Evidence for a maternal origin of spontaneous autopolyploidy in cultured white sturgeon (*Acipenser transmontanus*)

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ARTICLE INFO

ABSTRACT

Sturgeons (order Acipenseriformes) are members of an ancient evolutionary lineage that has experienced multiple rounds of whole-genome duplication. As a result, most extant species are polyploid and possess more than two complete sets of chromosomes. While interspecific variation in ploidy and overall genome size is common within this order, recent studies have also documented intraspecific variation in some captive bred sturgeon species, most notably in white sturgeon (*Acipenser transmontanus*). The unintentional duplication of one or more complete sets of chromosomes in a single species in the absence of hybridization is known as spontaneous autopolyploidization. The mechanism and the effects on viability and reproductive development of spontaneous autopolyploidy, however, are poorly understood. Sturgeons are valuable aquacultural species for production of boneless meat and caviar, and incidence of spontaneous autopolyploidy could have negative economic implications. In this study, we wished to determine whether there was a maternal or paternal origin of spontaneous autopolyploidy. We used flow cytometry to identify spontaneous autopolyploids and microsatellite genotyping to track the transfer of unique maternal and paternal alleles from broodstock to offspring across multiple full-sibling families in two populations of cultured white sturgeon. In all families, we found that the probability of transfer success of unique maternal alleles was greater than that of unique paternal alleles in spontaneous autopolyploid white sturgeon, indicating a maternal origin of spontaneous autopolyploidy. Secondly, as no spontaneous autopolyploid progeny shared all the same microsatellite alleles as the respective dam as would be expected with premeiotic endomitosis or apomixis, we believe that failure to extrude the second polar body in meiosis II is the most likely cause of spontaneous autopolyploidy in cultured white sturgeon.

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1. Introduction

Sturgeons are a valuable aquacultural resource as producers of boneless meat and highly prized caviar. All sturgeons belong to the order Acipenseriformes, an ancient lineage that has experienced multiple rounds of whole-genome duplication (Peng et al., 2007; Vasil'ev et al., 2010), or polyploidization (Otto, 2007), a process that is thought to facilitate species diversification and augment biological complexity over time (Ohno, 1970; Van de Peer et al., 2009). Polyploidy is a defining feature of Acipenserid fishes and the 25 extant sturgeon species have been sorted into three ploidy classes according to overall genome size, as determined by karyotype of somatic cells or nuclear DNA content measured by flow cytometry: Group A species possess approximately 120 chromosomes (3.2–4.6 pg of DNA); Group B species have approximately 250 chromosomes (6.1–9.6 pg of DNA); and Group C consists

of a single species (*Acipenser brevirostrum*) with approximately 360 chromosomes (13.1 pg of DNA; Birstein and DeSalle, 1998; Fontana et al., 2008). While these groups are widely upheld, the nature of sturgeon ploidy remains controversial due to conflicting cytogenetic and inheritance data. For this reason, sturgeon in Groups A, B, and C are classified as functional diploids (2N), tetraploids (4N), and hexaploids (6N) and also as evolutionary tetraploids (4N), octoploids (8N), and dodecaploids (12N), respectively (Birstein and Vasil'ev, 1987; Birstein et al., 1997; Fontana et al., 2008). For clarity in discussing the results of this study, we reference the evolutionary scale of sturgeon ploidy.

Recent studies have reported not only interspecific, but also intraspecific variation in the overall genome size of some sturgeons. The unintentional duplication of one or more haploid chromosome sets within a species is known as spontaneous autopolyploidization. Many natural mechanisms may cause spontaneous autopolyploidy in fishes, including the production of unreduced sperm or oocytes through apomixis or premeiotic endomitosis, the penetration of an oocyte by more than one sperm (polyspermy), retention of the second polar body in meiosis II, and unequal cleavage or suppression of the first cleavage after fertilization has occurred (Dawley, 1989; Nomura et al., 2013;

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Table 1
Occurrence of spontaneous autopolyploidy in sturgeon species.

Species	Expected		Spontaneous autopolyploid		References
	Evolutionary ploidy	DNA content (pg nucleus^{-1})	Evolutionary ploidy	DNA content (pg nucleus^{-1})	
A. ruthenus	4N	3.8–4.3	6N	6.0	Zhou et al. (2011); Havelka et al. (2013)
A. mikadoi	8N	8.1–9.2	12N	12.6–14.2	Zhou et al. (2011, 2013)
			16N	16.0–18.0	Zhou et al. (2011, 2013)
H. dauricus	8N	8.1–8.5	12N	12.5–13.3	Zhou et al. (2011)
A. transmontanus	8N	7.4–9.2	10N	10.4–11.4	Drauch Schreier et al. (2011); Schreier et al. (2013)
			12N	11.9–13.6	Schreier et al. (2013)
A. baerii	8N	8.3	6N	6.3	Bytyutskyy et al. (2012)
			10N	9.0	Bytyutskyy et al. (2012)
			12N	12.7	Bytyutskyy et al. (2012)
A. gueldenstaedtii	8N	7.9	12N	12.0	Bytyutskyy et al. (2012)

Piferrer et al., 2009). Spontaneous autopolyploid fish have been observed among artificially propagated sterlet (*Acipenser ruthenus*), Sakhalin sturgeon (*Acipenser mikadoi*), kaluga (*Huso dauricus*), white sturgeon (*Acipenser transmontanus*), Siberian sturgeon (*Acipenser baerii*), and Russian sturgeon (*Acipenser gueldenstaedtii*) (Table 1).

