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Histological Investigation on the Gonads of Artificially Induced Triploid Crucian Carp, *Carassius auratus*

Etsuro YAMAHA* and Hiroshi ONOZATO**

Abstract

The gonads of artificially induced triploid juveniles of crucian carp were observed histologically. The triploid testis consisted of many cysts including spermatogenic cells in several stages as seen in diploid gonads. Although the diploid ovaries were packed with a number of oocytes at the peri-nucleolus stage, degenerated oocytes of the chromatin-nucleolus stage were prominent and oocytes at peri-nucleolus stage were not found in the triploid ovaries.

Animals with triploid chromosome sets may be sterile because of the failure of the synapsis of homologous chromosomes during the early gametogenesis. Purdom (1972b, 1976) reported that the ovaries of the triploid plaice (*Pleuronectes platessa*) × flounder (*Platichthys flesus*) contained very few oocytes compared with the diploid hybrid. He also found that the gonadosomatic index of triploid fish was lower than that of diploids at both 10 months and 4 years of age. In amphibians, however, triploid individuals are not always sterile as seen in *Triturus viridescens* (Fankhauser, 1945) and axolotl (Fankhauser and Humphrey, 1950). So, it is likely that induced triploid animals are not always sterile.

Natural triploid fish belonging to genera such as *Poeciliopsis* (Shultz, 1967) and *Carassius* (Kobayasi and Ochi, 1972) were known to be all female fish and to reproduce gynogenetically. The origin of those fish is not known. It is an interesting subject from the viewpoint of their origin to know whether artificially induced triploids of crucian carp, *Carassius auratus*, generate gynogenetic reproduction or not.

In this paper we report the histological investigation of gonads of artificially induced triploids in crucian carp.

Materials and Methods

Ovulated female and spermiated male crucian carp, *Carassius auratus*, were collected by traps in Lake Junsainuma, 20 km north of Hakodate. Artificial triploid induction were carried out by means of hydrostatic pressure, according to Onozato (1984). Details will be described in another paper on a hydrostatic pressure treatment for adhesive crucian carp eggs. Induced triploids and control diploids were reared separately in aquariums with air-filtration systems. From six to seven months after hatching, whole bodies of juveniles were fixed with Bouin's

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solution for one day and then their body length, fork length, body weight and gonadal weight were measured. Their gonads and kidneys were embedded in Tissue Prep (Fisher Scientific Company) and sectioned at 6 μm thickness. The sections were stained with Delafield's hematoxylin and eosin. For determination of ploidy, nuclear sizes of 20 kidney cells were measured with a microscope Olympus model OSM-D_2.

Results

Measurements of 25 treated juveniles and 19 control juveniles, obtained from one female parent, are shown in Table 1. The kidney cell nuclear sizes of all the experimental fish were significantly larger than those of control fish (Table 1), and triple ratio of mean nuclear diameter between control and treated fish was 2 : 3.2. These results showed that the induction of triploids was succeeded.

Fork length of the triploid fish was significantly larger than that of diploid one ($0.01>t>0.005$), but body weight was not ($0.1>t>0.05$).

Both control diploid and experimental triploid fish were sexually differentiated and the sex ratios of both of them were approximately one to one (Table 1).

Ovary

Ovary cross sections of diploids and of triploids are shown in Plate I-Figure 1 and Plate I-Figure 2, respectively. They had distinct ovigerous lamelae and ovarian cavity. The gonadosomatic index of the triploids was slightly lower than that in diploids, though the body weight in triploids was slightly larger than in diploids (Table 1).

