The appearance of diploid-triploid and diploid-triploid-tetraploid mosaic individuals in polyploid fish, ginbuna (*Carassius auratus langsdorfii*)[1]

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Summary. Diploid-triploid and diploid-triploid-tetraploid mosaic individuals were found in the progeny of gynogenetic triploid fish, *Carassius auratus langsdorfii*. In the mosaic specimens, the blood contained diploid and triploid cells, or diploid, triploid and tetraploid cells. Mosaicism was also observed in the spermatids in the testis of the diploid-triploid-tetraploid mosaic specimen.
Key words. Polyploid; gynogenesis; chromosomal mosaic.

Polyploid species are rare in vertebrates but have been reported in fish, amphibians and reptiles[2]. In fish, a triploid form in a natural population is known in three genera of teleost, *Poecilia*, *Poeciliopsis* and *Carassius*[3]. A tetraploid form is also observed in the genus *Carassius;* ginbuna, *Carassius auratus langsdorfii*.

Ginbuna, the Japanese crusian carp, is widely distributed in Japan. There are three forms of females in the ginbuna: a bisexual diploid form with 100 chromosomes, a unisexual (all female) triploid with 156 chromosomes and a unisexual tetraploid (all female) with 206 chromosomes[4,5]. Unlike other triploid vertebrates such as *Poeciliopsis*[6] and *Ambystoma*[7], both unisexual ginbuna produce their progeny by gynogenesis, omitting a meiosis during the maturation process. Then the first polar body formation is always skipped in these specimens[8]. The polyploid ginbuna produce the eggs which have unreduced chromosome numbers through a single homoeotype meiosis. In fertilized eggs of triploid ginbuna, artificially inseminated with sperm from a diploid bisexual male, the sperm nucleus remained in a condensed condition without fusing with the female pronucleus throughout fertilization to early cleavage stages[9,10]. Then the polyploid fish always produce progeny which have the same chromosome numbers as the parental females.

In our laboratory, mating experiments of triploid or tetraploid ginbuna with male goldfish *(C. a. auratus)* which have a dominant genetical character, scale transparency, were performed from 1978 to 1983. We obtained several strains of polyploid ginbuna. In the mating of triploid ginbuna (designated 10S22), collected from Lake Suwa in Nagano Prefecture, with a male goldfish, we found the occurrence of diploid-triploid and diploid-triploid-tetraploid mosaicism in the progeny, and among them six of the seven individuals were male.

These offspring were without doubt produced by gynogenesis, because 1) scale transplants among them were accepted by each other, 2) in electrophoretic analysis of several enzymes and muscle proteins which are polymorphic proteins in polyploid ginbuna, no electrophoretic variant was found in any protein among the offspring, and 3) paternal morphological characters (scale-transparency) did not appear in the offspring[11]. These facts support the assumption that gene addition or loss had not occurred in the offspring.

The ploidy of the progeny was estimated after 2–3 years from the DNA content in the erythrocyte nucleus. Whole blood was obtained from the heart or ventral aorta. The erythrocyte nuclei were stained according to the method of Rasch et al.[12]. The DNA value of each erythrocyte nucleus was determined at 560 nm by a one-wavelength scanning method using a Olympus multi micro spectrophotometer, MMSP.

The results are shown in the table. The relative DNA values ranged from 14 to 23 in five (A–E) offspring. And the ratios of the mean DNA values of these offspring to the mean DNA value of control fish (goldfish) ranged from 1.00 to 1.40. Offspring (B) was triploid but (C) and (D) were diploid in the erythrocytes. The cells from offspring (A) and (E) were distributed from the diploid range to the triploid range, and therefore these offspring showed an intermediate DNA ratio (1.27) between diploid and triploid. Offspring (A) and (E) were diploid-triploid mosaics.

The DNA values of blood cells were further analyzed using a fluorescence activated cell sorter, FACS IV (Becton-Dickinson). The cell suspension for whole blood was washed twice in BSS (buffered saline solution), and stained in distilled water containing 0.005% propidium iodide and 0.1% sodium citrate for 30 min in a refrigerator.

Erythrocyte DNA values determined by micro spectrophotometry. 10 erythrocytes were measured in each specimen

Specimen designation	Gonad	Relative DNA values (mean)	Mean DNA value Control mean DNA value
A	Testis	16–22 (19)	1.27
B	Testis	18–23 (21)	1.40
C	Testis	14–18 (16)	1.07
D	Testis	14–17 (15)	1.00
E	Absence	16–21 (19)	1.27
Control (goldfish)		13–16 (15)	1

Flow cytometric analysis of blood from two additional offspring (both males) revealed that one (F) was triploid and the other (G) was a diploid-triploid-tetraploid mosaic (fig. 1). The histogram of the cells from the diploid control (goldfish) had a single peak at channel 92. The distribution of cells from offspring (F) showed a single peak at the triploid position (channel 134). The DNA value for this offspring was 1.46 times that of the control fish. The cells from offspring (G) were distributed among three peaks corresponding to diploid (channel 84), triploid (channel 130) and tetraploid (channel 167). The DNA values of each cell population were in the ratio of 1:1.55:1.99. The number of diploid cells (2918) was almost equal to that of tetraploid cells (2703). The triploid peak was low and broad in this mosaic offspring. These data, put together, indicated distinctly that

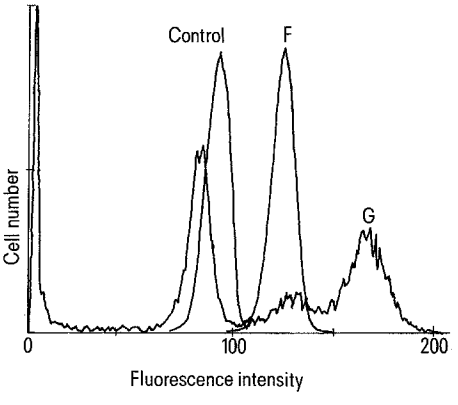

Figure 1. FACS profiles of two male specimens. 5×10^4 (diploid control and F) or 5×10^3(G) cells were counted.

