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Presence of gynogenetic males suggests a female heterogamety in sterlet *Acipenser ruthenus* L.

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ABSTRACT

Investigation of the heterogametic sex in sterlet *Acipenser ruthenus* L. was performed using meiotic gynogenesis and gonadal histology. Eggs from the albino females were activated by UV irradiated sperm of wild colored males and exposed to a heat shock. The resultant fish were all albino and exhibited exclusively maternal inheritance of the microsatellite DNA markers. Cytogenetic analysis indicated that gynogenetic progeny were diploids with 120 chromosomes. Based on the histological analysis, more than 86% of the gynogenetic individuals were found to be females. Moreover, some males (7%), sterile speciemens (3.5%) and fish with unidentified gonads (3.5%) were observed among the gynogenetic fish. Presence of both females and males in the gynogenetic offspring is indicative that the heterogametic sex in sterlets is female.

1. Introduction

Sturgeons (Acipenseridae) are the most valuable fish species and a source of meat and black caviar (Birstein et al., 1998). The high quality and price of sturgeon products have resulted in the overexploitation of natural stocks of sturgeon. Currently, most sturgeon populations are endangered or close to extinction. Preservation of wild stocks and improvement of sturgeon aquaculture will require the application of reproductive technologies including sperm crypreservation (Glogowski et al., 2002), and genome manipulation techniques (Van Eenennaam et al., 1996; Omoto et al., 2005; Flynn et al., 2006; Fopp-Bayat, 2010, Saber and Hallajian, 2014) and transplantation of primordial germ cells (Pšenička et al., 2015) among others.

Sturgeon are the long lived-fish that reach sexual maturity relatively late when compared with other fish species (Hurvitz et al., 2007; Mola et al., 2011). As late-maturing fish generate greater costs of production, it could be profitable to produce all-female sturgeon stocks for caviar aquaculture.

In fish, several techniques are applied to produce monosex fish stocks and most of these utilize information concerning genetic mechanisms of the sex determination process. Investigation into the sex determination systems in fish may be performed with application of gynogenesis which leads to the development of specimens with only maternal inheritance (Devlin and Nagahama, 2002). Induced gynogenesis requires activation of eggs by the UV-irradiated spermatozoa during which the nuclear genome is damaged. Diploidization of the gynogenetically activated eggs is performed using a thermal or hydrostatic pressure shock that inhibits extrusion of the second polar body (meiotic gynogenesis) or inhibits the first cell cleavage (mitotic gynogenesis) and results in the production of heterozygous and homozygous (Doubled Haploids) gynogenotes, respectively (Pandian and Koteeswaran, 1998). Gynogenetic experiments have been performed to describe genetic sex determination systems in several species from various fish families (Dabrowski et al., 2000; Lin et al., 2001; Campos-Ramos et al., 2001, 2003, among others).

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The most common mechanisms of genetic sex determination include male heterogamety in mammals (XX female; XY male) and female heterogamety in birds (ZZ male; ZW female; Ogata et al., 2003). In fish, several sex determination systems with both male and female heterogamety have been described (Penman and Piferrer, 2008). The presence of females and males among the gynogenetic specimens indicated similarities to the avian ZW/ZZ chromosomal sex determination system with the female heterogamety of several sturgeon species (Van Eenennaam et al., 1999; Omoto et al., 2005; Flynn et al., 2006; Fopp-Bayat, 2010, Saber and Hallajian, 2014). Crossing of gynogenetic females (WW) with normal males (ZZ) should result in the production of only female (ZW) offspring (Van Eenennaam et al., 1999).

Recently, meiotic and mitotic gynogenesis has been applied in sterlet *Acipenser ruthenus* (Fopp-Bayat et al., 2007; Fopp-Bayat and Ocalewicz, 2015, Fopp-Bayat et al., 2017). Although, only inheritance has been confirmed in sterlet gynogenetic individuals, no information concerning the gonadal development in such specimens has been provided. The main objective of the present study was, therefore, the application of gynogenesis and gonadal histology in order to determine the heterogametic sex in sterlets.

2. Material and methods

2.1. Fish reproduction and meiotic gynogenesis

This study was conducted in strict accordance with the recommendations in the Polish ACT of 21 January 2005 on Animal Experiments (Dz. U. of. 2005, No 33, item 289). The protocol was approved by the Local Ethical Committee for the Experiments on Animals of the University of Warmia and Mazury in Olsztyn, Poland (Permit Number: 75/2012).