While little research has been completed on the performance outcomes of spontaneous autopolyploidy in sturgeons, the presence of an additional set of chromosomes may have an effect on phenotypic, physiological, behavioral, or reproductive traits. Studies comparing relative performance of diploid and triploid individuals of other fish species have revealed differences in survival (e.g. Chiasson et al., 2009), growth (e.g. Cal et al., 2006), condition factor (e.g. Fraser et al., 2013), disease resistance (e.g. Benfey, 1999; Ching et al., 2010), and thermal tolerance (e.g. Fraser et al., 2012), although variable results among studies even within the same species suggest that factors other than ploidy influence these traits. Triploid (16N) shortnose sturgeon exhibited a reduced stress response relative to diploid (12N) individuals (Beyea et al., 2005). Therefore, the presence of large numbers of spontaneous autopolyploids in the broodstock pool or among their offspring therefore ultimately has the potential to negatively affect caviar production, sturgeon farming efficiency, and overall profit. Moreover, the generation of infertile or unfit spontaneous autopolyploid fish could undermine the success of conservation aquaculture programs that exist to promote the natural recruitment in endangered or threatened wild sturgeon populations. Although spontaneous autopolyploid sturgeon from Group B species are fertile (Drauch Schreier et al., 2011; Havelka et al., 2014; Pšenička et al., 2011), the reproductive potential of their 10N offspring (produced by crosses with 8N conspecifics) is unconfirmed and one would expect 9N and 11N offspring of 10N × 8N or 10N × 12N crosses, respectively, to suffer from impaired chromosome pairing during meiosis due to an odd chromosome number. If descendants of spontaneous autopolyploid sturgeon were infertile, the presence of these individuals in the broodstock could reduce caviar production and undermine goals of conservation aquaculture programs. Thus, the identification of the source of spontaneous autopolyploidy and how to control its production are a high priority for sturgeon aquaculture.

Spontaneous autopolyploidy has been documented most extensively in white sturgeon (Schreier et al., 2013; Zhou et al., 2011), an evolutionary octoploid, however the cause of genome size increase in this species has not yet been identified. Work by Schreier et al. (2013) suggests a paternal effect in the generation of spontaneous autopolyploids however additional lines of evidence that are consistent across multiple populations are needed to confirm this finding. Here, we use flow cytometry to identify and classify spontaneous autopolyploid white sturgeon in multiple populations. We then perform controlled crosses of white sturgeon of known ploidy as well as microsatellite genotyping of the broodstock and their progeny to identify a maternal or paternal origin of spontaneous autopolyploidy in the species. To best accomplish this aim, we chose to examine full-sibling families of white sturgeon of known parentage from two populations. However, we were somewhat constrained by the availability of this specific type of sample (known full-sibling families) and so we divided our study into three sub-experiments. In each case, we made the assumption, based upon the frequency of spontaneous autopolyploidy in white sturgeon reported elsewhere (Drauch Schreier et al., 2011; Schreier et al., 2013), that progeny with elevated ploidy would be detected in full-sibling families from these two populations.

2. Materials and methods

2.1. Experimental design

2.1.1. Experiment 1 (E1)
The eggs of 12N females were fertilized with the sperm of 8N males, both from a genetically diverse population of white sturgeon. We hypothesize that: 1) the 12N females are fertile and will produce viable eggs; 2) all progeny that are not spontaneous autopolyploids (i.e., those that did not experience an additional spontaneous genome duplication) will be 10N by flow cytometry; 3a) all progeny with elevated ploidy will be 14N by flow cytometry, indicating that an additional set of chromosomes has been donated by the male parent; and 4a) microsatellite genotyping of spontaneous autopolyploid progeny (14N individuals) will show a greater number of unique paternal alleles, suggestive of unreduced sperm or a polyspermy event. The alternative hypotheses are: 3b) spontaneous autopolyploid progeny will be 16N by flow cytometry, pointing to a maternal origin of the additional set of chromosomes; and 4b) microsatellite genotyping of progeny with elevated ploidy (16N individuals) will show a greater number of unique maternal alleles, indicating that unreduced oocytes or failure to extrude the second polar body during meiosis contributed to elevated ploidy in these individuals.

2.1.2. Experiment 2 (E2)
The eggs of 8N females were fertilized with the sperm of 8N males, both from the same genetically diverse population of white sturgeon as in *E1*. We hypothesize that: 1) all spontaneous autopolyploid progeny will be 12N; and 2a) microsatellite genotyping of spontaneous autopolyploid individuals will show a greater number of unique paternal alleles. The alternative hypothesis is: 2b) a greater number of maternal alleles will be evident from microsatellite genotyping of spontaneous autopolyploid progeny.

2.1.3. Experiment 3 (E3)
The eggs of several wild-caught 8N females were fertilized with the sperm of wild-caught 8N males to generate ten individual full-sibling families; all fish were from an endangered and genetically depauperate population (Schreier et al., 2012) of white sturgeon. We make the same hypotheses for each full-sibling family as in *E2*.

2.2. Fish specimens

2.2.1. E1

In February 2013, we collected blood and fin clip samples from all broodstock ($n = 126$) at a white sturgeon caviar farm in California. The overall genome size of each fish was determined by flow cytometry according to the methods described below. From this analysis (data not shown), we identified seven fish with abnormal ploidy: five females (average genome size = 12.9 pg) and two males (average genome size = 10.5 pg). The genome size of the five females is indicative of spontaneous autopolyploidy is consistent with that of dodecaploid-derived (13.1 pg; 12N) sturgeon species described by Blacklidge and Bidwell (1993a). An average genome size similar to that of the two males has been observed before in white sturgeon (10.9 pg; Drauch Schreier et al., 2011) and suggests that these fish were of intermediate ploidy, or 10N, and were the result of a fertilization event between a 12N spontaneous autopolyploid and a parent of normal 8N ploidy.

