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Ploidy of Backcross Hybrids of Largemouth Bass and Smallmouth Bass

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Abstract.—Interspecies hybridization in fish may trigger the transformation of oogenesis in hybrid females, resulting in formation of eggs with unreduced diploid chromosome number. This phenomenon is revealed by the triploidy of backcross offspring obtained from crossing F_1 hybrid females with males of either parental species. To determine whether the F_1 hybrids of largemouth bass *Micropterus salmoides* and smallmouth bass *M. dolomieu* produce diploid eggs, we designed a study for evaluating the ploidy of backcross offspring (F_1 hybrid females × largemouth bass males). Fish ploidy was determined by flow cytometry measurement of DNA content. The results of flow cytometry analysis showed that backcross hybrids had the same amount of DNA as largemouth bass and F_1 hybrids, which suggests diploidy (2n) of backcross hybrids. This indicates that the F_1 hybrid females between largemouth bass and smallmouth bass generate haploid eggs and do not experience the transformation of oogenesis that has been described in several other distant fish hybrids. Results of this study will be used to evaluate hybrids of largemouth bass and smallmouth bass as potential candidates for aquaculture.

Interspecies hybridization frequently impacts the reproductive performance of fish. Incompatibility of the haploid chromosome sets of two different species results in disturbances in the process of meiosis, which can lead to complete or partial sterility of F_1 hybrids (Chevassus 1983; Purdom 1993). Sometimes, interspecies hybridization in fish triggers the transformation of oogenesis, which can overcome incompatibility of chromosome sets from two species. This transformation is usually observed in hybrid females and results in formation of eggs with an unreduced diploid chromosome number. This phenomenon was described for several interspecies F_1 fish hybrids belonging to different taxonomic groups, including hybrids of silver crucian carp *Carassius auratus gibelio* and common carp *Cyprinus carpio* (Cherfas al. 1994), brown trout *Salmo trutta* and Atlantic salmon *S. salar* (Johnson and Wright 1986; Galbreath and Thorgaard 1995; Galbreath et al. 1997), and pumpkinseeds *Lepomis gibbosus* and green sunfish *L. cyanellus* (Dawley et al. 1985; Dawley 1987). Cherfas et al. (1994) and Shimizu et al. (2000) showed that generation of unreduced diploid eggs by hybrid females results from the occurrence of premeiotic endomitosis (i.e., doubling of chromosomes without cytokinesis) in early oogenesis; the resulting tetraploid oocytes undergo two normal, consecutive meiotic divisions. Production of unreduced diploid eggs by hybrid females is usually revealed by the triploidy of backcross offspring obtained from crossing F_1 hybrid females with males of either parental species.

The present study was conducted to determine the ploidy of backcross hybrids produced by crossing F_1 hybrid females (female largemouth bass *Micropterus salmoides* × male smallmouth bass *M. dolomieu*) with male largemouth bass, which would allow determination of whether F_1 hybrids produce diploid eggs. Currently, studies to evaluate F_1 hybrids of female largemouth bass and male smallmouth bass as possible candidates for aquaculture are being conducted at the Aquaculture Research Center, Kentucky State University, Frankfort.

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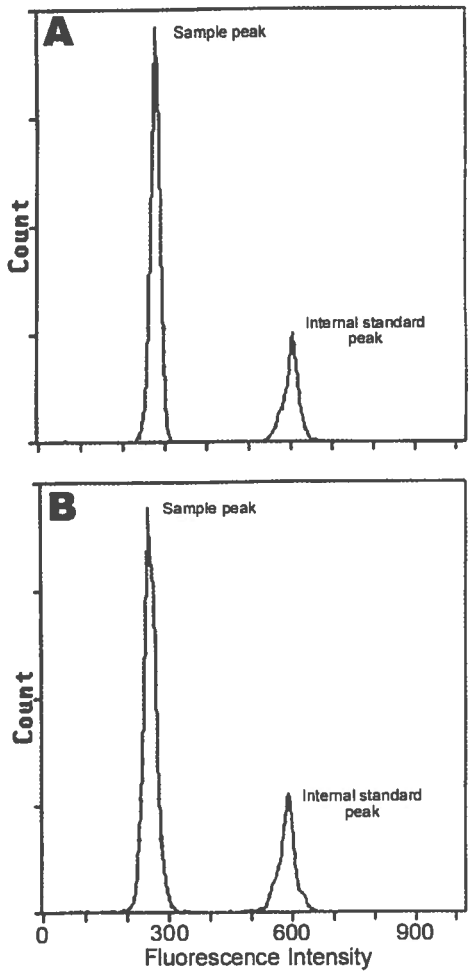

