124. A Note on Triploidy of the Funa (Cyprinidae, Pisces)*)

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The Funa (*Carassius auratus*) distributed in Japan has been subdivided into five subspecies, such as Kinbuna (*Carassius auratus* subsp.), Ginbuna (*C. auratus langsdorffii* Temminck et Schlegel), Nagabuna (*C. auratus burgeri* Temminck et Schlegel), Nigorobuna (*C. auratus grandoculis* Temminck et Schlegel) and Gengorobuna (*C. auratus cuvieri* Temminck et Schlegel) (Nakamura 1969). Unusual sex-ratio, or female excess, is a common feature in the Funa (Kobayashi and Mizutori 1960). Extreme female excess has been reported in the Ginbuna from certain Kanto districts of Japan. They were characterized by a 3n-range number of chromosomes, being 156 at mode (Kobayashi *et al.* 1970). Kobayashi (1971) has expressed the view that the chromosomes transmitted from the triploid-range Ginbuna mother by gynogenesis might result this situation.

There is a question whether the unusual sex ratio in the Funa is caused merely by gynogenesis. The present study deals with the triploidy problem in the Funa by investigating comparatively the chromosomes of several subspecies of the Funa.

Materials and methods. Specimens, ♀42 and ♂27, for study were collected in the Lake Biwa in Shiga Prefecture, in the Lake Mikata and the Lake Kitagata in Fukui Prefecture, and in the Ishikari River in Hokkaido. Kidney cells from the colchicized fish exclusively provided the material for study in samples from the Lake Biwa, the Lake Mikata and the Lake Kitagata (1), while spleen-and/or kidney-cultured cells were used for the Ishikari River specimens (2). (1): Each fish received an intramuscular injection of colchicine (7–10 µg/lg body weight) for 4 to 5 hours prior to sacrifice. Small pieces of the kidney were put into 0.075 M KCl for 20 minutes at 37°C. They were fixed in Carnoy's fixative. Flame-drying procedure was applied 1 hour after fixation, and Giemsa-stain was used. (2): Living fish was put into 0.01% potassium permanganate solu-

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tion for about 1 hour in order to remove surface bacteria. Kidney and/or spleen were washed in five changes of saline solution containing both penicillin (120 IU/ml) and kanamycin (100 µg/ml). They were cut and put into 0.25% trypsin solution for 20 second, and then an equal amount of Eagle's essential minimum medium was added and mingled. Tissue fragments were cultivated at 20–22°C in 20% fetal calf serum in 30 ml Falcon flasks. Two drops of colcemid solution (10 µg/ml) were added for fifteen to seventeen hours before harvest when more than 20 mitotic metaphases were observed with the Olympus inverted microscope. The trypsin solution was used to remove cells from the flasks. The same procedure applied to the non-cultured material followed.

Heidenhain's hematoxylin-eosin testis-section slides were made for histological study.

Results. The Ginbuna, Gengorobuna and specimens presumably identified as Ginbuna from four localities (Table I) were chromosomally investigated. The diploid chromosome number was determined as 100: they were characterized by 10 pairs of metacentrics, 20 pairs of submetacentrics and 20 pairs of acrocentrics, showing a gradual size diminution in each group except one largest pair of submetacentrics (Fig. 1). Meiotic metaphases from one male of the Ginbuna contained 50 bivalents. No visible morphological difference was detected in the 2n karyotype between two sexes and among subspecies.

The metaphases showing a 3n-range number were observed in

Table I. Localities and numbers of the Funa studied

Funa	Honshu						Hokkaido	
	Shiga Pref. Lake Biwa ♀ ♂	Fukui Pref. Lake Mikata ♀ ♂	Fukui Pref. Lake Kitagata ♀ ♂	Ishikari River ♀ ♂				
<i>Carassius auratus langsdorfi</i> (Ginbuna)				22 (11)	8 (3)			
<i>Carassius auratus gran- doculis</i> (Nigorobuna)	2	4						
<i>Carassius auratus cuvieri</i> (Gengorobuna)	3	4	3 (1)	3	1			
Funa (possibly Ginbuna)	2 (2)	4	10 (5)	3				
Total	7 (2)	12	13 (6)	6	1	22 (11)	8 (3)	

The number of individuals having triploid-range chromosomes is indicated in parentheses.