In May 2013, mature eggs were obtained from two of the above 12N females and fertilized with the sperm of two of the above males with normal ploidy (average genome size = 8.8 pg; 8N). Milt from the two males was pooled to fertilize the eggs of one female while separate one by one crosses were conducted with the milt of the same two males and the second female. Artificial spawning procedures for white sturgeon are described elsewhere (Conte et al., 1988). The families created from the second female were held in separate tanks by paternal half-sibling family. In September 2013, we collected blood and fin clip samples from the four month-old progeny. We sampled 50 fish from each maternal family ($n = 100$ total).

2.2.2. E2

Six sires and six dams from the large broodstock pool described above were used to generate the 2011 year-class of white sturgeon at the caviar farm in California. All progeny from these crosses were pooled and randomly distributed among tanks meaning that it was possible for a single tank to contain full-siblings, half-siblings, and unrelated individuals. Blood and fin clip samples were collected from the twelve broodstock and a random sampling of their two year-old offspring ($n = 57$) in February and April of 2013, respectively. Of the six female broodstock, four were of normal ploidy (average genome size = 8.7 pg; 8N; data not shown) and two demonstrated elevated ploidy (average genome size = 12.6 pg; 12N; data not shown). All male broodstock were of normal ploidy (average genome size = 8.9 pg; 8N; data not shown).

2.2.3. E3

In May 2012, we obtained fin clip samples from eight female and six male wild-caught white sturgeon from the Kootenai River in Idaho. Natural recruitment has not been known to occur in this federally listed endangered population of white sturgeon since the construction of the Libby Dam in 1972 (Anders et al., 2002). The Kootenai Tribe of Idaho operates a conservation hatchery to preserve Kootenai River white sturgeon by spawning wild-caught fish in the hatchery and releasing their young back into the river, thus facilitating recruitment. Also in May, we collected blood samples from six of these broodstock that were held temporarily in the hatchery; all remaining broodstock were not held in the hatchery and so blood samples were not collected from these individuals. The sampled broodstock exhibited normal ploidy (average genome size = 8.9 pg; 8N) by flow cytometry analysis according to the methods described below (data not shown). In September 2013, we obtained fin clip and blood samples from 15 individuals from each of ten families ($n = 150$ total; age 14–16 months) created from the wild broodstock; all full-sibling families were reared separately. Some sires and dams were used more than once to generate multiple paternal and maternal half-sibling families.

2.3. Flow cytometry

We utilized flow cytometry to estimate genome size and identify spontaneous autopolyploid progeny. Total nuclear DNA content (genome size) of all blood samples was measured using flow cytometry according to the methods described by Blacklidge and Bidwell (1993a, 1993b). A detailed description of our flow cytometry protocol can be found in Schreier et al. (2013). Stained erythrocyte and TEN control samples were vortexed and total PI fluorescence was immediately evaluated on a flow cytometer. Blood samples from California white sturgeon were analyzed on a FACScan (Becton-Dickson, San Jose, CA) at the Optical Biology Shared Resource facility at the University of California Davis. Blood samples from Kootenai River white sturgeon were analyzed on a FACSAria (Becton-Dickson, San Jose, CA) at the Optical Imaging Core Facility at the University of Idaho Moscow. Mean PI fluorescence was measured using FlowJo analysis software (Tree Star, San Carlos, CA). We estimated the genome size of each white sturgeon by comparing the mean PI fluorescence of an experimental sample to that of the TEN control. We calculated genome size by solving the following equation:

$$\text{white sturgeon genome size (pg)} = (5.240\,\text{pg}) \times (S/T)$$

where S and T correspond to the mean PI fluorescence of the experimental blood sample and the TEN control, respectively.

2.4. Microsatellite genotyping

We performed microsatellite genotyping for the purpose of parentage analysis in E1 and E2 and also to determine whether the additional set of chromosomes in spontaneous autopolyploids could be traced to the male or female parent in all three sub-experiments. Genomic DNA was extracted from fin clip tissue using a Puregene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN), according to the manufacturer's instructions. The concentration of genomic DNA was measured using an FLA-5100 fluorescent image analyzer (Fuji, Stamford, CT); all samples were then normalized to a concentration of 20 ng/µl in preparation for amplification by PCR. We conducted PCR with 12 fluorescently labeled primers specific to polymorphic sturgeon microsatellite loci: AciG 2, AciG 35, AciG 52, AciG 53, AciG 110, AciG 140, AS 015, Atr 105, Atr 107, Atr 109, Atr 117, and Atr 1173 (Börk et al., 2008; Rodzen and May, 2002; Zhu et al., 2005). Details of PCR conditions and thermal cycler profiles for all microsatellite loci can be found in Schreier et al. (2012). Microsatellite fragment analysis was performed on a 3730x1 ABI Genetic Analyzer (Applied Biosystems, Foster City, CA) with a GeneScan ROX 400 fluorescent size standard (Applied Biosystems, Foster City, CA) and genotypes were resolved by eye using GeneMapper v.4.0 software (Applied Biosystems, Foster City, CA).

2.5. Parentage analysis

Microsatellites are considered codominant genetic markers in diploid species. However, in the polyploid white sturgeon, microsatellite loci are frequently found in four to eight copies (Drauch Schreier et al., 2011). The complexity of the highly duplicated white sturgeon genome and the nature of current microsatellite genotyping technology make it impossible to reliably determine the number of copies of any allele at a given locus. Therefore for the purpose of parentage analysis, we treated each microsatellite allele as binary dominant data: if an allele was present, it was given a score of 1; if an allele was absent, it was given a score of 0 (Drauch Schreier et al., 2011; Israel et al., 2009; Pfeiffer et al., 2011; Rodzen and May, 2002). We generated such binary genotypes of the 12 microsatellite loci for all of the progeny created from pooled milt in E1 and all of the offspring in E2 as well as the associated broodstock in both sub-experiments.

