**83. Tetraploidy appeared in the Offspring of Triploid
Ginbuna, *Carassius auratus langsdorffii*
(Cyprinidae, Pisces)**

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The ginbuna (*Carassius auratus langsdorffii*) is a member of the funa (*C. auratus*) having a wide distribution in Japan, with an inclination to the female in the sex ratio. In the Kanto district, the ginbuna so far obtained were all females having 3n-range (about 150) or 4n-range (about 200) chromosomes (Kobayashi *et al.*, 1970). These 3n-individuals propagate unisexually by gynogenesis (Kobayashi, 1971). Also, the ginbuna from Lake Biwa were mostly female with 3n-range chromosomes produced by gynogenesis (Ojima and Asano, 1977). Recent investigations have revealed that 3n-individuals of the ginbuna distribute widely through Japan accompanied with bisexual diploid individuals of the ginbuna or the other subspecies (Kobayashi, 1978; Takai and Ojima, 1982). On the other hand, 4n-individuals were sometimes found only in the limited areas as the Kanto district. The present paper deals with the chromosomal and electrophoretical investigations of the tetraploid ginbuna, appeared in the offspring of the triploid ginbuna developed from the fertilized eggs treated by refrigeration after the artificial insemination with the sperm of the gengorobuna (*C. a. cuvieri*).

Materials and methods. The spawned eggs from a single specimen of the ginbuna were artificially inseminated with the sperm of a single gengorobuna. Fifteen minutes after the insemination, fertilized eggs were exposed to low temperature at 1°C for 15 min. Ten individuals developing from the eggs thus treated, and the parental species, the ginbuna and the gengorobuna, were used for the present study. The chromosome preparations were made from cultured cells from fins (Ojima, 1978) as well as directly prepared kidney cells (Ojima and Takai, 1979) following the routine air-drying method with conventional stain. The BSG method by Sumner (1972) was employed for the C-banding analyses.

For electrophoretic analysis, specimens dissected for the chromosome studies were stored frozen at -20°C until the use. Sampling of the muscle tissue and the horizontal starch-gel electrophoresis for

muscle protein followed the methods after Ojima and Takai (1979). Electrophoresis for the non-specific esterase of the muscle, followed the horizontal acrylamide-gel method, was carried out at 20 mA for 6 h at 4°C in 5% gel with 2 mm thickness and 20 cm width. BN-buffer (0.3 M Boric acid + 0.05 M NaOH, pH 8.45) for electrode, and the mixed buffer of TC- (0.2 M Tris + 0.035 M citric acid, pH 8.3) and BN-buffer (3:1) for gel, were used. After the end of run, the gel was stained for the non-specific esterase in the staining solution containing 1 ml of 1% α -naphthyl acetate, 50 mg of Fast blue RR salt, and 50 ml of 0.2 M Tris buffer, pH 7.0.

Results and discussion. The maternal ginbuna had 3n-range chromosomes with a modal number of 154 consisting of 84 meta- and submetacentrics, 66 acrocentrics, and 4 minute elements, and the C-banding pattern was characterized by two larger submetacentrics with intensely stained short arms and four deeply stained minute elements. These results were identical with those of the

Fig. 1. The conventional stained karyotype and the C-banding pattern of the 4n-offspring from the triploid ginbuna. Allows show the characteristic C-bands found in the short arms of two larger submetacentrics and in minute elements.

ginbuna reported by Ueda and Ojima (1978). The paternal gengorobuna had 100 diploid chromosomes consisting of 6 pairs of meta-centrics, 18 pairs of submetacentrics, and 26 pairs of acrocentrics and they showed no characteristic C-banding pattern, as shown by Ojima *et al.* (1966), and Ueda and Ojima (1978).

Ten offspring examined in this experiments were all females. Four from among ten offspring showed 4n-range chromosomes, having 204 in modal number, with 108 meta- and submetacentrics, 92 acrocentrics, and 4 minute elements (Fig. 1). These C-banding patterns were characterized by two larger submetacentrics with intense C-bands at the short arms, and four C-banded minute elements, as well as those of the maternal ginbuna, in spite of the difference of their chromosome number (Fig. 1). The other offspring, however, showed 3n-ranged chromosomes with the same karyotypic constitution and C-banding pattern as those of the maternal ginbuna. In comparison with the karyotype constitution and the C-banding pattern between the 4n-offspring and their parents, it was clear that the 4n-offspring had a haploid chromosome set of the paternal gengorobuna and the triploid chromosome set of the maternal ginbuna.

In electrophoretic patterns of the muscle protein, the maternal ginbuna and the 3n-offspring showed the same pattern (Fig. 2-1) as a common pattern in the triploid ginbuna (Takai and Ojima, 1982). The 4n-offspring showed a pattern similar to that of the maternal ginbuna, but the density of the 3rd band in the 4n-offspring was higher than that of the maternal ginbuna (Fig. 2-2). This 3rd band

Figs. 2-3. 2: The electrophoretic patterns of the muscle protein. 2-1, the maternal ginbuna and the 3n-offspring; 2-2, the 4n-offspring; 2-3, the paternal gengorobuna. 3: The electrophoretic patterns of non-specific esterase of the muscle. 3-1, the maternal ginbuna and the 3n-offspring; 3-2, the 4n-offspring; 3-3, the paternal gengorobuna.

was characterized in the pattern of the paternal gengorobuna (Fig. 2-3). In non-specific esterase patterns of the muscle, the 3n-offspring had an identical pattern with that of the maternal ginbuna (Fig. 3-1). However, there was some difference in pattern of the maternal ginbuna and the 4n-offspring. Obviously, the 5th band in the 4n-

offspring was not found in the maternal ginbuna (Fig. 3-2), but was characterized by the pattern of the paternal gengorobuna (Fig. 3-3). Thus, the electrophoretic patterns of the 4n-offspring apparently expressed hybrid specificities between the paternal and the maternal characters. These electrophoretic findings could support the suggestion from the chromosomal study as stated above.

Usually the gynogenetic form produces ova with non-reduced chromosomes and they develop by an activation of spermatozoa derived from the bisexual species, without the participation of the paternal genome in fertilization (Ojima and Asano, 1977). From the chromosomal and electrophoretic results it is evident that the 4n-offspring has a haploid genome derived from the paternal gengorobuna, in addition to a triploid genome of the maternal ginbuna. This fact indicates that the 4n-offspring have been induced by the occurrence of unexpected fertilization by the union of the sperm nucleus and the egg nucleus through the gynogenetic reproduction, probably caused by the refrigeration of the fertilized eggs.

Thus, the present study revealed that tetraploidy was induced from the triploid ginbuna under the artificial condition. Such phenomenon would be possible to occur accidentally also under the natural condition. Kobayashi *et al.* (1977) suggested that the tetraploid form in the Kanto district was produced by gynogenesis. The artificial 4n-individuals of our experiment were all females. So far as our findings were concerned, at least, the hybrid between the triploid ginbuna and the bisexual diploid funa as shown in this study would be an origin of the tetraploid ginbuna in nature.

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