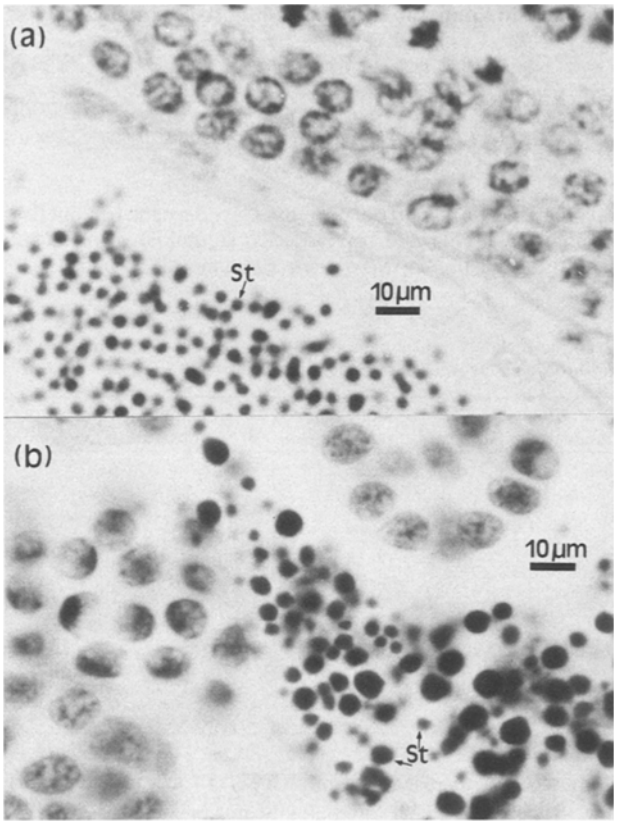

Figure 2. Photomsicrographs of histological sections from testes of bisexual diploid male (a) and diploid-triploid-tetraploid mosaic specimen, G (b). St: spermatid.

diploid-triploid or diploid-triploid-tetraploid mosaics were present in the progeny.

Mosaicism was also found in the testis of the offspring (G). Figure 2(a) shows a cross-section of the testis of bisexual diploid male ginbuna. Many spermatids that had undergone meiosis were observed and the mean diameter of spermatids was 2.7 ± 0.3 μm (mean ± standard deviation, n = 20). On the other hand, the spermatids produced in the mosaic offspring (G) apparently differed in size (fig. 2, b). In addition to the haploid size spermatids (2.2–3.0 μm), spermatids 1.4–2.3-fold larger (3.8–6.2 μm) than haploid ones were observed.

Though it is obscure why the mosaicism occurred under the present experimental conditions, a possible explanation is that the mosaicism arose as a result of some mitotic errors after chromosome replication. A diploid cell may arise by the loss of four sets of chromosomes from a triploid cell during mitosis, and a tetraploid cell may be produced as a result of unequal mitotic division of a triploid into diploid and tetraploid cells. The numbers of diploid and tetraploid blood cells were almost equal in the offspring (G), which may reflect the above possibility. If it occurs early in development, many types of cells will be mosaics or if it occurs late in development, some organs or tissues may involve mosaic cells while others may not. The offspring (C,D) were diploid in erythrocytes, but they accepted the grafts from triploid offspring, and showed the same isozyme phenotypes as triploid ones in electrophoretic analysis. They might also be mosaics, and contain triploid cells in other types of cells.

Examples of diploid-triploid and diploid-tetraploid mosaicism have also been reported in some mammalian species[13], and recently, diploid-triploid mosaic individuals have been found in natural populations of side-necked turtles *(Platemys platycephala)*[14]. In the mosaic turtles, only diploid cells underwent meiosis in males and haploid gametes were produced. In the mosaic ginbuna (G), germ cells might also have consisted of diploid and polyploid (triploid and/or tetraploid) cells. Diploid germ cells might undergo meiosis and develop into haploid gametes during spermatogenesis, whereas polyploid cells might lack meiosis as reported in the oogenesis of polyploid ginbuna. An alternative hypothesis is that aneuploid spermatids might result from unequal meiotic division. This possibility has been proposed by our co-workers[15]. They have observed rod-shaped bi-, tri-, and multi-valent chromosomes in some metaphases during the spermatogenesis of naturally produced triploid male and artificially sex-reversed male ginbuna. Unequal separation of chromosomes might produce aneuploid spermatids.

The sex-determining mechanism in some lower vertebrates is still not well established[16]. In the progeny of triploid ginbuna (10S22), six of seven individuals were male, and two-thirds of the males had diploid cells. In the goldfish *(C. a. auratus)*, it has been ascertained that the male shows a heterogamety of the XX/XY type[17], and a YY male is viable[18]. But as the sex chromosome composition of triploid ginbuna has not been analyzed, it is obscure why male individuals were produced in this progeny.

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