For each individual progeny, we computed an LOD score for every possible sire and dam as well as the associated delta (δ) statistic in the program Parent.exe (Rodzen et al., 2004). The LOD score was estimated according to the method developed by Gerber et al. (2000) and is the log of the likelihood that animal k is the parent of animal j divided by the log of the likelihood that animal k is not the parent of animal j. The δ value is a measure of confidence of each LOD score and is the difference between the LOD score of the most probable parent and that of the second most probable parent (e.g. if $\delta = 3$, the most probable parent is 10^3, or 1000 times more likely to be the true parent than the second most probable parent). Using the LOD scores in conjunction with the δ statistic, we determined the most probable sire for the pooled milt offspring in *E1* and the most probable sire and most probable dam for all progeny in *E2*.

2.6. Analysis of unique maternal and paternal alleles

We wished to ascertain whether spontaneous autopolyploidy in white sturgeon was of maternal or paternal origin, or both. Once spontaneous autopolyploid progeny were identified in each sub-experiment and parentage of all individuals was known, we examined the dominant binary microsatellite genotypes of the broodstock and their progeny; each full-sibling family was evaluated separately. Included in this analysis were four families generated from wild broodstock from the previous year class from the Kootenai River in Idaho described in Schreier et al. (2013) that contained spontaneous autopolyploids. Genotypes from the fifth family that contained spontaneous autopolyploids (family B4DA × C6EB; Schreier et al., 2013) were incomplete and were therefore excluded from the analysis. As in *E3*, there were 15 individuals in each family ($n = 60$ total). If an allele was found in both the sire and the dam, we considered it to be uninformative and it was discarded from this analysis. If an allele was observed only in the sire or the dam, it was retained as a unique and informative paternal or maternal allele. If the unique paternal or maternal allele was observed in the offspring, the transmission of the allele was considered successful and was given a value of 1. Conversely, if the allele was not witnessed in the offspring, the transmission of the allele did not occur and was coded as 0.

The fundamental observation of the data set was, therefore, the successful transmission (or absence of transmission) of an allele from parent to offspring. Accordingly, the basic unit of measurement was a binomial variable: the allele was transmitted or it was not. The binomial observation was further classified, not just by the ploidy of the offspring, but also by the origin of the marker: from the male parent or from the female parent. In this way, we considered the success of allele transfer to be a Bernoulli (π_{ijk}) random variable Y_{ijk} such that

$$P\left(Y_{ijk} = y_{ijk}\right) = \pi_{ijk}^{y_{ijk}}\left(1 - \pi_{ijk}\right)^{(1-y_{ijk})}$$

where i denotes the ploidy states of the offspring and j denotes the parents of origin for the k-th marker within that parental classification. As this data set implies, we believe that the probability of an allele, π_{ijk}, is a function of the parental origin and that there may be a difference in the probability of a successful allele transfer from dam or sire to offspring across the two ploidy classes (i.e. expected ploidy class, 10N in *E1* and 8N in *E2* and *E3*, and spontaneous autopolyploid class, 16N in *E1* and 12N in *E2* and *E3*). We relate these sources of information (ploidy status, origin, and marker within origin) to the probability of success, π_{ijk} through a link function for a set of covariates ($\mathbf{x'_{ijk}}$) and unknown coefficients (β, a vector representing unknown ploidy and origin, their interaction, and marker effects) in a logistic regression approach.

$$\theta_{ijk} = \mathbf{x'_{ijk}}\beta \tag{1}$$

$$= \mu + \text{Ploidy}_i + \text{Origin}_j + \text{Ploidy} * \text{Origin}_{ij} + \text{Marker}_{jk} \tag{2}$$

$$= \log\left(\frac{\pi_{ijk}}{1 - \pi_{ijk}}\right). \tag{3}$$

In this case, we treated the marker effect, nested within the origin identifier, as a random effect. Prediction of the probability of transfer success can then take the form:

$$\Pr[\text{Transfer} = 1] = \pi_{ijk} = \frac{e_{ijk}^{\theta}}{\left(1 + e_{ijk}^{\theta}\right)}. \tag{4}$$

Estimation of the unknown model effects, along with variances for the random effects of the models, was facilitated through the lme4 package (Bates, 2010) of the public domain programming language R (R Core Team, 2013).

Three separate analyses were undertaken: one for each experiment (*E1*–*E3*). Model (2) is appropriate for two of the data sets (*E1* and *E2*). However, data collected in *E3* was collected across 10 families: four families from the 2012 year class (Schreier et al., 2013) and six families from the 2013 year class. Thus, model (2) was expanded to include an additional term for each family and the family term was included as a random effect.

3. Results

3.1. Fertility of spontaneous autopolyploids

In *E1*, we discovered that the 12N females produced viable eggs that, when fertilized by the milt of 8N males, successfully developed into juvenile white sturgeon. However, hatchery personnel noted that fry from the pooled milt cross exhibited poor survival relative to other crosses from the same year and many fry developed a half moon body shape and abnormal swimming (swimming in small circles in place; E. Phillips, pers. comm.). Deformed fry did not survive to the time of sample collection for ploidy determination and therefore they were not included in our analyses. While the progeny of this 12N female were seemingly not as robust as those produced by 8N females in the same year, several hundred of these offspring being held at an on-campus aquaculture facility have survived to age 15 months. In addition, offspring of other 12N individuals have been observed in the broodstock at the caviar farm in California indicating that the progeny of 12N fish are capable of surviving to adulthood (D. Gille, unpublished data).

