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# Chromosome Synapsis and Recombination during Meiotic Division in Gynogenetic Triploid Ginbuna, *Carassius auratus langsdorfii*

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**Abstract** Electron microscope examinations of primary oocytes of gynogenetic triploid ginbuna (*Carassius auratus langsdorfii*) and diploid gengoroubuna (*Carassius auratus cuvieri*) were carried out. Typical synaptonemal complexes were observed in both subspecies. In addition, clear differences in PGM-A electrophoretic patterns between a female parent ginbuna and her offspring were detected. It was concluded that synapsis and recombination occur between at least some homologous chromosomes in triploid ginbuna.

Ginbuna (*Carassius auratus langsdorfii*) has a wide distribution throughout Japan, and a high female to male ratio has often been observed in this fish (Okada and Nakamura, 1948). Kobayashi et al. (1970) made a karyological study of ginbuna and concluded that populations in the Kanto district might comprise both triploid ($3n=156$) and tetraploid ($4n=206$) forms. They discussed the mechanism of gynogenesis with respect to the persistence of unisexual polyploid populations. Subsequently, it was observed that during oogenesis of triploid ginbuna the first meiotic division did not occur, but rather the oocyte underwent a homotypic nuclear division as in somatic cells (Kobayashi, 1976). In addition, the egg developed by gynogenesis, without normal fertilization (Kobayashi and Ochi, 1972). Ojima and Asano (1977) observed similar patterns in triploid ginbuna from Lake Biwa. On the other hand, Kojima et al. (1984) observed the partial pairing of homologue-like chromosomes at the zygotene stage in triploid ginbuna.

In the present study we tried to clarify whether or not homologous chromosomes undergo synapsis and recombination during the meiotic divisions in triploid ginbuna.

## Materials and methods

Ginbuna and gengoroubuna (*Carassius auratus cuvieri*) were obtained from the Fisheries Experimental Station of Saitama Prefecture in 1987. Loach (*Misgurnus anguillicaudatus*) were bought from a fish market in Tokyo.

The level of ploidy was ascertained by the chromosome number and/or DNA content of somatic cells. Chromosome preparations were made from leucocyte cultures after Ojima et al. (1970). DNA content of red blood cells (RBC) was measured by flow cytometry after propidium iodide (PI)-staining (Allen, 1983).

Artificial insemination was performed between the eggs of triploid ginbuna and the sperm of loach. The offspring of gengoroubuna were collected at the same time. The hatched fry were reared until 100 days after hatching. The young fish were dissected and their gonads removed carefully under a stereoscopic microscope. Small pieces of the gonad were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 12 hours at 4°C, and post-fixed in 2% osmic acid in the same buffer for 1 hour at 4°C. Ultrathin sections were made after dehydration through a graded ethanol series and embedding in Queto 812. These sections were stained with uranyl acetate and lead citrate, and observed with a Model H-7000 (Hitachi Co. Ltd.) electron microscope. The electrophoretic patterns of PGM(phosphoglucomutase)-A were analysed. Liver tissue was removed and homogenized with 10 mM Tris-HCl (pH 6.8). The supernatants were electrophoresed on a horizontal agarose gel (0.9%). Buffer systems employed and enzyme staining followed Sakaizumi et al. (1983), and Shaw and Prasad (1970), respectively.

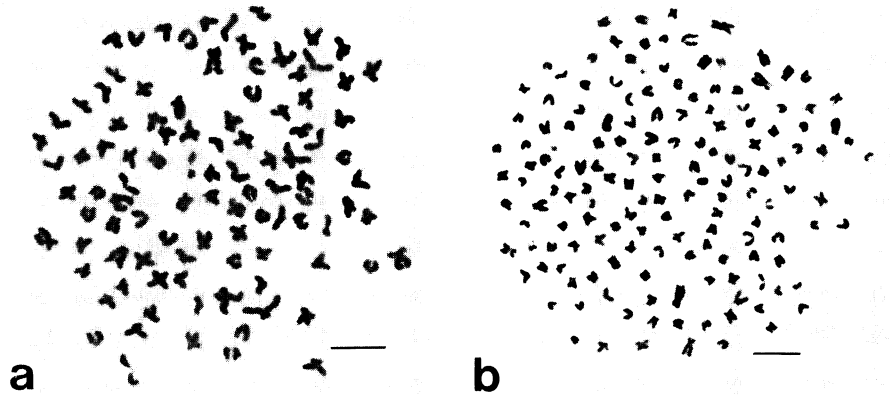


Fig. 1. Mitotic metaphase chromosomes from (a) gengoroubuna (*Carassius auratus cuvieri*) and (b) ginbuna (*Carassius auratus langsdorfii*). Bar indicates 5 μm.