In the control diploid ovary, oocytes of the peri-nucleolus stage were observed to be distributed throughout the ovary. Clusters composed of several oogonia were found among the oocytes (PL. I-Fig. 1). The cytoplasm of oogonia were slightly stained with hematoxylin and their nuclei ranged from 5.69 to 7.48 μm in diameter. In the triploid ovary, however, there were no eggs at the peri-nucleolus stage, but three types of younger germ cells were found. They corresponded to oogonia, and oocytes of synaptic and cromatin-nucleolus stages. Oogonia were very few in number and their nuclei, with a diameter varying from 5.99 to 9.86 μm, did not differ in histological appearance from those of diploid oogonia (PL. I-Fig. 3). The oocytes of the synaptic stage had oval nuclei and a part of the nucleus was deeply stained with hematoxylin (PL. I-Fig. 3). Oocytes at the synaptic stage were not found in diploid ovary. A number of oocytes at chromatin-nucleolus stage were found in triploid ovaries. Their nuclei were oval in shape, vacuolated in texture and contained many chromatin-like particles in their peripheral regions (PL. I-Fig. 4). It seemed to stop their advanced oogenesis at the chromatin-nucleolus stage.

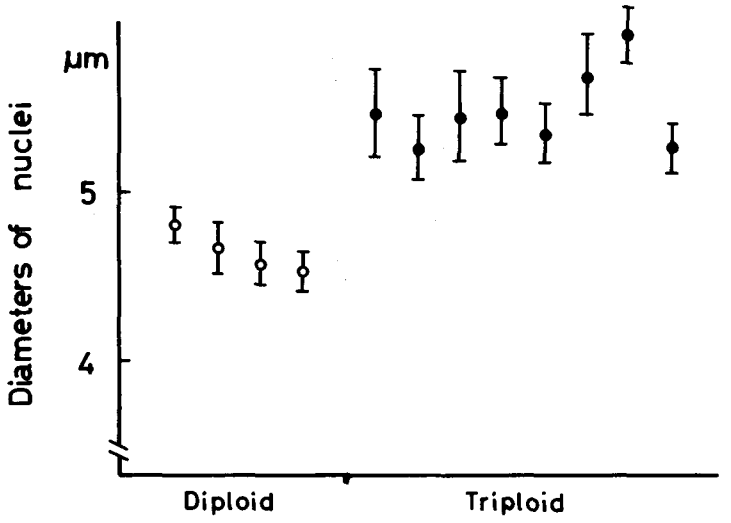
Testis

Testes of diploids consisted of many cysts which included spermatogenic cells of several stages (PL. I-Fig. 5).

Spermatogonia, stained slightly with eosin, were found among the cysts in control testes. Their nuclei were large and oval shaped, having two or three nucleoli, and were surrounded by a small amount of cytoplasm (PL. II-Fig. 9). In

Table 1. Measurements of body length (B.L.), fork length (F.L.) body weight (B.W.), gonadal weight (G.W.), relative gonadosomatic index (GSI) and diameter of kidney cell nuclei of control diploid and experimental triploid crucian carp.

