Fertility of Triploid Backcross Progeny, (Gengoroubuna Carassius auratus cuvieri ♀ × Carp Cyprinus carpio ♂)F1 ♀ × Carp or Gengoroubuna ♂

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Abstract  The fertility of triploid backcross progeny, (gengoroubuna Carassius auratus cuvieri ♀ × carp Cyprinus carpio ♂)F1 ♀ × gengoroubuna or carp ♂ is reported. In females, spawning could be induced by the injection of pituitary extracts. The fertilization rates were from 52.4 to 88.6%, and free swimming rates from 2.6 to 34.4%. Many normal larvae were obtained after crossing with the sperm of gengoroubuna. However, after crossing with sperm of the loach Misgurnus anguillicaudatus, most of the embryos died before hatching and no normal larvae were obtained.

Sperm could not be stripped from the progeny of either type of triploid backcross after the injection of pituitary extracts. Under light microscope, each seminal lobule in the testis was mainly occupied by spermatocytes in various stages. Some spermatids were found in part of the lobules, but no spermatozoa were observed.

These facts suggest that the laboratory-reared, triploid backcross progeny may represent an intermediate step in the evolution of a gynogenetic triploid form.

Materials and Methods

Experimental fish. Common carp, Cyprinus carpio, and goldfish, Carassius auratus auratus, from Yoshida Research and Training Station, Tokyo University of Fisheries and gengoroubuna, C. auratus cuvieri, from the Fisheries Experimental Station, Saitama Prefecture were used in this study. Loach, Misgurnus anguillicaudatus, were purchased from the fish market in Tokyo. Hybrids (F1) between female gengoroubuna and male carp were produced in 1985. In 1988, fresh egg of a F1 female were fertilized with fresh sperm of gengoroubuna or carp, and two types of triploid backcross progeny were produced. These were reared in indoor tanks until maturation and used for this study.

Ploidy determination and sex distinction. The ploidy levels were determined by DNA content and chromosome number of somatic cells. DNA content of red blood cells (RBC) was measured by flow cytometry after propidium iodide (PI)-staining (Allen, 1983). Chromosome preparations were made from leucocyte cultures (Ojima et al., 1970).
Fig. 1. Mitotic metaphase chromosomes from backcross progeny (gengoroubuna ♀ × carp ♂) F₁ ♀ × gengoroubuna ♂.
Fig. 2. Eggs from backcross progeny (gengoroubuna ♀ × carp ♂) F₁ ♀ × gengoroubuna ♂. Small size eggs (se) and large size eggs (le) were observed. Bar indicates 1 mm.
Fig. 3. Cytological observation of testis in triploid backcross progeny (gengoroubuna ♀ × carp ♂) F₁ ♀ × gengoroubuna ♂. The primary spermatocyte (psc), secondary spermatocyte (ssc), and spermatid (spt) are delimited. Bar indicates 30 µm.
Fig. 4. Cytological observation of testis in a diploid goldfish. The sperm (spt) and secondary spermatocyte (ssc) are shown. Scale bar indicates 30 µm.

The sex of backcross progeny was determined by observation of secondary sexual characters, such as the pearl organ or genital pore, and by inspecting ova in the ovary by means of cannulation.

Fertility check. Females. In 1991, eggs could be stripped from some of the female triploid backcross progeny after intraperitoneal injection of pituitary extracts from the silver carp, Hypomichthys molitrix. Sperm of gengoroubuna and squashed testes of loach were each diluted to four times their original volume with Ringer's solution. Eggs were divided into two groups and inseminated with diluted sperm of gengoroubuna and loach, respectively. Fertilization rate, eyed egg rate, hatching rate and free swimming (A free swimming eleutheroembryo is defined as an individual that has developed beyond the period of attachment to the substrate) rate were examined in each group (50–600 eggs) used in the
crossing experiments.

**Males.** The bellies of 20 male triploid backcross progeny were squeezed whether or not sperm could be stripped out. For histological observations, testes were removed and fixed with Bouin's solution for 12 hrs. After dehydration with an ascending ethanol series and finally with toluene, the testes tissues were embedded in paraplast. Sections 7μm in thickness were stained with Mayer’s haematoxylin and eosin. Testes of diploid goldfish were also investigated for comparison.

**Backcross progeny.** The ploidy levels of the offspring of backcross progeny were also determined by flow cytometry, as mentioned above.

**Results**

**Ploidy level and sex ratio of backcross progeny.** Chromosome spreads of backcross progeny are shown in Fig. 1, the chromosome number being 150. When the DNA content of diploid goldfish measured by flow cytometry was adjusted to a channel number of 100, the relative values of the triploid backcross progeny (carp and gengoroubuna♂) were 136.3±6.5 (n=6) and 143.0±1.2 (n=6), respectively.

The sex ratio is shown in Table 1. In the case of the backcross (gengoroubuna♀×carp♂)F₁♀×carp♂♂, 3 females and 6 males resulted. On the other hand, the backcross (gengoroubuna♀×carp♂)F₁♀×gengoroubuna♂ resulted in 11 females and 26 males.

**Fertility of female backcross progeny.** Spawning was induced in 4 out of 11 progeny from the backcross (gengoroubuna♀×carp♂)F₁♀×gengoroubuna♂ after injection of pituitary gland extracts. Two egg sizes were distinguished, being about 1.25 mm and 1.10 mm in diameter (Fig. 2). As shown in Table 2, after insemination with sperm of gengoroubuna, normal fry were obtained. When two backcross progeny (nos. 1 and 2) were inseminated with...
sperm of the loach, some free swimming, actively feeding larvae were obtained, but all of them showed extreme deformities and died within 3 months after hatching. At no time were normal fry obtained from crossing with loach sperm.