FIGURE 1.—Representative fluorescence distributions of (A) pure largemouth bass and (B) backcross hybrids produced by crossing F_1 hybrid females (female largemouth bass × male smallmouth bass) with male largemouth bass.

Methods

The ploidy of three groups of fish—largemouth bass (LMB), F_1 hybrids of largemouth bass and smallmouth bass, and backcross (F_b) hybrids (female F_1 hybrids × male largemouth bass)—was determined by flow cytometry measurement of DNA content. The production and rearing of fish progenies were conducted at the Aquaculture Research Center, Kentucky State University. Flow cytometry analysis was performed at the School of Natural Resources, Ohio State University, Columbus.

All three groups of fish were obtained by induced spawning. To obtain pure largemouth bass and F_1 hybrids, largemouth bass eggs were fertilized with sperm of largemouth bass and smallmouth bass, respectively. To obtain F_b hybrids, eggs produced by mature F_1 hybrid females were fertilized with largemouth bass sperm. Two-year-old female and male largemouth bass used in crosses were purchased at Mayer's Fish Farm, Bardstown, Kentucky. The mean weight (±SD) of largemouth bass females was 938 ± 208 g and that of males was 884 ± 173 g. Male smallmouth bass (544 ± 312 g) were collected by electrofishing in Dale Hollow Lake, Kentucky; the age of these fish was not determined. The F_1 females (female largemouth bass × male smallmouth bass) were obtained from previous experiments on hybrid production by induced spawning at the Aquaculture Research Center. The mean weight of 2-year-old F_1 females used in crosses was 124 ± 40 g; until sexually mature, the females were raised in earthen ponds with a recirculating water system.

To stimulate ovulation, we injected mature females with human chorionic gonadotropin (HCG) at a dosage of 4,000 IU per kilogram of body weight (IU/kg); to stimulate spermiation, we injected mature males with HCG at a dosage of 2,000 IU/kg. Eggs were stripped 30–36 h after injection. Largemouth bass and smallmouth bass males did not release sperm by stripping. Therefore, males were killed, and their testes were dissected and washed with 0.85% saline solution; the resulting sperm suspension was used for egg fertilization. Eggs were fertilized in glass dishes or metal bowls. Small batches of eggs were incubated in aerated tanks; large batches of eggs were incubated in McDonald jars. After transition to active feeding, larvae were fed with brine shrimp *Artemia* spp. nauplii and then trained to accept artificial feed.

Flow cytometry analysis of DNA content was performed in F_b hybrids obtained in two crossing experiments (designated as experiments 1 and 2). In experiment 1, eggs taken from one F_1 female were fertilized with sperm taken from one largemouth bass male; larvae obtained in experiment 1 were fixed for flow cytometry analysis at 7 d after hatching. In experiment 2, a mixture of eggs taken from three F_1 females were inseminated with a mixture of sperm taken from two largemouth bass males; samples of blood from fish obtained in experiment 2 were taken at 70 d after hatching, when the mean total length (±SD) of fingerlings was 7.9 ± 1.1 cm. Samples of pure largemouth bass and F_1 hybrids of the corresponding ages were taken from large batches of fish obtained by fertilization

TABLE 1.—Flow cytometry determination of DNA content in pure largemouth bass (LMB), F_1 hybrids (female largemouth bass × male smallmouth bass), and backcross (F_b) hybrids (female F_1 hybrids × male largemouth bass). The mean (±SD) fluorescence intensity of each sample peak, mean intensity of each internal standard peak (derived from rainbow trout red blood cells), mean relative DNA content (ratio of sample peak intensity to standard peak intensity), and ploidy level are shown. Relative DNA content was not significantly different among groups within experiments.