Fig. 1. Karyotype with 100 chromosomes, from a non-cultured kidney cell of a Gengorobuna female; diploid strain.

Fig. 2. Karyotype with 153 chromosomes and 4 microchromosomes, from a cultured spleen cell of a Ginbuna female; triploid strain. Definite trisomic chromosomes are shown by black lines. Arrows indicate microchromosomes.

eight females collected in Honshu: four of them showed 153 at mode, while the remaining four had more than 150 chromosomes, the correct count being not made due to overlapping. The presence of the microchromosome could not be observed in these specimens at all.

Eleven females and three males derived from the Hokkaido samples were characterized by the chromosome number lying in a $3n$ -range. Among them, there were one female and one male which provided 157 and 165 chromosomes, respectively. Rough counts were available in the remaining ten females and two males due to overlapping. One metaphase (♀) with 157 chromosomes consisted of 32 metacentrics, 59 submetacentrics, 62 acrocentrics and 4 micro-

chromosomes. Karyotype analyses in the 157-chromosome cells revealed a partial trisomic constitution for the one pair of the well-defined largest submetacentrics and another pair of satellited acrocentrics, whereas any clear-cut evidence for the trisomic condition was not obtained in the remaining elements because of the paucity of characteristic configuration (Fig. 2). One male showed rather 156 macrochromosomes and 9 microchromosomes (Fig. 3). Histologically, the testis of this triploid specimen corresponded fairly to that of the normal diploid male, no appreciable difference being visible (Fig. 4). Interestingly, the occurrence of the microchromosomes observed was limited to the Funa from Hokkaido. No such element was shown in Honshu specimens.

Fig. 3-4. 3: Metaphase plate showing 148 chromosomes and 9 microchromosomes, from a cultured kidney cell of a Ginbuna male. Arrows indicate microchromosomes. 4: Histological figure of the testis from the same individual as Fig. 3.

Discussion. The triploid-range chromosomes were observed in the Funa from both Honshu and Hokkaido, supplementing the findings with the Ginbuna by Kobayashi and associates (1970). The present study has shown the triploid-range specimens to occur not only in both sexes of the Ginbuna but also in the Gengorobuna. Kobayashi (1971) has adopted the mechanism of gynogenesis for the occurrence of the triploid-range females. Several triploid females presumably produced by gynogenesis or parthenogenesis have been reported: in fish such as *Poecilia formosa* (Rasch *et al.* 1970) and *Poeciliopsis lucida* (Cimino and Schultz 1970); in salamander such as *Ambystoma jeffersonianum* (Macgregor and Uzzell 1964); and in lizards, such as *Cnemidophorus velox*, *C. tessellatus* and *C. exsanguis* (Pennock 1965). Cimino (1972) has stated that triploid gynogen, *Poeciliopsis*, reproduce by the formation of hexaploid primary oocytes

through premeiotic doubling of the chromosome number by endomitosis. The occurrence of the triploid-range males in the Ginbuna and their regular sperm-formation are not consistent with the above view. Sex reversal can be conceivable under the hormonal influence in the Funa, referring to the finding of Yamamoto (1975) showing that reversal in sex differentiation of the medaka (*Oryzias latipes*) was induced by the application of heterologous sex hormone. Another possible critique for the appearance of the triploid-range males of the Ginbuna should be that mating between tetraploid females or males and diploid ones would result in the triploid offspring. The occurrence of some tetraploid Ginbuna specimens as reported by Kobayashi *et al.* (1970) will be interpreted favourably by this assumption.

Final conclusion should be postponed until further cytogenetic studies and breeding experiments provide sufficient data essential for the elucidation of the mechanism involved.

Summary. The occurrence of male and female specimens of the Funa with the triploid-range complement of chromosomes is described. They showed an inter-chromosomal variation from 153 to 165, while diploid males and females have invariably 100 chromosomes. The production of triploid specimens is discussed.

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