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Short communication

Meiotic gynogenesis revealed not homogametic female sex determination system in Siberian sturgeon (*Acipenser baeri* Brandt)

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ABSTRACT

Meiotic gynogenesis was induced in a Siberian sturgeon (*Acipenser baeri* Brandt) by heat shock after egg activation with UV-irradiated sperm of a hybrid Siberian sturgeon × Russian sturgeon (*Acipenser gueldenstaedti* Brandt). Microsatellite DNA analysis was applied for verification of unipaternal inheritance in the gynogenetic diploid group of fish. All the analyzed gynogenetic diploids possessed only maternal genotype in the examined experimental group of fish. Histological analysis of gonads of 50 gynogenetic diploids, obtained from one family, showed 81% of females and 19% of males. The observed sex ratio suggested that the Siberian sturgeon does not have a female homogametic sex determination system.

1. Introduction

Genome manipulation, especially gynogenesis, is attractive for studying the sex determination mechanism in sturgeons and for production of all-female progeny of this fish (Mims et al., 1997; Pandian and Koteeswaran, 1998; Devlin and Nagahama, 2002). All-female stocks of sturgeon could be very useful for farmed black caviar production despite the decline of natural sturgeon populations.

Genome manipulations should be verified by the ploidy level investigation and unipaternal inheritance confirmation. In recent years, precise and sensitive methods based on microsatellite DNA analysis have been applied to monitor uniparental inheritance after meiotic gynogenesis on shortnose sturgeon, bester, sterlet and Siberian sturgeon (Flynn et al., 2006; Omoto et al., 2005; Fopp-Bayat et al., 2007; Fopp-Bayat, 2007). In such kind of study, a minimum of two carefully selected diagnostic loci in the offspring are sufficient to confirm exclusively maternal contribution. Selection of 2 loci is recommended, taking into consideration the mutation rate at these loci and the possible existence of paternal residual transmission in some offspring analyzed (Thorgaard et al., 1985). Molecular techniques based on microsatellite DNA analysis are non-invasive because they require only a small piece of tissue (e.g., a fin clip).

Gynogenesis in fish has been studied in the past few decades with respect to its potential value in experimental genetics and aquaculture (Thorgaard et al., 1985; Pandian and Koteeswaran, 1998; Arai, 2001). In Acipenseridae, gynogenesis has been successfully induced in some species, for example in white sturgeon *Acipenser transmontanus* (Van Eenennaam et al., 1996), shovelnose sturgeon *Scaphirhynchus platorynchus* (Mims and Shelton, 1998), Russian sturgeon *Acipenser gueldenstaedti* (Recoubratsky et al., 2003), stellate sturgeon *Acipenser stellatus* (Recoubratsky et al., 2003; Saber et al., 2008), bester *Huso huso* × *Acipenser ruthenus* (Omoto et al., 2005), shortnose sturgeon *Acipenser brevirostrum* (Flynn et al., 2006), sterlet *Acipenser ruthenus* (Fopp-Bayat et al., 2007) and Siberian sturgeon *Acipenser baeri* (Fopp-Bayat, 2007). The sex determination system has been described only in white sturgeon and bester (Van Eenennaam et al., 1996; Omoto et al., 2005). These two species are characterized by the ZW female heterogametic sex determination system.

The objective of the present study was to study the sex determination system in gynogenetic diploids of Siberian sturgeon *Acipenser baeri*.