3.2. Flow cytometry

In *E1*, we found that all progeny originating from the two maternal half-sibling families in which fertilization was conducted separately showed evidence of intermediate ploidy, or 10N (Table 2). This finding also held true for the progeny of the pooled milt cross except for one fish whose total nuclear DNA content was approximately $1.6\times$ that of the other full-siblings, or 16N (Table 2) — twice the total DNA content of a normal octoploid white sturgeon. In *E2*, we identified three spontaneous autopolyploid individuals all with $1.5\times$ the expected total nuclear DNA content (12 N) in a single full-sibling family (Table 2). The one offspring detected from family 6158 × 2808 was 10N, however this result was expected as the female parent (2808) was a known spontaneous autopolyploid (12N; Table 2). In this case, the 10N fish was not considered to be a spontaneous autopolyploid. The overall genome sizes of all other individuals in the remaining *E2* families were consistent with that of octoploid white sturgeon described previously (other studies are summarized in Table 1; Table 2). In *E3*, six of the ten families examined showed evidence of spontaneous autopolyploidy, again in individuals with $1.5\times$ the expected total nuclear DNA content (12N; Table 2). The prevalence of spontaneous autopolyploidy was similar between each

Table 2
In each sub-experiment (E1–E3) and for each full-sibling family, the number of progeny of expected ploidy and the number of spontaneous autopolyploid progeny with the associated total nuclear DNA content (pg).

Experiment	Male parent	Female parent	Progeny (expected ploidy)				Progeny (spontaneous autopolyploids)			
			n	Range (pg)	Mean (pg)	Ploidy	n	Range (pg)	Mean (pg)	Ploidy
E1	R8	B12	39[a]	10.5–11.6	11.1	10N	1	–	18.0	16N
	W61	B12	8[a]	10.8–11.5	11.1	10N	0	–	–	–
	R8	G20	25	10.2–11.6	10.9	10N	0	–	–	–
	W61	G20	25	10.0–11.2	10.7	10N	0	–	–	–
E2[b]	OE5E	0610	32	8.6–9.7	9.2	8N	3	14.2–14.6	14.4	12N
	OE5E	234A	1	–	8.7	8N	0	–	–	–
	6158	2E4C	5	9.1–9.6	9.3	8N	0	–	–	–
	6158	2808	0	–	–	–	1	–	11.5	10N
	6F6B	0610	4	8.8–9.3	9.0	8N	0	–	–	–
	6F6B	2E4C	2	8.9–9.1	9.0	8N	0	–	–	–
	2179	0610	4	7.8–8.9	8.7	8N	0	–	–	–
	2179	2E4C	2	8.7–9.1	8.9	8N	0	–	–	–
	5C49	0610	1	–	9.2	8N	0	–	–	–
	5C49	7627	1	–	9.4	8N	0	–	–	–
E3	F04B	1043	13	7.4–9.3	8.5	8N	2	12.9	12.9	12N
	F04B	FBF1	14	7.9–9.2	8.7	8N	1	–	13.8	12N
	C523	0A06	14	8.1–8.9	8.6	8N	1	–	11.6	12N
	C523	629B	13	7.5–9.0	8.5	8N	2	12.0–13.1	12.3	12N
	5021	E47A	15	7.3–8.7	8.2	8N	0	–	–	–
	5021	629B	13	8.3–9.1	8.6	8N	2	12.9–13.1	13.0	12N
	2800	1043	15	8.8–9.3	9.1	8N	0	–	–	–
	2800	86C1	15	8.9–9.4	9.1	8N	0	–	–	–
	2C27	F4FA	8	8.1–9.4	9.0	8N	7	12.1–13.8	13.1	12N
	13DB	F621	15	8.5–9.4	9.1	8N	0	–	–	–

[a] Two 8N individuals from the pooled milt cross could not be reliably assigned to either sire and were excluded from the table.
[b] The parentage of one individual from this year class could not be reliably assigned and so this fish was excluded from the table.

of three paternal half-sibling families: 13.3% and 6.7% in families F04B × 1043 and F04B × FBF1, 6.7% and 13.3% in families C523 × 0A06 and C523 × 629B, and 0% in 2800 × 1043 and 2800 × 86C1, respectively. The proportion of spontaneous autopolyploids identified in paternal half-sibling families here is comparable to that found in the previous year class of the same population described by Schreier et al. (2013). However, in two paternal half-sibling families, 5021 × E47A and 5021 × 629B, the percentage of spontaneous autopolyploids was not entirely congruent at 0% and 13.3%, respectively. One family for which there was no corresponding paternal half-sibling family, 2C27 × F4FA, showed the greatest prevalence of spontaneous autopolyploids at 46.7%. The overall genome sizes of individual fish that were not spontaneous autopolyploids were similar to octoploid white sturgeon in the E2 population and of those described in earlier studies (other studies are summarized in Table 1; Table 2).

3.3. Parentage analysis

We determined that 40 of the offspring from the pooled milt cross in E1 were sired by male R8 while only 8 were sired by male W61 (Table 2). Two offspring from the pooled milt cross (all 10N fish) could not be reliably assigned to either sire and so were excluded from further analyses (Table 2). Parentage analysis of E2 progeny revealed the presence of ten families (Table 2). The family with the greatest representation in our sample, OE5E × 0610, consisted of 35 individuals, of which three were spontaneous autopolyploids (12N; Table 2). The most successful dam was female 0610 (8N) that produced 44 offspring while the most successful sire was male OE5E (8N) that generated 36 offspring (Table 2). The parentage of one individual from this year class could not be reliably assigned and was therefore not incorporated in further analyses.

