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COMMUNICATION

Koi × Goldfish Hybrid Females Produce Triploid Progeny when Backcrossed to Koi Males

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Abstract

Hybrids of koi (an ornamental variant of the common carp *Cyprinus carpio*) and goldfish *Carassius auratus auratus* were produced by artificial spawning. All 3-year-old F_1 hybrid males examined were sterile, whereas some F_1 hybrid females were fertile and produced eggs after hormonal injection. Backcross progeny were obtained by using intact koi sperm to inseminate eggs from F_1 hybrid females; gynogenetic progeny were obtained by inseminating eggs from F_1 hybrid females with koi sperm that was genetically inactivated by ultraviolet irradiation. Flow cytometric analysis of DNA content indicated that the backcross progeny were triploid, while the gynogenetic progeny, pure koi, pure goldfish, and F_1 hybrids were all diploid. The triploidy of backcross progeny obtained without application of any treatment to the eggs demonstrates that the koi × goldfish hybrid females produce diploid eggs.

The common carp *Cyprinus carpio* and goldfish *Carassius auratus auratus* are nonnative cyprinid species that naturally reproduce in North America (Panek 1987; Schofield et al. 2005). Ornamental forms of common carp (i.e., koi) and goldfish are popular decorative fish in many countries throughout the world, including the United States. Goldfish are also raised in the United States for use as bait fish and as forage in fish hatcheries (Schofield et al. 2005).

Common carp naturally hybridize with goldfish in their native range and in many areas where both species have been introduced (Trautman 1957; Bardach et al. 1972; Taylor and Mahon 1977; Pullan and Smith 1987; Schofield et al. 2005). Cases of hybridization between koi and goldfish have also been described in the aquarium fish and koi hobbyist literature (Hemdal 2003; Muha 2007). Hybrids of common carp or koi and goldfish have been produced artificially for different types of studies (e.g., Suzuki 1962; Hedrick et al. 2006). The morphometric and meristic characteristics of common carp (or koi) × goldfish hybrids have been widely reported (Taylor and Mahon 1977; Pulan and Smith 1987; Schofield et al. 2005). In contrast to many interspecies fish hybrids, the hybrids of common carp and goldfish are not sterile, and the backcrossing of hybrids with parental species has been reported (Trautman 1957; Bardach et al. 1972).

Previous studies on hybridization of common carp with two other subspecies of *C. auratus* (Japanese crucian carp *C. auratus cuvieri* and silver crucian carp *C. auratus gibelio*) revealed that F_1 hybrid females produced triploid progeny when backcrossed to males of parental species (Ojima et al. 1975; Cherfas et al. 1994). Triploidy of backcross hybrids resulted from the diploidy of eggs produced by hybrid females. The present study was performed to investigate whether koi × goldfish hybrid females have the same feature. For this purpose, the ploidy of backcross progeny obtained from hybrid females was determined.

METHODS

Fish spawning and rearing were conducted at the Aquaculture Research Center, Kentucky State University, Frankfort. Koi × goldfish hybrids were produced by artificial spawning in spring 2008. To induce final oocyte maturation and ovulation in females and spermiation in males, broodfish were given intramuscular injections of carp pituitary extract (Sigma Chemical, St. Louis, Missouri) at a concentration of 3 mg/kg of body weight. Males received a single injection approximately 16 h before stripping of sperm. Females received two injections (10% and 90% of the total dose) 12 h apart. After injections, broodfish were kept in tanks at a water temperature of 21.5°C; ovulation was observed 11–12 h after the resolving injection. Eggs and sperm were collected from broodfish by hand stripping. A mixture of eggs from six koi females of different colors (white–red, white–yellow, and

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solid white) was fertilized with a mixture of sperm taken from two solid-red goldfish males. Eggs were artificially inseminated in plastic bowls according to standard techniques for common carp breeding (Horvath et al. 2002) and were treated with a solution of water : pasteurized cow's milk (volumetric ratio = 8:1) to remove adhesiveness. Embryos were incubated in McDonald hatching jars.

The resulting F_1 hybrids were raised in 20-m^3 outdoor tanks during their first summer and then were transferred to earthen ponds, where they were reared for an additional 2.5 years. The functional fertility of 3-year-old hybrids was investigated in spring 2011. For this purpose, 10 selected hybrid females and 20 hybrid males were injected with carp pituitary extract by using the dosages and methodology described above for koi and goldfish breeders.

After hormonal injection, hybrid males did not release any sperm. To determine whether the testes of hybrid males contained any viable spermatozoa, we sacrificed the fish, removed their testes, and thoroughly dissected and washed the testes with a saline solution (0.85% NaCl). The resulting suspension was used for insemination of koi eggs.

Five koi × goldfish hybrid females produced ovulated eggs after hormonal injection and were used in crosses. Two separate groups of backcross progeny were obtained by crossing F_1 hybrid females with koi males. Backcross progeny group 1 was obtained by using sperm from a single koi male to inseminate a mixture of eggs collected from four hybrid females. Backcross progeny group 2 was produced by crossing a single hybrid female with a single koi male. Gynogenetic progeny were also obtained by using genetically inactivated (by ultraviolet [UV] irradiation) koi sperm to inseminate eggs from the same F_1 hybrid female that was used to produce backcross progeny group 2. Sperm was irradiated with a UV crosslinker (FB-UVXL-1000; Fisher Scientific) at 4,000 J/m^2. This dosage of UV irradiation was chosen based on previously performed experiments involving the induction of gynogenesis in koi (Alsaqufi 2011). Before irradiation, the sperm was diluted (1:9) in a saline solution (0.85% NaCl). The backcross progeny groups and the gynogenetic progeny were obtained without application of any treatment to the eggs. After transition to active feeding, the larvae were stocked in 20-m^3 outdoor tanks for rearing.