2. Material and methods

2.1. Experimental design

The experiments described in this paper were conducted in 2006–2009. The first step of this research involved induction of meiotic gynogenesis in Siberian sturgeon. For activation of eggs, UV-irradiated sperm of the hybrid of Siberian sturgeon × Russian sturgeon was used. One female of Siberian sturgeon and one male of the hybrid of Siberian sturgeon × Russian sturgeon provided all the gametes used for this study. Induction of ovulation and spermiation of fish were described by Fopp-Bayat (2007). The dose of UV irradiation (288.75 Jm^{-2}) applied in the present study was similar to maximum effective dose of UV irradiation described by Recoubratsky et al. (2003) for Russian sturgeon. The procedure for obtaining the gynogenetic offspring of Siberian

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sturgeon was described by Fopp-Bayat (2007). The induction of gynogenesis and egg incubation were conducted in Wasosze Fish Farm near Konin, Poland. The experiment included: diploid control group, triploid control group, gynogenetic haploid group (with the UV-irradiation dose of 288.75 Jm^{-2}), and gynogenetic diploid group of fish (with the UV-irradiation dose of 288.75 Jm^{-2}, heat shock of 37 °C for 2 min was applied at 18 min after fertilization in the gynogenetic group). The eggs were maintained at temperature of 15 °C between fertilization and the initiation of the heat shock. The survival in the experimental group was monitored during the fertilization, gastrulation and hatching. Hatched larvae from each treatment were divided into two batches and one batch of all the experimental groups of fish was transported to a rearing system at the Inland Fisheries Institute in Olsztyn, where fish were reared from two days post-hatch (PH) until three months PH. After the three months, fish were transported to the Dgal Experimental Fish Farm in Gizycko, Poland (IRS in Olsztyn, Poland) for culture continuation. The other batches from all the experimental groups of fish were transported to experimental aquaria in a rearing system at the Department of Ichthyology, University of Warmia and Mazury in Olsztyn, Poland. The fish were reared for cytogenetic and molecular analysis for 3 months. Larval feeding was initiated on the ninth day post-hatch in all the experimental groups of fish.

2.2. Cytogenetic and molecular analysis

Twenty randomly sampled fish from the gynogenetic haploid, gynogenetic diploid, triploid control and diploid control groups were sacrificed prior to seven days PH in order to determine their ploidy. Chromosomes were prepared according to Woznicki et al. (1998) with modifications (the gill epithelium was used instead of the head kidney). At least five well-spread metaphase plates from each specimen were analyzed.

Fin clips were sampled from a female — the mother of the gynogenetic offspring, and a male — the sperm donor. During the experiment, the fin clips were sampled randomly from specimens of all the experimental groups for molecular analysis. All dead fish from gynogenetic haploid and gynogenetic diploid groups were also analyzed to determine their parentage using microsatellite DNA analysis. Genetic analysis was conducted on: 30 specimens from "diploid control" group, 30 specimens from "triploid control" group, 30 specimens from "haploid" group, and 168 specimens from "gynogenetic" group. Genomic DNA of the female — the mother of the gynogenetic offspring, and the male — the sperm donor, was extracted using Chelex 100 (Walsh et al., 1991). Eleven microsatellite loci [*Afu-39*, *Afu-68*, *AfuB-68* (May et al., 1997), *Spl-104*, *Spl-105*, *Spl-113*, *Spl-163*, *Spl-168* (McQuown et al., 2000), *Aox-45* (King et al., 2001) and *AfuG-9*, *Afu-G122* (Welsh and May, 2006)] were amplified for identification of the difference between the female — the mother of the gynogenetic offspring and the male — the sperm donor. The procedure of microsatellites amplification was described by Fopp-Bayat (2009). Aliquots containing PCR products and reaction buffer were electrophoresed using 6% polyacrylamide gel, and DNA bands were visualized by the silver staining method (Tegelström, 1986). Electrophoresis was conducted on a Bio-Rad SequiGen Sequencing Cell-system, and the gel size was 38×30cm. Amplified fragments were sized by comparing migration with two DNA standards: ϕX 174 DNA/*Hinf* I DNA Step Ladder (Promega, Madison, WI, USA) and 25 bp DNA Step Ladder (Promega, Madison, WI, USA). Every gel analyzing sample included two lanes containing the appropriate parental microsatellite PCR amplification products. Specific microsatellite profiles for parents were noted and compared to those from analyzed specimens.