3.4. Origin of spontaneous autopolyploidy

Across all full-sibling families in E1–E3, logistic regression showed that the probability of transfer success of unique maternal alleles was greater than that of unique paternal alleles in spontaneous autopolyploid white sturgeon (16N fish in E1; 12N fish in E2 and E3; Fig. 1). The probability of transfer success of unique maternal and paternal alleles was roughly the same among full-siblings of expected ploidy (10N fish in E1; 8N fish in E2 and E3; Fig. 1). However in E1, the higher probability of transfer success of unique maternal alleles in spontaneous autopolyploids was not significant likely due to small sample size as only a single 16N white sturgeon was discovered in a sample of 40
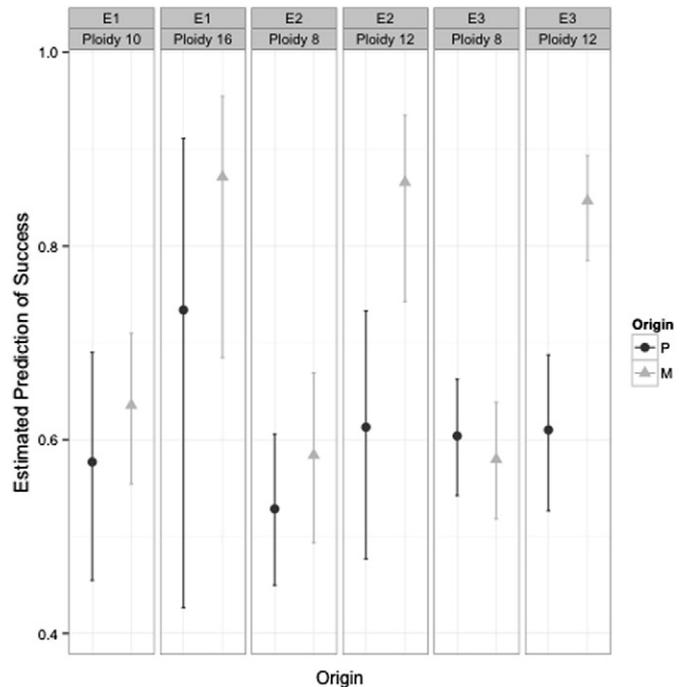

Fig. 1. In each sub-experiment (E1–E3), the probability of transfer success of unique paternal alleles (P; circles) and unique maternal alleles (M; triangles) in progeny with normal ploidy (10N in E1; 8N in E2 and E3) and in spontaneous autopolyploid progeny (16N in E1; 12N in E2 and E3).
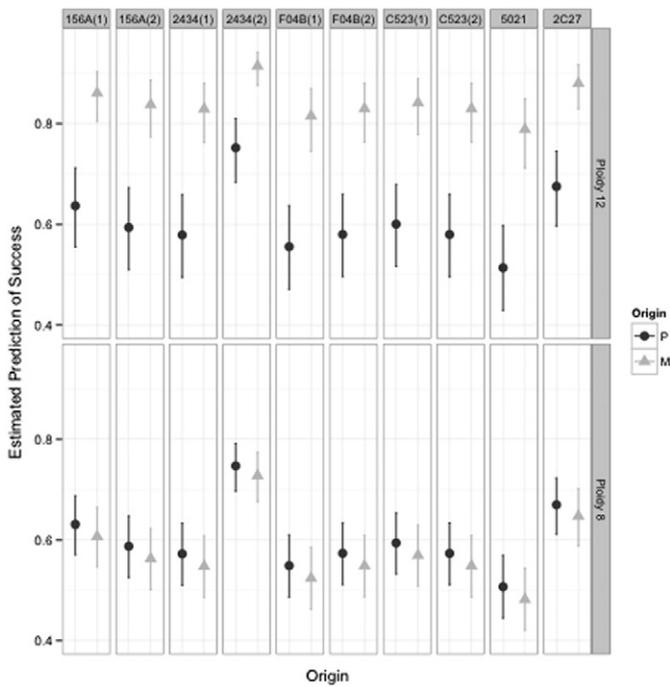
Fig. 2. In *E3*, the probability of transfer success of unique paternal alleles (P; circles) and unique maternal alleles (M; triangles) in progeny with normal ploidy (8N) and spontaneous autopolyploid progeny (12N) from each full-sibling family. The following families originated from Schreier et al. (2013): 156A(1) denotes family 156A × E703, 156A(2) denotes family 156A × 5E6D, 2434(1) denotes family 2434 × 2E93, and 2434(2) denotes family 2434 × AEFC. The following families originated from this study: F04B(1) denotes family F04B × 1043, F04B(2) denotes family F04B × FBF1, C523(1) denotes family C523 × 0A06, C523(2) denotes family C523 × 629B, 5021 denotes family 5021 × 629B, and 2C27 denotes family 2C27 × F4FA.

full-siblings (Fig. 1). Additionally in *E3*, some male and female parents were more diverse relative to one another and thus the overall transmission success of microsatellite alleles was higher in these families (family 2434 × AEFC from Schreier et al., 2013 and family 2C27 × F4FA in Fig. 2) compared to other families.