		No.	B.L. (mm)	F.L. (mm)	B.W. (g)	G.W. (mg)	GSI (%)	Diameter of kidney nuclei (mean±S.E.)
DIPLOID	MALE	1	37.3	43.7	1.92	0.7	0.04	5.70±0.09
		2	—	44.1	2.23	11.3	0.51	5.13±0.06
		3	39.3	46.3	2.54	4.0	0.16	5.24±0.08
		4	—	46.9	2.67	5.9	0.22	5.06±0.08
		5	41.6	49.0	2.90	—	—	5.45±0.15
		6	45.4	54.0	3.85	23.3	0.61	5.29±0.05
		7	48.2	56.1	4.14	18.4	0.44	5.27±0.07
		8	49.3	56.4	4.70	28.0	0.59	5.12±0.08
		mean±S.E.	43.5±2.0	49.6±1.8	3.12±0.35	13.1±3.9	0.37±0.09	5.28±0.07
	FEMALE	1	34.1	38.4	1.28	1.5	0.12	5.47±0.07
		2	35.1	40.5	1.60	3.2	0.20	5.34±0.07
		3	38.0	44.8	2.15	4.0	0.19	5.57±0.11
		4	38.1	44.6	2.13	4.2	0.20	5.49±0.12
		5	36.8	43.3	1.89	2.8	0.15	5.51±0.06
		6	38.4	45.5	2.07	3.9	0.19	5.41±0.06
		7	—	45.7	2.58	20.0	0.78	5.15±0.05
		8	40.8	48.3	2.96	1.7	0.06	5.20±0.07
		9	—	48.5	2.88	20.5	0.71	5.20±0.04
		10	—	49.1	3.02	12.5	0.41	5.27±0.06
		11	47.7	55.5	4.45	15.2	0.34	5.18±0.06
		mean±S.E.	38.6±1.5	45.8±1.4	2.46±0.26	8.1±2.2	0.30±0.07	5.34±0.05
		Mean of total	40.7±1.3	47.4±1.1	2.73±0.22			5.31±0.04
TRIPLOID	MALE	1	38.3	46.8	2.61	6.1	0.23	6.10±0.07
		2	41.1	48.7	2.67	2.9	0.11	6.11±0.09
		3	41.9	49.9	3.54	3.0	0.08	6.31±0.13
		4	43.0	50.5	2.74	39.2	1.43	6.18±0.10
		5	43.3	51.8	3.57	5.4	0.15	6.50±0.12
		6	44.0	52.5	3.36	2.8	0.08	6.01±0.09
		7	44.2	52.9	3.65	10.9	0.30	6.15±0.11
		8	44.7	53.5	3.59	2.2	0.06	6.35±0.10
		9	45.1	54.4	4.11	14.2	0.34	6.10±0.10
		10	45.4	53.7	3.26	15.5	0.48	6.13±0.09
		11	48.4	56.8	4.25	6.7	0.16	6.29±0.11
		12	50.2	58.7	4.70	98.1	2.09	6.16±0.15
		13	50.3	58.1	5.14	17.8	0.35	6.28±0.09
		mean±S.E.	44.6±1.0	52.9±1.0	3.63±0.21	17.3±7.3	0.45±0.17	6.21±0.04
	FEMALE	1	34.8	41.7	1.60	1.1	0.07	5.94±0.10
		2	38.3	46.1	2.28	3.5	0.15	6.12±0.10
		3	39.1	47.9	2.43	2.8	0.12	6.06±0.08
		4	39.6	48.1	2.49	7.0	0.28	6.08±0.16
		5	40.7	48.0	2.71	2.5	0.09	6.62±0.10
		6	41.8	49.2	2.64	1.6	0.06	6.37±0.10
		7	42.3	49.4	2.38	1.0	0.04	6.09±0.10
		8	43.4	50.6	2.59	1.5	0.06	5.83±0.11
		9	44.0	51.3	2.72	0.6	0.02	6.24±0.10
		10	44.5	52.3	3.37	6.5	0.19	6.08±0.15
		11	45.5	54.6	3.71	4.2	0.11	5.87±0.10
		12	48.4	56.8	4.16	9.4	0.23	6.51±0.12
		mean±S.E.	41.9±1.1	49.7±1.1	2.76±0.20	3.48±0.8	0.12±0.02	6.15±0.07
		Mean of total	43.3±0.7	51.4±0.8	3.21±0.17			6.18±0.04



Text-Fig. 1. Nuclear size of the first spermatocyte of the diploid (○) and the triploid (●) crucian carp. Vertical bars indicate confidence limits at 99%.

triploid testes there were only a few normal spermatogonia (PL. II-Fig. 10). Most of the spermatogonia had vacuolated nuclei. Vacuolated spermatogonia were predominant in number in the testes of some triploid individuals (PL. I-Fig. 7), but more advanced spermatogenic cells were predominant in other triploids (PL. I-Fig. 8).

In the synaptic stage, a part of the nucleus of spermatocytes was deeply stained with hematoxylin. These cells were found in both diploid and triploid testes (PL. II-Figs. 11 and 12). First and second spermatocyte and spermatid were observed throughout diploid and triploid testes (PL. II-Figs. 13–16). There were no prominent differences in appearance except the larger size in triploids (Text-Fig. 1).