Fish	Number of analyzed fish	Intensity of sample peak	Intensity of standard peak	Relative DNA content	Ploidy
		Experiment 1			
LMB	4	253 ± 5	625 ± 19	0.40 ± 0.02	2n
F_1 hybrids	3	262 ± 11	632 ± 9	0.41 ± 0.01	2n
F_b hybrids	10	263 ± 11	635 ± 14	0.41 ± 0.01	2n
		Experiment 2			
LMB	5	253 ± 16	596 ± 16	0.42 ± 0.02	2n
F_1 hybrids	5	247 ± 5	588 ± 7	0.42 ± 0.01	2n
F_b hybrids	20	247 ± 8	590 ± 8	0.42 ± 0.01	2n

of largemouth bass eggs with sperm of largemouth bass and smallmouth bass, respectively.

The flow cytometry technique that we employed was similar to that described by Gomelsky et al. (2000). Larvae or blood samples were fixed in 5% DMSO solution in Isoton II (Coulter Balanced Electrolyte Solution, Beckman-Coulter, Miami Florida) and kept frozen until analysis. Samples were transferred into 12-mm × 75-mm, sterile plastic tubes with snap caps (Fisher Scientific, Pittsburgh, Pennsylvania). The tubes contained 800 μL of propidium iodide stain and 10 μL of blood from rainbow trout *Oncorhynchus mykiss* (internal standard). The solution was incubated overnight at 4°C, gently syringed, and filtered through 60-μm Nitex filters. Flow cytometry analysis was performed on an EPICS Elite flow cytometer (Coulter Corp., Miami, Florida) equipped with a 448-nm, 15-mW, air-cooled Argon laser. A minimum of 10,000 gated cells was collected at a rate of 500 events/s. The propidium iodide signal was measured with a 610-nm-long pass transmission filter and was represented in linear mode. Single-parameter statistics were generated by Elite Workstation Software (Coulter Corporation 1996). Relative DNA content was calculated and expressed as the ratio of the fluorescence intensity of the sample peak to the fluorescence intensity of the rainbow trout red blood cells (internal standard). The diploid DNA content of rainbow trout is 5.84 pg/cell (Schmidtke et al. 1976). The significance of differences in relative DNA content among the different groups of fish was evaluated by single-factor analysis of variance.

Results

The results of flow cytometry determination of DNA content are presented in Table 1. In both experiments, the relative DNA contents did not differ significantly ($P > 0.05$) among fish of all three groups (LMB, F_1 hybrids, and F_b hybrids) and were consistent with diploidy (2n). Figure 1 shows the similarity of representative fluorescence distributions of largemouth bass (panel A) and F_b hybrids (panel B).

Discussion

Production of F_1 and F_b hybrids of largemouth bass and smallmouth bass and the inheritance of polymorphic proteins in these hybrid forms were described previously by Wheat et al. (1974) and Childers (1975). Data on the ploidy of F_b hybrids of largemouth bass and smallmouth bass have never been reported. The purpose of the present study was to determine the ploidy of F_b hybrids in connection with the possibility of F_1 hybrid females producing eggs with an unreduced diploid chromosome number.

The results of the flow cytometry analysis showed that F_b hybrids had the same amount of DNA as largemouth bass and F_1 hybrids, corresponding to a diploid level. This indicates that F_1 hybrid females produce haploid eggs and do not undergo the transformation of oogenesis that has been described in several other distant fish hybrids. Apparently, the incompatibility of haploid chromosome sets between largemouth bass and smallmouth bass is insufficient to trigger this transformation.

The results of our study will be used in the evaluation of F_1 hybrids of largemouth bass and smallmouth bass as potential candidates for aquaculture. It is known that rearing of fertile distant hybrids may result in genetic contamination of parental species, since escaped hybrids may cross with fish

from natural populations. If F_1 females produced diploid eggs, the F_b progeny would have been represented by sterile triploids. However, the diploidy of backcross hybrids of largemouth bass and smallmouth bass revealed in the present study indicates that these fish will be fertile, and therefore the rearing of F_1 hybrids may have negative genetic consequences.

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