The results of the crossing experiments using backcross progeny [(gengoroubuna ♀× carp♂)F₁ ♀× carp♂] are shown in Table 3. Two out of 3 individuals spawned after the injection of pituitary gland extracts. In a cross with gengoroubuna♂, a fertilization rate of 77.9–88.6% and free swimming rate of 29.7–34.4% were achieved. Although more than half of the embryos died before hatching, a large number of normal cross progeny were obtained. On the other hand, the hatching rate using loach sperm was lower than that with gengoroubuna sperm. All of the larvae showed extreme deformities and died in early stages before free swimming and feeding.

Fertility of male backcross progeny. The males of both types of triploid backcross progeny had many pearl organs on the pectoral fins, but did not produce sperm on stripping after the injection of pituitary gland extracts. However, it was found that the testes had developed to a certain extent and that the gonado-somatic index (GSI) attained 1.39±0.71% (n=20) in the breeding season. Under light microscopy, each seminal lobule of the testes was occupied mainly by primary spermatocytes, secondary spermatocytes and spermatids, but no spermatozoa were observed (Fig. 3). On the other hand, the seminal lobules of the testes of diploid goldfish were occupied by spermatozoa (Fig. 4).

Ploidy level of the offspring of backcross progeny. The relative DNA content of 6 offspring of triploid backcross progeny are shown in Table 4. When the DNA content of diploid carp measured by flow cytometry was adjusted to a channel number of 200, the relative DNA contents of 4 individuals (nos. 1–4) showed more than 350, the others showing about 230. It is suggested that the first 4 individuals were tetraploids, and the other 2, hyperdiploids (approximately 2.5n).

**Discussion**

Hubbs (1955) gave evidence that Poecilia formosa, a well-known gynogenetic, all-female species, was a hybrid, by crossing P. sphenops with P. latipinna. Reciprocal crosses of the latter species under laboratory conditions produced bisexual hybrids, which in turn produced intermediate offspring following backcrossing.

Schultz (1969) reported a hybrid complex from the Rio Fuerte of northwestern Mexico. The complex consisted of two bisexual species, Poeciliopsis monacha and P. lucida, and 3 unisexual derivatives, 2 of which were triploid (P. 2 monacha-lucida and P. monacha-2lucida) and the other, diploid (P. monacha-lucida). The karyotype of the unisexual diploid approximated that of the hybrid of P. monacha and P. lucida. During oogenesis the paternally derived P. lucida chromosomes were lost, the P. monacha set being transmitted to the egg. Since the all-female form mated mainly with males of P. lucida, the hybrid nature of the fish was maintained. Schultz coined the term “hybridogenesis” for this mode of reproduction. Subsequently, Schultz (1973) bred unisexual hybrids of P. monacha and P. lucida in the laboratory, these being all-female diploids that were indistinguishable from wild P. monacha-lucida.

Cherfas (1980) obtained triploid hybrids by backcrossing carp and diploid silver crucian carp, Carassius auratus gibelio, and suggested that the evolutionary development of gynogenetic, triploid populations of the latter species might have originated from hybridization, as in Poeciliopsis.

In the present study, we also obtained triploid, backcross progeny of gengoroubuna and carp, which were bisexual. The females were fertile, but the males could not produce sperm.

Populations of triploid silver crucian carp, Carassius auratus gibelio, in northeast China include only 5–25% males (Shen et al., 1983), which cannot produce functional sperm (Jiang et al. 1982). Likewise, males constitute about 40% of a family of
gynogenetic, triploid ginbuna, kept in our laboratory, but nevertheless cannot produce sperm (unpublished data). It is very interesting that triploid backcross females are fertile, while the males are not, similar properties to those of the triploid ginbuna population mentioned above.

On the other hand, the offspring of backcross progeny might be composed of tetraploid and hyper-diploid (approximately 2.5n) individuals. Triploid backcross females may produce two types of ova; 3n ova, which combine with 1n sperm to make 4n larvae, and 1.5n ova, which combine with 1n sperm to make 2.5n larvae. Apparently, the triploid backcross progeny did not reproduce by gynogenesis, because perfect fertilization occurred. However, some triploid backcross progeny produced 3n ova, indicating that meiotic division did not occur during oogenesis as in natural triploid ginbuna. It is possible that the triploid backcross progeny may represent an intermediate step in the evolution of a gynogenetic triploid form.

Literature Cited


Cherfas, N. B. 1966. Natural triploid in female of the diploid (approximately 2.5n) individuals. Triplaid progeny might be composed of tetraploid and hyper-diploid (approximately 2.5n) individuals. Triploid backcross females may produce two types of ova; 3n ova, which combine with 1n sperm to make 4n larvae, and 1.5n ova, which combine with 1n sperm to make 2.5n larvae. Apparently, the triploid backcross progeny did not reproduce by gynogenesis, because perfect fertilization occurred. However, some triploid backcross progeny produced 3n ova, indicating that meiotic division did not occur during oogenesis as in natural triploid ginbuna. It is possible that the triploid backcross progeny may represent an intermediate step in the evolution of a gynogenetic triploid form.


(Received March 13, 1992; accepted May 11, 1992)