In diploid testes, spermiogenesis proceeded but spermatozoa were not yet produced. The nuclei of the diploid spermatid became smaller and were deeply stained with hematoxylin. At that point, no cytoplasm was found around the periphery of the nucleus (PL. II-Fig. 17). Triploid spermatid were polygonal in shape and seemed to be covered with a small amount of cytoplasm (PL. II-Fig. 18).

Discussion

The present study showed that artificially induced triploids of crucian carp were not unisexual, rather both sexes were found at almost the same ratio. The results showed that the triploidy of natural gynogenetic crucian carp was not caused by retention of the second polar body at miosis II.

Yamamoto, *et al.* (1967) reported that, in goldfish, the male was heterogametic (XY) based on the sex ratio of the progeny of sex-reversed fish. Crucian carp used in the present study was of the same species as goldfish, *Carassius auratus*. Accordingly, the induced triploid fish in this experiment must have the zygotes with the formulas XXY (male) and XXX (female).

In recent years, the application of genetic techniques to aquaculture has been greatly improved. Purdom (1972a) pointed out the importance of the application of genetic techniques, irrespective of plants or animals, and proposed the following three points: 1) selection, 2) hybridization, and 3) chromosome engineering.

Purdom said that polyploids could be useful in fish cultivation in the following ways. First, their growth rate might be greater than that of equivalent diploids. This tends to agree with Valenti's results (1975). Valenti pointed out that polyploid *Tilapia aurea* grew larger than diploid siblings when measured at 14 weeks of age. Whereas Swarup (1959) reported that triploid sticklebacks, *Gasterosteus aculeatus*, did not grow faster than diploids. Swarup insisted that increased cell size was compensated for a decrease in the number of cells maintained in normal total body length. In present study, the fork length of the triploid fish was significantly larger than that of the diploid one, but the body weight was not. The discrepancy between these two results is not known at present.

The second advantage of polyploid fish, especially of triploids, is that of sterility. Sexual maturation consumes a lot of energy for the formation of gonads, and the growth rate of body size is limited at the mating season. In sterile fish, reproductive energy may be transferred to growth functions, thererfore it is expected that they will show more rapid growth and greater longevity.

In the present study it was suggested that the triploid females seemed to be sterile. In males, on the other hand, spermiogenesis went on, though we have not determined their fertility. Therefore, the production of all female triploids may be useful for crucian carp fish culture. It may differ from species whether triploid fish sterile or not. More detailed studies are desirable in each species.

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PLATE I

Fig. 1. Ovary of the diploid crucian carp. It contains many eggs at peri-nucleolus stage. Scale indicates 0.01 mm.

Fig. 2. Ovary of the triploid crucian carp. No oocytes at peri-nucleolus stage are found. Scale indicates 0.01 mm.

Fig. 3. Oogonia (O) and the oocytes of synaptic stage (SO) in the triploid ovary. Scale indicates 0.01 mm.

Fig. 4. Oocytes at the chromatin-nucleolus stage observed in the triploid ovary. Scale indicates 0.01 mm.

Fig. 5. Histological features of the diploid testis. Scale incicates 0.02 mm.

Fig. 6. Histological features of the triploid testis. Scale indicate 0.02 mm.

Fig. 7. Triploid testis with mainly spermatogonia. Scale indicates 0.02 mm.

Fig. 8. Triploid testis with cysts at several stages. Scale indicates 0.02 mm.