2.3. Gonad examination

For gonad examination, fish were cultured for three years. The animals were measured (total length accuracy ± 1 mm and live weight, accuracy ± 0.01 g), anaesthetized with 2-phenoxyethanol, and euthanized by decapitation; the entire gonad were then sampled and fixed in Bouin's fluid. The gonads were processed for routine paraffin embedding, and 6-μm thick sections were stained with the haematoxylin and eosin method (Zawistowski, 1986). The gonad histology was analyzed in total 50 fish from the gynogenetic diploid group and in total 30 specimens from the diploid control group.

3. Results

Hatching rates of gynogenetic haploid group 14% while hatching rates of gynogenetic diploid group 22% (Table 1).

All microsatellite loci were amplified for the parents of the gynogenetic group of fish and three primers pair (*Afu-68*, *Spl-113* and *Spl-168*) were classified as reliable tools for identification of gynogenetic offspring. From the three loci (*Afu-68*, *Spl-113* and *Spl-168*), two (*Afu-68* and *Spl-168*) were selected for gynogenetic offspring identification. As a result, 30 fish from the diploid control group, 30 fish from the triploid control group, 60 fish from the gynogenetic haploid group, and 238 fish from the gynogenetic diploid group were screened using microsatellite DNA analysis.

The locus *Spl-168* was the most reliable tool for genetic verification of fish from the present experiment because in this locus the mother of the gynogenetic diploids was characterized by all alleles being different from those of the male sperm donor. Alleles *212* and *224* base pairs (bp) were observed at locus *Spl-168* in the female of Siberian sturgeon (the mother of gynogenetic offspring) while alleles *90* and *114* bp were characteristic for the male sperm donor at the same locus. All verified gynogenetic offspring possessed alleles identical to the female used for meiotic gynogenesis and the three genotypes were observed (212/212, 212/224 and 224/224). The observed genotypes suggested that locus *Spl-168* is situated in an interstitial position of chromosome. At locus *Afu-68* alleles: *228*, *156* and *152* were observed in the female of Siberian sturgeon while alleles *228*, *244*, *216* and *152* were characteristic for the male sperm donor. In the gynogenetic group of fish only one genotype (*228/150/152*) were observed. Locus *Afu-68* was identified as a duplicated locus and the observed genotype suggested that it is located on the distal end of a chromosome arm.

Based on the cytogenetical and molecular analysis in "haploid" group of fish, 23% of spontaneous diploid gynogens, 70% of haploid gynogens and 7% normal diploid fish were detected. Among dead fish in the haploid groups, morphologically abnormal larvae were observed. The cytogenetic analysis shows that all the analyzed gynogenetic diploids possessed ~240 chromosomes in metaphase plates (from 231 to 240 chromosomes were noted in particular methaphases).

In total, 108 gynogenetic specimens of Siberian sturgeon aged 3+ were obtained using the described methods. All gynogenetic specimens were screened using the microsatellite DNA analysis and uniparental inheritance was detected. For gonad examination, 50 fish were exploited and 60 gynogenetic diploids are still being cultured. After the gonad examination in the gynogenetic group of fish, 39 females (81%) and 9 males (19%) were identified. Fig. 1 shows an ovary in a gynogenetic female and testis in a gynogenetic male. In

Table 1
Survival rate (number of specimens and percent) of the diploid control, triploid control, haploid and diploid gynogens of Siberian sturgeon (*Acipenser baeri*).

Experimental group	Egg (number)	Fertilization (%)	Gastrulation (%)	Hatch Larvae (%)
Diploid control	5200	4628 (89)	4004 (77)	3432 (66)
Triploid control	5200	3640 (70)	2860 (55)	2080 (40)
Haploid	5200	3380 (65)	2340 (45)	728 (14)
Gynogen	5200	3224 (62)	2132 (41)	1180 (22)