4. Discussion

The principal aim of this investigation was to identify whether the additional set of chromosomes in spontaneous autopolyploid white sturgeon were paternal or maternal in origin. Similar prevalence of 12N individuals in paternal half-sibling families in a previous study (Schreier et al., 2013) led us to the initial hypothesis that spontaneous autopolyploidy was the result of a paternal effect, most likely polyspermy. Sturgeon eggs possess multiple micropyles and polyspermic embryos may be produced at high sperm concentrations, such as would be found under artificial spawning conditions (Cherr and Clark, 1985). However, we discovered that a significantly greater number of unique microsatellite alleles were likely to be transferred from dams to their spontaneous autopolyploid offspring across multiple full-sibling families in two separate white sturgeon populations. To the best of our knowledge, our study is the first to show conclusive evidence of a maternal origin of spontaneous autopolyploidy in white sturgeon.

Several mechanisms may generate maternal spontaneous autopolyploids in fish including premeiotic endomitosis, apomixis, failure to extrude the second polar body in meiosis II, unequal cleavage, and suppression of the first cleavage once meiosis has occurred (Nomura et al., 2013; Piferrer et al., 2009). Premeiotic endomitosis is a process by which chromosome doubling occurs without cytokinesis before an egg enters meiosis and is thought to be a consequence of hybridization between two genetically disparate species (Dawley, 1989; Itono et al., 2006). In apomixis, an abortive first meiotic division prevents synapsis

and subsequent segregation of homologous chromosomes (Arai and Fujimoto, 2013; Itono et al., 2006). The result of both premeiotic endomitosis and apomixis is unreduced oocytes that are genetically identical to the mother. We were able to rule out both mechanisms as causes of spontaneous autopolyploidy in white sturgeon as no spontaneous autopolyploid offspring possessed all of the same microsatellite alleles as that of the respective female parent. The result of unequal cleavage is differential partitioning of chromosomes among daughter cells following meiosis II. However, it is improbable that different sets of homologous chromosomes would all be partitioned in the same way between daughter cells, moreover offspring produced by unequal cleavage would be aneuploid and aneuploid organisms are often inviable and infertile. Here we have shown that 12N females are fertile and produce viable offspring, which suggests that there is a complete additional set of chromosomes present in spontaneous autopolyploid white sturgeon. Suppression of the first cleavage directly following meiosis in fish would result in tetraploid offspring (Piferrer et al., 2009); total DNA contents of spontaneous autopolyploid white sturgeon measured by flow cytometry in this study are not consistent with a tetraploid (here, 20N in *E1* or 16N in *E2* and *E3*) genome size. Finally, failure to extrude the second polar body in meiosis II would generate triploid (here, 16N in *E1* or 12N in *E2* and *E3*) offspring that do not share all the same microsatellite alleles as the female parent (i.e., recombination has occurred). Our data are congruent with this mechanism and we therefore find failure to extrude the second polar body in meiosis II to be the most likely cause of spontaneous autopolyploidy in cultured white sturgeon. Retention of the second polar body has been identified as the cause of spontaneous triploidy in other fishes including rainbow trout (*Oncorhynchus mykiss*; Thorgaard and Gall, 1979), carp (*Cyprinus carpio*; Cherfas et al., 1995), tench (*Tinca tinca*; Flajšhans et al., 2007), and Japanese eel (*Anguilla japonica*; Nomura et al., 2013).

Induced triploidy via retention of the second polar body is a common practice in aquaculture to produce sterile individuals. Sterility may be desirable in fish as bodily resources are shunted to somatic growth, survival, and meat quality rather than sexual maturation (Maxime, 2008). Typically, a physical (thermal or pressure) or chemical shock is applied during embryonic development that prevents the release of the second polar body (molecular mechanisms of induced triploidy are reviewed in Maxime (2008)). Interestingly, spontaneous autopolyploid sturgeon studied here (16N in *E1* or 12N in *E2* and *E3*) were produced in the absence of known physical or chemical stressors. Furthermore, Schreier et al. (2013) found no correlations among spawning conditions such as time to ovulation and spawning temperature and the tendency to produce spontaneous autopolyploid white sturgeon. In other aquaculture species, the unintended generation of spontaneous autopolyploids via second polar body retention has been linked to post-ovulatory oocyte aging (Aegerter and Jalabert, 2004; Flajšhans et al., 1993, 2007; Nomura et al., 2013). In these species as in white sturgeon, ovulation is induced via hormonal injection. Female white sturgeon are then monitored from 18 to 24 h post-injection (F. Conte, pers. comm.; Conte et al., 1988) for the spontaneous release of eggs indicating the initiation of ovulation; eggs are then stripped and inseminated shortly thereafter. It is possible that the up to 6 hour time lapse from ovulation to fertilization in white sturgeon may cause ova to over-ripen and thus inhibit release of the second polar body in meiosis II. In Nomura et al. (2013), the percentage of Japanese eel larvae of abnormal ploidy increased from 5.8%, when fertilization occurred directly after ovulation, to 43.3% in vivo and 35.8% in vitro when eggs were inseminated 4 h post-ovulation. The amount of time between ovulation and fertilization was not recorded for the full-sibling families generated for use in *E1–E3* but will be an important measurement for future studies as a potential contributing factor to spontaneous autopolyploidy in cultured white sturgeon.

