Japanese Journal of Ichthyology
Vol. 39, No. 3 1992

魚類学雑誌
39巻3号 1992年

Fertility of Triploid Backcross Progeny, (Gengoroubuna *Carassius auratus cuvieri* ♀ ×Carp *Cyprinus carpio* ♂)F_1 ♀ ×Carp or Gengoroubuna ♂

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Abstract The fertility of triploid backcross progeny, (gengoroubuna *Carassius auratus cuvieri* ♀ ×carp *Cyprinus carpio* ♂)F_1 ♀ ×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.

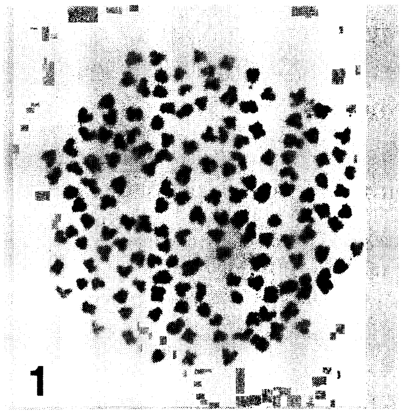
Several teleost species produce all-female offspring by gynogenesis (Hubbs and Hubbs, 1932; Cherfas, 1966; Schultz, 1967; Kobayasi, 1971; Cimino, 1972; Echelle et al., 1988; Fan and Shen, 1990; Dawley et al., 1987). Most of them may have originated from hybrids of related species (Schultz, 1967, 1971; Abramoff et al., 1968).

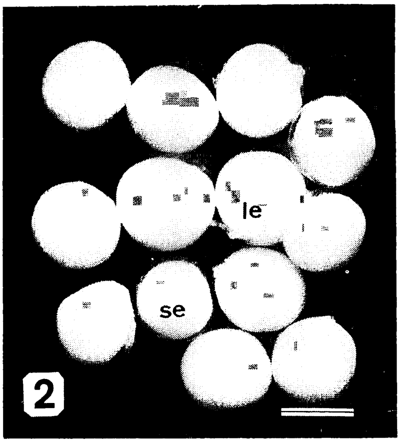
Gynogenetic triploid ginbuna, *Carassius auratus langsdorfii*, in Japan are also considered to have originated from hybrids of different species or subspecies (Sekiya and Honma, 1973, 1975; Shimizu et al., 1991.). Ojima et al. (1975) reported that triploid larvae, all females and morphologically similar to ginbuna, were produced by backcrossing of (funa ♀ ×carp ♂)F_1 ♀ ×carp ♂. Furthermore, Cherfas and Ilyasova (1980) reported that the backcross of (silver crucian carp ♀ ×carp ♂)F_1 ♀ ×carp ♂ resulted in triploids. In these studies, however, the fertility of triploid backcross progeny of crucian carp and carp was not reported. In the present study, we examined the fertility of triploid backcross progeny of gengoroubuna, *Carassius auratus cuvieri*, and carp to clarify the evolutionary history of gynogenetic triploid ginbuna.

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 (F_1) between female gengoroubuna and male carp were produced in 1985. In 1988, fresh egg of a F_1 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).





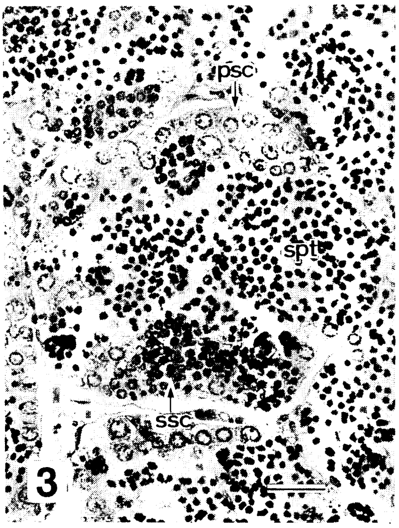


Fig. 1. Mitotic metaphase chromosomes from backcross progeny (gengoroubuna ♀ × carp ♂) F_1 ♀ × gengoroubuna ♂.

Fig. 2. Eggs from backcross progeny (gengoroubuna ♀ × carp ♂)F_1 ♀ × 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 ♂) F_1 ♀ × 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 (spm) 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, *Hypophthalmichthys 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 7 m 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 ♂)F_1 ♀ × carp ♂, 3 females and 6 males resulted. On the other hand, the backcross (gengoroubuna ♀ × carp ♂)F_1 ♀ × 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 ♂)F_1 ♀ × 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

Table 1. Sex ratios of backcross progeny

Crossing	F_1 ♀ × Carp ♂		F_1 ♀ × Gengoroubuna ♂	
	Female	Male	Female	Male
Number	3	6	11	26

* F_1 was a gengoroubuna ♀ × carp ♂ cross.