## Results

**Ploidy level.** Mitotic figures of gengoroubuna and ginbuna are shown in Fig. 1. The chromosome number was 100 in gengoroubuna and 154 in ginbuna. In flow cytometry, the DNA content of RBC of triploid ginbuna and diploid gengoroubuna was 136.17±2.25 (n=12) and 100±1.76 (n=10), respectively, relative to diploid goldfish (*C. auratus auratus*) adjusted to a channel value of 100.

**Electron microscopy.** Electron microscope examinations of primary oocytes of triploid ginbuna and diploid gengoroubuna showed synaptonemal complexes between pairs of bivalent (Fig. 2) or trivalent chromosomes. The primary oocytes of both fish with synaptonemal complexes were at the chromatin-nucleolus stage, with a diameter of 10–15 μm. Viewed under light microscope, they had a large nucleus, including a single, central nucleolus, with chromosome strands distributed throughout the former (Fig. 2a). The synaptonemal complex in both fish consisted of two lateral components and a central region. The central region was about 80 nm in width, each lateral component being about 25 nm in width. (Fig. 2b–d). Clear differences in the synaptonemal complexes were not seen between triploid ginbuna and diploid gengoroubuna.

**Zymograms.** The electrophoretic patterns of the monomeric enzyme PGM-A were analysed in triploid ginbuna and diploid gengoroubuna (Fig. 3). The liver tissues of triploid ginbuna showed one, two or three bands, whereas in diploid gengoroubuna, they showed either one or two bands, either one seemingly corresponding to one of the three bands of the triploid ginbuna. These facts suggested that triploid ginbuna and diploid gengoroubuna had a similar PGM-A gene locus. Thus, diploid gengoroubuna, having two sets of chromosomes, carried at most two alleles (a1 and a2) on the PGM-A locus, one band showing the homozygous condition and two bands indicating the heterozygous condition. On the other hand, triploid ginbuna, with three sets of chromosomes, carried at most three alleles (a1, a 2 and a3) on the PGM-A locus. Therefore, the triploid ginbuna showing two or three bands were probably heterozygous, those showing one band being homozygous.

The comparison between the PGM-A electrophoretic pattern of the mother ginbuna and her offspring are shown in Fig. 4. In this case the mother (P) seemed to be heterozygous having three bands. Eight of the 10 offspring were very similar to the mother, having three bands, with the remaining two (Nos. 3 and 8) having only two bands.

## Discussion

Cherfas (1966) analysed the cytological process of meiosis in the silver crusian carp *C. auratus gibelio*, and assumed that at the synapsis, crossing-over and reductional division of homologous chromosomes did not occur. In *Poeciliopsis*, Cimino (1972) observed the formation of hexaploid oogonia by endo-