PLATE I

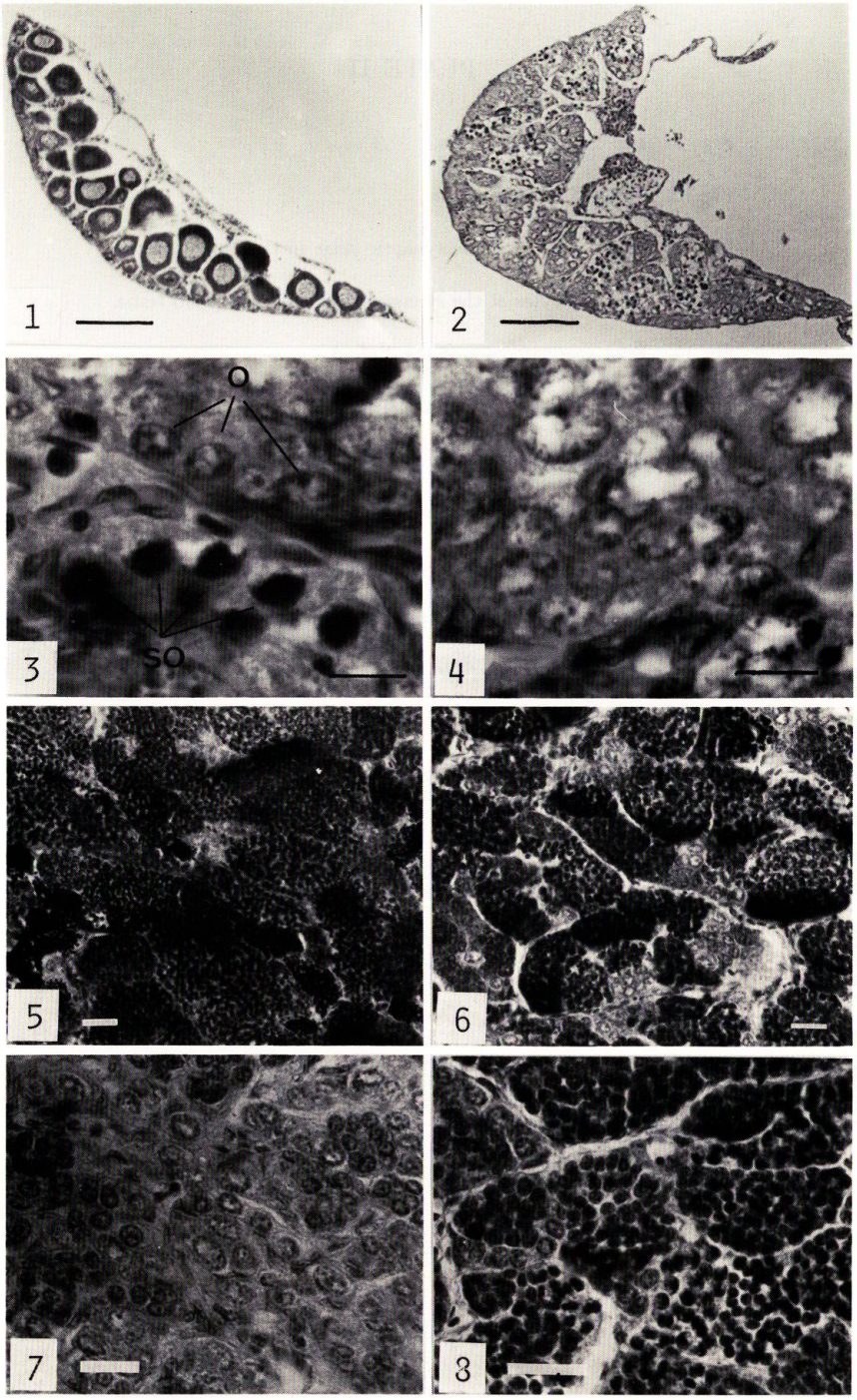

PLATE II

Fig. 9. Diploid spermatogonia.

Fig. 10. Triploid spermatogonia.

Fig. 11. Spermatocytes at the synaptic stage in the diploid testis.

Fig. 12. Spermatocytes at the synaptic stage in the triploid testis.

Fig. 13. First spermatocytes of the diploid.

Fig. 14. First spermatocytes of the triploid.

Fig. 15. Spermatids at the early spermiogenesis in the diploid.

Fig. 16. Spermatids at the early spermiogenesis in the triploid.