Gamete donors for this study were derived from the sturgeon broodstocks maintained at the Wasosze Fish Farm, Poland. Three albino sterlet females at the age of 6 years with the average body weight of 3000 g (Female 1, Female 2 and Female 3), one wild colored sterlet male (Male 1; sperm donor) with a body weigth of 3000 g and one wild colored bester (*Huso huso* × *Acipenser ruthenus*) male (Male 2; seminal fluid donor) at the age of 14 years with the body weight of 8000 g were used to provide gametes and seminal fluid. Collection of gametes and assessment of quality were performed as previously described (Fopp-Bayat et al., 2007; Fopp-Bayat and Ocalewicz, 2015). From each female a portion of 200 g of ovulated eggs was obtained. Approximately 160 and 60 ml of sperm from the besters and sterlets (respectively) were obtained during a single suction performed with a syringe. Milt from the bester was centrifuged and its seminal fluid transferred to a plastic falcon to be used further for dilution of sterlet spermatozoa. Diluted sperm (1:9) was exposed to UV-radiation at a dose of 135 J/m^{-2} (Fopp-Bayat et al., 2007). Sterlet eggs in three portions of 100 g from Female 1, Female 2 and Female 3 were activated with the irradiated wild colored sterlet spermatozoa and three groups (Gyno 1, Gyno 2 and Gyno 3, respectively) of the gynogenetic haploid zygotes were produced. To avoid photoreactivations, the sperm and embryos were stored in the dark for 5 h. Additionally, three control groups (Control 1, Control 2 and Control 3) were provided by independent fertilization of ova from each female with the untreated sterlet sperm. The egg de-sticking was conducted using NaCl-tannic acid solutions (Feledi et al., 2011).

At 18 min post-activation, a heat shock of 34 °C was applied for 2 min to block the second polar body extrusion and to restore diploid state in the meiotic gynogenetic zygotes (Fopp-Bayat et al., 2007). The incubation temperature of 15 °C was maintained between insemination and exposure to heat shock.

2.2. Eggs incubation and fish rearing

The eggs were incubated in McDonald Jars at the temperature of 15 °C. The survival in the experimental groups was monitored and recorded at the different stages of development: fertilization, neurulation, hatching, larvae, juvenile until 19 months.

At 5–6 day post-fertilization (dpf) the number of normal, free-swimming larvae was counted for four treatments: Gyno 1. Gyno 2, Control 1 and Control 2, and the remainder of the abnormal larvae and unhatched eggs were removed from the tank and counted. Hatched larvae (without abnormal specimens) from each of the individual treatments (Gyno 1, Gyno 2, Control 1 and Control 2) were transferred from the hatching system to a rearing RAS system (16 °C), where these were kept until sampling for the molecular, cytogenetic and histological analysis. The gynogenetic individuals from Gyno 1 and Gyno 2 groups were pooled and maintained in one tank as “the Gynogenetic group”. The same procedure was applied for the fish from the control groups 1 and 2. Gynogenetic and control fish were reared in two separate tanks in the RAS system for 19 months post fertilization (mpf). The rearing procedure was described by Fopp-Bayat and Ocalewicz (2015), and Laczynska et al. (2017).

2.3. Molecular verification of gynogenetic diploids

Fin-clips from the parental individuals and 54 randomly selected 19-month-old fish from the gynogenetic group and 54 control specimens were sampled and stored in 96% ethanol. Genomic DNA was isolated using a Sherlock DNA purification kit (A&A Biotechnology, Poland) according to the manufacturer’s procedure. Three microsatellites: *Afu-68*, *AfuB-68* and *Spl-163* (May et al., 1997; McQuown et al., 2000) were amplified and genotyped as described by Fopp-Bayat and Ocalewicz (2015). The lengths of the amplified DNA fragments were determined using an Applied Biosystems 3130 Genetic Analyser with the GeneScan 500 [LIZ] size standard (Life Technologies, California, USA). The size of the DNA fragments and alleles were determined using the GeneMapper 4.0 and the Genetic Analyser software (Life Technologies, California, USA) according to the manufacturer’s recommendations.