In studies of spontaneous triploidy in rainbow trout, Cuellar and Uyeno (1972) and Thorgaard and Gall (1979) found the greatest frequency of spontaneous autopolyploidy in certain strains or familial

lines leading both sets of authors to theorize that there is a genetic predisposition for the production of unreduced ova. Work by Flajšhans et al. (1993) corroborated this theory by also finding high frequencies of spontaneous triploids in only particular lines of tench. Flajšhans et al. (1993) extended the original hypothesis and proposed that fish that produce a great many number of triploids are likely to carry a recessive allele that contributes to the failure of second polar body extrusion or egg overripening when present in a homozygous state. In our study, by chance two sets of maternal half-sibling families were produced in E3 (F04B × 1043 and 2800 × 1043; C523-2 × 629B and 5021-2 × 629B). The frequency of spontaneous autopolyploidy was dissimilar in the two half-sibling families generated by female 1043 (13.3% and 0%, respectively) but identical in those families generated by female 629B (13.3% in both). It is difficult to draw meaningful conclusions about a potential genetic predisposition to produce unreduced eggs with the small sample size of two sets of maternal half-sibling families. However, a similar examination of the frequency of spontaneous autopolyploidy in a greater number of individuals and among multiple maternal half-sibling families using flow cytometry could be very telling. A more in depth investigation into the genetic causes of polar body retention in white sturgeon is warranted.

It is clear from this study and others (Drauch Schreier et al., 2011; Schreier et al., 2013) that white sturgeon can tolerate some degree of ploidy plasticity. More importantly from the results of E1, 12N female white sturgeon are fertile and produce offspring of elevated ploidy. It remains uncertain how spontaneous autopolyploids will fare over time, whether viable offspring will be produced after several generations of mating with white sturgeon of normal (8N) ploidy, and what impact the production of 12N fish may have on white sturgeon aquaculture programs. From E1, we have seen that 12N white sturgeon are capable of surviving into adulthood. It is still possible that there are other morphological, behavioral, or metabolic differences that we have not detected and that 12N white sturgeon are less fit than 8N fish. In general, there is a higher incidence of deformities of triploid fish in comparison to their diploid counterparts. For example, triploid Atlantic salmon (Salmo salar L.) exhibit a higher rate of lower jaw and gill filament deformities (Sadler et al., 2001) and eye cataracts (Cotter et al., 2002) than diploid salmon. However, triploidy was artificially induced in each of these studies and harsh gamete manipulations may have been the root cause of the physical abnormalities rather than the triploid state itself (Piferrer et al., 2009). The production of a large number of spontaneous autopolyploids that do not survive to adulthood or whose physical deformities make them undesirable for harvest and subsequent meat or caviar production could affect the efficiency and success of white sturgeon aquaculture.

Perhaps the most troubling consequence of spontaneous autopolyploidy in sturgeon aquaculture is the potential for reduced fertility or sterility several generations after a chromosome doubling event has occurred. This concern should not be limited to white sturgeon aquaculture as fertile spontaneous autopolyploid individuals of other Group B sturgeon species have been documented (Havelka et al., 2014; Pšenička et al., 2011). In E1, we showed that 12N white sturgeon females were fertile and when crossed with 8N males, produce 10N offspring. If 10N sturgeon are capable of producing viable gametes, mating with 8N and 12N fish would generate 9N and 11N offspring, respectively. Organisms with odd ploidy are relatively infertile or sterile as there is an uneven distribution of homologous chromosomes among daughter cells during meiosis. If hatchery-generated 12N sturgeon are recruited back into natural populations, as would be the case in conservation aquaculture programs, it is possible that over a minimum of two generations, their progeny would be unable to reproduce. This scenario is less of a concern for caviar farmers, who rear sturgeon for eggs and meat, unless a great proportion of spontaneous autopolyploid individuals are present in the broodstock pool. However, an increase in genome size may affect the quality and output of meat and caviar. Also, infertile females that do not produce viable eggs are a waste of time and

resources for caviar farmers. Finally, no information exists regarding survival, phenotype, or fertility of white sturgeon of intermediate ploidy (here, 10N fish in E1); we are currently holding a large group of 10N progeny from E1 on which to conduct such experiments.

While there is much evidence to suggest that the propagation of spontaneous autopolyploids will be detrimental to white sturgeon aquaculture, it is also conceivable that there will be benefits associated with an increase in genome size. For example, eggs of the triploid loach (Misgurnus aguillicaudatus) tend to be larger than haploid eggs (Matsubara et al., 1995; Zhang and Arai, 1999) and the eggs of hexaploid loach are significantly larger than those of diploid and tetraploid conspecifics (Arai et al., 1999). Similarly, eggs produced by spontaneous autopolyploid white sturgeon should be larger than eggs produced by 8N individuals to accommodate an additional set of chromosomes and thus a larger nucleus. In addition, triploidy induction in fish species with female heterogametic sex determination, including white sturgeon, can produce a female biased sex ratio in triploid offspring (e.g. Cal et al., 2006; Van Eenennaam et al., 1999), which has obvious benefits for caviar production. The potential for large egg size and production of female-biased populations should be further examined in spontaneous autopolyploid white sturgeon to allow hatchery managers to make informed decisions about whether to cull or retain individuals with elevated genome size in their cultured stock.

5. Conclusions

Contrary to our previous work indicating a paternal effect (Schreier et al., 2013), we discovered a maternal origin of spontaneous autopolyploidy in white sturgeon. Failure to extrude the second polar body in meiosis II is the most likely mechanism of spontaneous autopolyploidy in white sturgeon. Future work in this area should focus on identifying the causal mechanism of maternal spontaneous autopolyploidy so that hatchery managers may be able to control the production of individuals with elevated total genome size. It will also be essential to quantify the phenotypic effects of spontaneous autopolyploidy and to determine whether fish of elevated genome size are more or less desirable in an aquaculture setting. The fertility of offspring from spontaneous autopolyploid and normal ploidy fish crosses over multiple generations (i.e., 9N and 11N individuals) is a particular concern. Our study is the first step towards the identification of the ultimate cause and factors responsible for spontaneous autopolyploidy in this valuable aquaculture species.

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