Table 2. Crossing experiments with females of backcross progeny [(gengoroubuna ♀ × carp ♂) F_1 ♀ × gengoroubuna ♂]

Specimen number	Male	Fertilization rate (%)	Eyed egg rate (%)	Hatching rate (%)	Free swimming rate (%)
1	Gengoroubuna	78.8	51.5	20.4	2.6
	Loach	77.5	27.5	8.9	2.2
2	Gengoroubuna	79.5	47.7	24.1	11.8
	Loach	83.1	27.2	15.4	8.7
3	Gengoroubuna	77.6	64.2	37.3	11.9
	Loach	80.0	8.3	3.3	0
4	Gengoroubuna	52.4	34.0	20.2	10.3
	Loach	64.2	21.4	9.0	0

Table 3. Crossing experiments of females with backcross progeny [(gengoroubuna ♀ × carp ♂)F_1 ♀ × carp ♂]

Specimen number	Male	Fertilization rate (%)	Eyed egg rate (%)	Hatching rate (%)	Free swimming rate (%)
1	Gengoroubuna	88.6	57.0	45.6	29.7
	Loach	83.5	54.8	6.0	0
2	Gengoroubuna	77.9	64.9	38.2	34.4
	Loach	36.5	13.2	3.1	0

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 ♂)F_1 ♀ × 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 spermatozoom 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).

Table 4. Relative DNA contents of the offspring of triploid backcross progeny

No.	Crossing	Relative DNA contents
1	(F_1[*1] ♀ × funa[*2] ♂) ♀ × carp ♂	359
2	(F_1 ♀ × funa ♂) ♀ × carp ♂	352
3	(F_1 ♀ × funa ♂) ♀ × funa ♂	385
4	(F_1 ♀ × funa ♂) ♀ × funa ♂	356
5	(F_1 ♀ × funa ♂) ♀ × carp ♂	226
6	(F_1 ♀ × carp ♂) ♀ × carp ♂	231
7	carp (2N)	200
8	carp (2N)	191
9	carp (3N)	273
10	carp (3N)	279

[*1] F_1 was a gengoroubuna ♀ × carp ♂ cross.
[*2] Funa was a gengoroubuna.

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 hyperdiploid (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.

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

フナ・コイ3倍体雑種の生殖能力

張　峰・尾城　隆・隆島史夫

天然3倍体雌性発生ギンブナの雑種起源説を検討するため，3倍体交雑種（ゲンゴロウブナ♀×コイ♂）F_1♀×ゲンゴロウブナ♂またはコイ♂の生殖能力について検討した．雌は脳下垂体ホルモン注射によって人工採卵でき，得られた卵をゲンゴロウブナまたはドジョウの精子で再交雑した．その結果，ゲンゴロウブナの精子で媒精した場合の受精率は52.4–88.6%，自由遊泳率は2.6–34.4%に達し，正常な稚魚が多数得られた．一方，ドジョウ精子で媒精した場合，孵化率は3–15%に達したものの，ほとんどの個体は奇形のまま死亡し，正常稚魚は得られなかった．雄は，ホルモン注射を行なっても採精できなかった．精巣の光顕観察の結果，精小葉に精母細胞と少数の精細胞が観察されたものの，精子は認められなかった．これらの結果から，ここで作った人為3倍体交雑種は自然雌性発生集団へ進化する中間ステップに相当するものと考えられた．

（張・尾城・隆島：108 東京都港区港南4–5–7　東京水産大学資源育成学科；張：中国湖北省沙市市中国水産科学研究院長江水産研究所）

うえ，統一にご協力ください．その他の，論文の形式的な点については CBE Style Manual, 5th edition を入手してお読みくださると助かります（私も勉強中ですが...）．

フロッピーによる初校の作成も，皆様のご協力によりずいぶん定着してきました．ご存じのように校正の手間は著者にとっても編集サイドにとっても生半可なものではありません．もちろん最近はそんなことはありませんが，その昔に手で組んでいた頃は，著者の見つける誤植は編集サイドの見つける誤植の3分の1程度だったということです．迅速な出版を維持していくためにも皆様のご協力をよろしくお願いいたします．

(MM)

訂　正・Errata

魚類学雑誌39巻3号に下記の訂正があります．

Japanese Journal of Ichthyology, 39(3), Zhang et al.: page 231, 8th line, read “7 m” for “7 μm.”