic studies. J. Appl. Ichthyol. **22,** 337–344.

Wolfe, K.H., 2001: Yesterday's polyploids and the mystery of diploidization. Nat. Rev. Genet. **2,** 333–341.

Wright, J. E.; Johnson, K.; Hollister, A.; May, B., 1983: Meiotic models to explain classical linkage, pseduolinkage, and chromosome pairing in tetraploid derivative salmonid genomes. In: Isozymes: current topics in biological and medical research. Vol 10: genetics and evolution. B. J. Rattazzi, J. G. Scandalios, G. S. Whit (Eds). Alan R. Liss, New York, NY, pp. 239–260.

Zhou, H.; Fujimoto, T.; Adachi, S.; Yamaha, E.; Arai, K., 2011: Genome size variation estimated by flow cytometry in Acipenser mikadoi, Huso dauricus in relation to other species of Acipenseriformes. J. Appl. Ichthyol. **27,** 484–491.

Zhu, B.; Liao, X.; Shao, Z.; Rosenthal, H.; Chang, J., 2005: Isolation and characterization of microsatellites in Chinese sturgeon, *Acipenser sinensis*. Mol. Ecol. Notes **5,** 888–892.

**Author's address**: Andrea Drauch Schreier, University of California Davis, One Shields Ave, Davis, CA 95616, USA. E-mail: amdrauch@ucdavis.edu