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COMMUNICATION

Evidence of Female Heterogamety in Largemouth Bass, Based on Sex Ratio of Gynogenetic Progeny

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Abstract

Meiotic gynogenetic progeny in largemouth bass *Micropterus salmoides* have been obtained by inseminating largemouth bass eggs with UV-irradiated sperm from white bass *Morone chrysops* or striped bass *Morone saxatilis* and suppressing the second meiotic division by hydrostatic pressure. The sex composition of gynogenetic progeny was determined by dissection or ultrasound investigation of 1-year-old fish. Among the 21 fish analyzed, 7 fish (33.3%) were male and 14 fish (66.7%) were female. The presence of males in meiotic gynogenetic progeny suggests the existence of female heterogamety (WZ females, ZZ males) in largemouth bass.

The largemouth bass *Micropterus salmoides* is the most popular game fish in the United States. Females of this species exhibit faster growth and attain larger size than do the males (Padfield 1951). Manipulating the sex ratio of largemouth bass populations is of great interest because of its potential to produce higher ratios of females or monosex female populations for the purposes of creating larger and faster growing fish for aquaculture and trophy sport fishing. Several studies have evaluated hormonal sex reversal with the goal of shifting sex ratios under influence of androgens or estrogens in largemouth bass (Garrett 1989; Porter 1996; Al-Ablani and Phelps 2001; Arslan et al. 2009). Induced triploidy has also been tested in this species for reproduction control and obtaining potentially larger fish (Garrett et al. 1992; Fries et al. 2002; Neal et al. 2004).

The optimal method for production of monosex fish populations involves crossing previously obtained sex-reversed fish with normal breeding fish (Purdom 1993; Donaldson 1996). The scheme of crosses directed to production of monosex progenies depends on the type of heterogamety in given fish species. In fishes, both male (XY males, XX females) and female (WZ females, ZZ males) heterogamety are described; sometimes different types of heterogamety are revealed in closely related species (Dabrowski et al. 2000; Devlin and Nagahama 2002). Until now, there were no data on sex determination mechanism in largemouth bass.

One of the basic methods for determining the type of heterogamety in fish is based on sex composition of gynogenetic progenies. In the case of male heterogamety (XY/XX), gynogenetic progenies usually consist of females only; the presence of both females and males indicates female heterogamety (WZ/ZZ; Thorgaard 1983; Van Eenennaam et al. 1999; Devlin and Nagahama 2002). This article presents data on sex composition of a gynogenetic progeny in largemouth bass that suggests the existence of female heterogamety in this species.

METHODS

Experiments on production of gynogenetic progeny in largemouth bass were conducted at the facilities of J. M. Malone and Son, Inc. (Lonoke, Arkansas) in April 2009. To induce gynogenetic development, largemouth bass eggs were inseminated

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with UV-irradiated sperm from white bass *Morone chrysops* or striped bass *Morone saxatilis*. For production of gynogenetic diploids, the second meiotic division in eggs was suppressed by application of hydrostatic pressure.

Ovulation was induced in mature largemouth bass females by a single intramuscular injection of human chorionic gonadotropin (HCG, 4,000 IU/kg), after which they were placed into separate 246-L fiberglass tanks. The time between hormonal injection and ovation was 36–40 h. White bass and striped bass males were each given one intramuscular HCG injection (1,000 IU/kg). The sperm obtained was irradiated based on techniques previously used for white bass sperm irradiation in experiments on induced gynogenesis in black crappie *Pomoxis nigromaculatus* (Gomelsky et al. 2000). Specifically, sperm was irradiated at a dose of 1,000 J/m^2 with a FisherBiotech UV microprocessor-controlled Crosslinker (FB-UVXL-1000; Fisher Scientific). For irradiation, 2 mL of sperm diluted 10-fold with 0.85% NaCl solution was placed in 6-cm-diameter glass Petri dishes. Parameters of pressure shock for suppression of the second meiotic division in largemouth bass were chosen based on published data from experiments on induced triploidy (Garrett et al. 1992; Neal et al. 2004); pressure shock of 8,000 psi for 1 min was initiated 5 min after the eggs were inseminated with irradiated sperm. The fertilized eggs were placed into a 1-L hydrostatic pressure chamber and the desired pressure was achieved using a 20 ton hydraulic jack.

In total, ovulated eggs were produced from 12 females. Nine trials on insemination of eggs with irradiated sperm and application of hydrostatic pressure were performed; in some trials eggs from several females were mixed. After pressure treatment, eggs were put into separate 246-L fiberglass tanks for embryo incubation and hatching of larvae. The resulting swim-up gynogenetic larvae were reared in the same tanks in which embryos had been incubated and fed with live zooplankton supplied from filtered pond water for 30 days after hatching.

Two hundred and sixty gynogens survived to the fry stage. Sixty gynogenetic fish were randomly selected and reared in 1 m^3 tanks at a separate facility on live foods (minnow and bluegill) for 1 year. The remaining 200 gynogenic fish were moved to a different facility for hormonal sex reversal by androgen treatment; the results of that study will be reported in a separate publication.