Fig. 17. Spermatids at the late spermiogenesis in the diploid.

Fig. 18. Spermatids at the late spermiogenesis in the triploid.
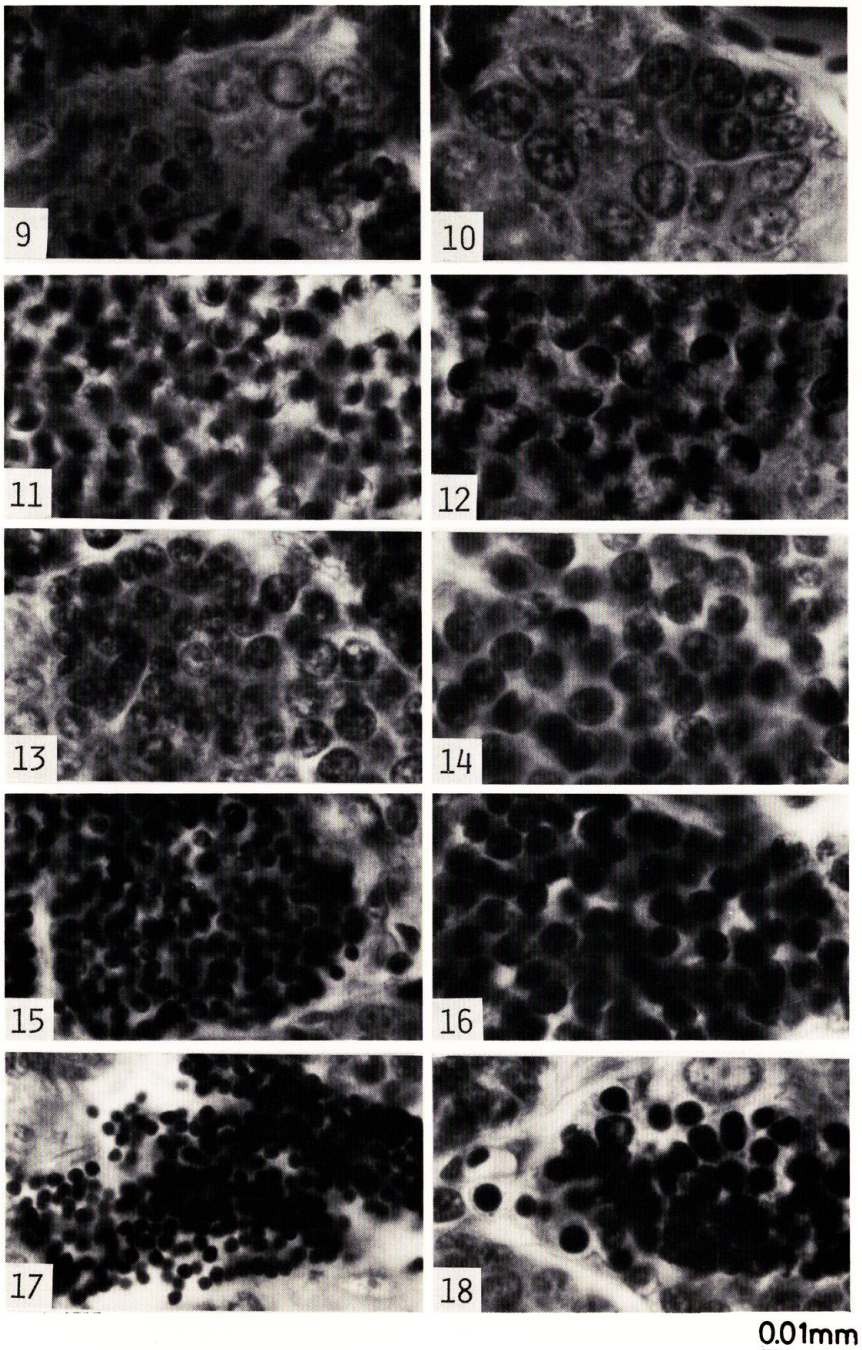
PLATE II