Ploidy of 4-month-old backcross progeny (mean total length ± SD = 13.8 ± 0.85 cm) and gynogenetic progeny (12.8 ± 0.75 cm) was determined by flow cytometric analysis of DNA content. The analyses were performed at AquaBounty Canada, Inc., Prince Edward Island, by using a Becton Dickinson (BD) FACSCalibur flow cytometer. The ploidy of some koi, goldfish, and F_1 hybrids was also analyzed for comparison. Instrument quality control for DNA quantitation was performed using CellQuest Pro software and DNA QC particles (BD, catalog number 349523) to assess resolution and linearity. Blood samples were drawn from each fish by caudal venipuncture and were collected in 3.0-mL Vacutainer tubes containing lithium heparin (BD, catalog number 366667). For each sample, two drops of heparinized blood from a syringe were collected in 500 μL of sheath fluid (BioSure, catalog number 1019). An 80-μL quantity of the blood–sheath fluid mixture was stained in 500 μL of propidium iodide solution containing a detergent for lysing (BioSure, catalog number 1021) along with 40 μL of chicken red blood cells (BioSure, catalog number 1005) as an internal staining control. Samples were incubated in propidium iodide solution in the dark for 10 min prior to analysis. For each sample analyzed, 10,000 events were recorded and the relative DNA content was determined as the ratio of sample fluorescence peak intensity to internal standard (i.e., chicken red blood cells) fluorescence peak intensity.

RESULTS

The koi × goldfish hybrids exhibited dark coloration that is typical of wild-type common carp and goldfish. After eggs from female koi were mixed with the suspension of dissected testes from F_1 hybrid males, examination of eggs under a dissecting microscope indicated that none of the eggs was fertilized.

The results of ploidy analysis for backcross progeny, gynogenetic progeny, the parental species, and the F_1 hybrids are

TABLE 1. Ploidy of koi, goldfish, F_1 hybrids, and backcross and gynogenetic progeny groups as determined by flow cytometric analysis. The relative DNA content was calculated as the ratio of sample fluorescence peak intensity to internal standard fluorescence peak intensity.

Group	Number of fish analyzed	Relative DNA content		Fish ploidy
		Mean (SD)	Range	
Koi	4	2.56 (0.04)	2.53–2.62	2n
Goldfish	2	2.52	2.51–2.52	2n
F_1 (♀ koi × ♂ goldfish)	10	2.56 (0.05)	2.49–2.60	2n
Backcross progeny group 1 (♀ F_1 × ♂ koi)	15	3.75 (0.08)	3.61–3.84	3n
Backcross progeny group 2 (♀ F_1 × ♂ koi)	12	3.84 (0.07)	3.73–3.96	3n
Gynogenetic progeny (♀ F_1 × ♂ koi [inactivated sperm])	12	2.51 (0.04)	2.43–2.59	2n

presented in Table 1. The mean relative DNA content was similar for koi, goldfish, F_1 hybrids, and gynogenetic progeny and varied from 2.51 to 2.56. The mean relative DNA content was 3.75 in backcross progeny group 1 and 3.84 in backcross progeny group 2. The ratio of DNA content in backcross progeny to the DNA content in the other groups was approximately 1.5, indicating that the backcross progeny were triploid (3n) fish, whereas the gynogenetic progeny, parental species, and F_1 hybrids were all diploid (2n).

DISCUSSION

The results of the present study reveal the functional genetic sterility of F_1 males produced by the hybridization of koi and goldfish. These males developed testes, but their gonads did not contain spermatozoa with the capability of fertilizing eggs. Sterility of goldfish × common carp hybrid males was also reported by Yamaha et al. (2003).

Flow cytometric analysis indicated that the DNA content in koi and goldfish was similar. These results concur with data reported by Ohno et al. (1967), who indicated that common carp and goldfish had comparable amounts of DNA comprising 50–52% of the typical DNA content in placental mammals. Similar mean values of relative DNA content were observed in F_1 hybrids, whereas backcross progeny appeared to be triploid.

The triploidy of backcross progeny obtained from hybrid females (without application of any treatment to eggs) indicates that these females produced diploid eggs. Diploidy of eggs from hybrid females resulted in diploid gynogenetic progeny, which were produced by use of genetically inactivated spermatozoa chromosomes but without application of any treatment to the eggs. Hybrid females obtained by crossing common carp males with females of two other *C. auratus* subspecies (Japanese crucian carp and silver crucian carp) have previously been shown to produce diploid eggs (Ojima et al. 1975; Cherfas et al. 1994). The results of the present study demonstrate that the same phenomenon is observed in hybrids obtained from two popular ornamental fishes, the koi and goldfish. Specifically, the hybrid females producing diploid eggs were obtained by crossing koi females with goldfish males.

The ability of interspecies F_1 hybrids to produce diploid eggs has been described for several other fish taxa in addition to *Carassius* and *Cyprinus*; these include hybrids of the brown trout *Salmo trutta* and Atlantic salmon *S. salar* (Johnson and Wright 1986; Galbreath and Thorgaard 1995) and hybrids of the pumpkinseed *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.

The koi × goldfish hybrids had a color pattern that is typical of wild-type common carp and goldfish, which indicates that the alleles causing melanin depigmentation in parental forms are not expressed in the hybrids. The analysis of color inheritance in hybrids will be presented in a separate publication.

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