Twenty-one gynogenetic fish from the untreated group survived to the age of one year. On April 19, 2010, 15 fish were lost due to equipment failure; they were dissected for sex determination by macroscopic observation of gonads and their total length was recorded. The six surviving gynogenetic fish from this group were sexed 3 days later by ultrasound investigation using Tela-Vet 1000 equipped with a 5–8 MHz linear transducer (Classic Medical, Tequesta, Florida). Ultrasound is a noninvasive method that has been shown to accurately determine the sex in several species of fish (Martin et al. 1983; Reimers et al. 1987; Shields et al. 1993; Blythe et al. 1994; Colombo et al. 2004; Masoudifard et al. 2011). The fish were anesthetized with quinaldine sulfate and placed in a pan of water. Each fish was held submerged in the water against a styrofoam pad to prevent double imaging while the ultrasound was performed. The probe was placed near the abdomen to determine the sex of each fish. Female fish were identified by the presence of eggs in the ovarian sacs; males had no eggs but showed the presence of testes structures.

RESULTS

From 15 dissected fish, 7 fish were male and 8 fish were female. Males and females had well-developed maturing testes and ovaries, respectively, with normal gonad morphological structure and color for this species. Mean lengths (±SD) of males and females were 27.09 (± 1.17) cm and 25.44 (± 0.95) cm, respectively.

The six remaining 1-year-old fish were identified as females by ultrasound investigation. In the summer of 2011, at 2 years of age, further observations of these fish confirmed their identification as females; when pressed on the abdomen, no sperm was expressed and other traits typical for females were observed (e.g., swollen abdomen).

In total, of the 21 gynogenetic fish analyzed, 7 (33.3%) were male and 14 (66.7%) were female.

DISCUSSION

The use of heterologous sperm, instead of sperm from the same species, is a very effective method for experiments on diploid gynogenesis in fish. If hybrids between two species are nonviable, survivors are exclusively of gynogenetic origin (Chourrout 1987; Mims et al. 1997; Dabrowski et al. 2000; Gomelsky et al. 2000). Preliminary experiments conducted at the Aquaculture Research Center of Kentucky State University in 2008 have shown that white bass spermatozoa were capable to fertilizing largemouth bass eggs, but the hybrids obtained were nonviable and perished before or soon after hatching. Since the present study used only irradiated sperm of *Morone* species for induction of gynogenetic development in largemouth bass eggs, all obtained larvae could be only of gynogenetic origin. In an earlier study, irradiated sperm of white bass was used for induced gynogenesis in another species of the family Centrarchidae, black crappie (Gomelsky et al. 2000).

Usually the presence of males in meiotic gynogenetic progenies suggests the existence of female heterogamety (WZ/ZZ) in given species. Other possible factors such as autosomal and environmental influences on sex determination can also result in appearance of gynogenetic males (Devlin and Nagahama 2002; Flynn et al. 2006). Males in meiotic gynogenetic progenies were described in plaice *Pleuronectes platessa* (Purdom and Lincoln 1973), blue tilapia *Oreochromis aureus* (Penman et al. 1987), common barbel *Barbus barbus* (Castelli 1994), muskellunge *Esox masquinongy* (Dabrowski et al. 2000), white sturgeon *Acipenser transmontanus* (Van Eenennaam et al. 1999), shortnose sturgeon *A. brevirostrum* (Flynn et al. 2006), and

some other fish. The frequency of heterozygotes in a meiotic gynogenetic progeny obtained from a female that is heterozygous for some gene depends on the frequency of crossing over between the gene and centromere during prophase of the first meiotic division; the proportion of heterozygotes increases with increasing crossing over frequency (Thompson 1983; Thorgaard et al. 1983). Similarly, when meiotic gynogenetic progeny is obtained from heterogametic female (WZ), the resulting sex ratio depends on the frequency of crossing over between a sex-determining gene and centromere. If WW fish are viable, then in the absence of crossing over, the meiotic gynogenetic progeny obtained from WZ female will consist of WW females (so called super-females) and ZZ males with ratio 1:1. Crossing over between the sex-determining gene and the centromere will result in the appearance of WZ females and, with increasing recombination rates, the phenotypic sex ratio will be shifted towards prevalence of females. If all three categories are viable, the proportion of ZZ males should be approximately equal to the proportion of WW super-females (Van Eenennaam et al. 1999; Devlin and Nagahama 2002).

One third (33%) of the meiotic gynogenetic progeny of largemouth bass were males. This is similar to the proportion of males in meiotic gynogenetic progenies observed in plaice (37%; Purdom and Lincoln 1973), muskellunge (40%; Dabrowski et al. 2000), and shortnose sturgeon (35%; Flynn et al. 2006). If WW super-females in largemouth bass are viable, their proportion in gynogenetic progeny should be approximately 33%, the same as proportions of ZZ males and WZ females. The WW super-females are of special interest with regard to possible genetic sex regulation, since crossing them with normal ZZ males should produce all-female progeny (WZ). Crossing of WW super-females with phenotypic WW males obtained by sex reversal will allow for production of WW super-females in mass quantities.

The existence of female heterogamety in largemouth bass should be confirmed later by identification of WW fish in test crosses.

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