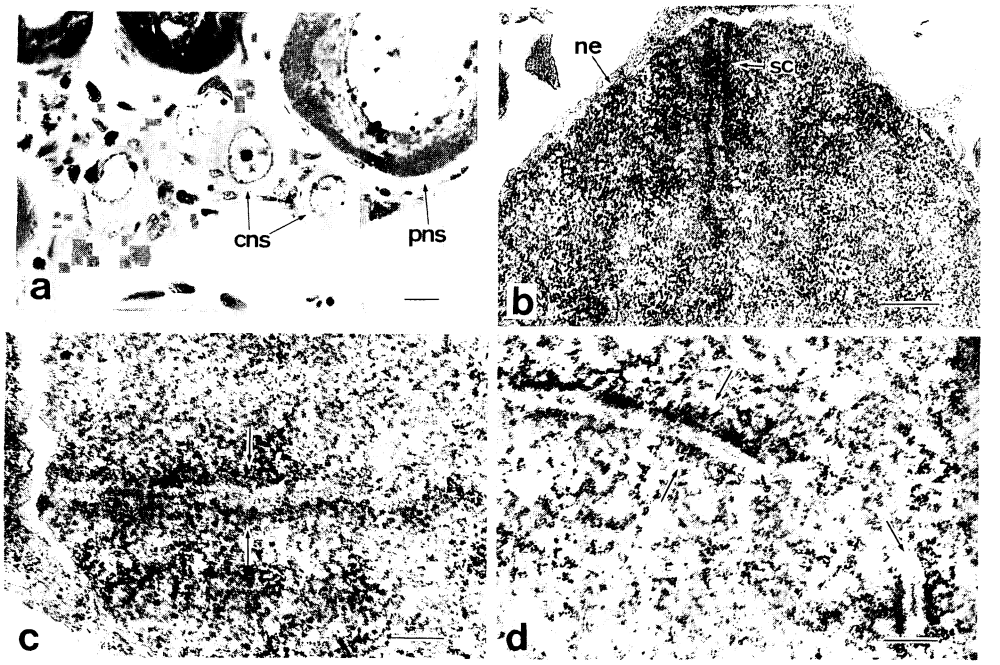


Fig. 2. Cytological observations of primary oocytes of triploid ginbuna and diploid gengoroubuna. (a) Light microscopy of primary oocyte of triploid ginbuna. Chromatin-nucleolus stage (cns) and peri-nucleolus stage (pns). Bar indicates 10 $\mu$m. (b) Electron microscopy of a primary oocyte (chromatin-nucleolus stage) of triploid ginbuna. The synaptonemal complexes (sc) are attached to the nuclear envelope (ne). Scale indicates 0.4 $\mu$m. (c) Higher magnification of (b). Arrows show the synaptonemal complexes. Bar indicates 0.2 $\mu$m. (d) Electron micrograph of primary oocyte of diploid gengoroubuna. Arrows show the synaptonemal complexes. Scale bar indicates 0.2 $\mu$m.

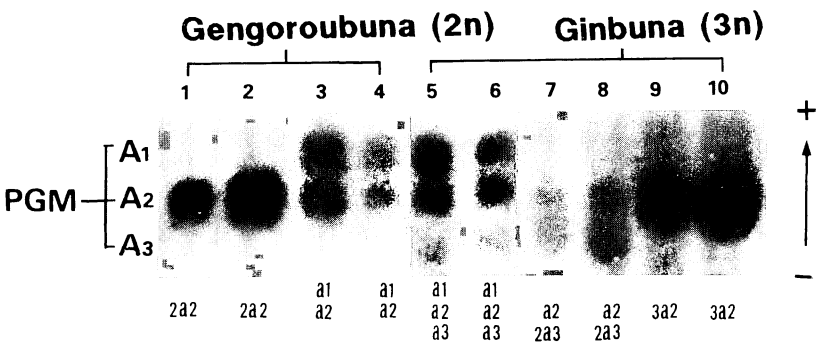


Fig. 3. Typical electrophoretic patterns of phosphoglucomutase (PGM-A) from the liver of triploid ginbuna (no. 5–10) and diploid gengoroubuna (no. 1–4).

mitosis. Thereafter, conventional meiosis proceeded, with triploid chromosomes being restored. In this way, sister-replicate pairing conserved the genotype of the clone. Monaco et al. (1984) undertook electron microscope examinations of primary oocytes of unisexual *Poecilia formosa*, but found only single chromatids and no synaptonemal complexes. On the other hand, Kobayashi (1976) reported that

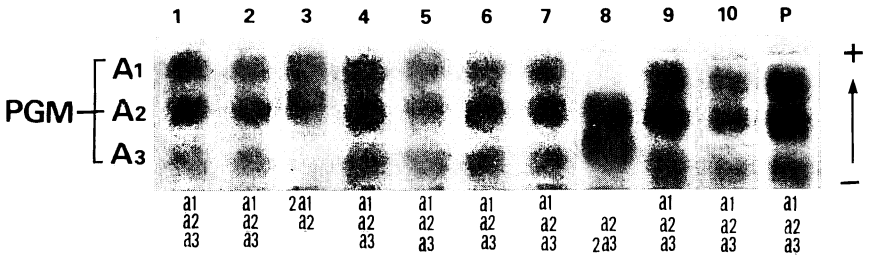


Fig. 4. Comparison of electrophoretic patterns of PGM-A in the liver of mother (P) and offspring (no. 1–10) triploid ginbuna.

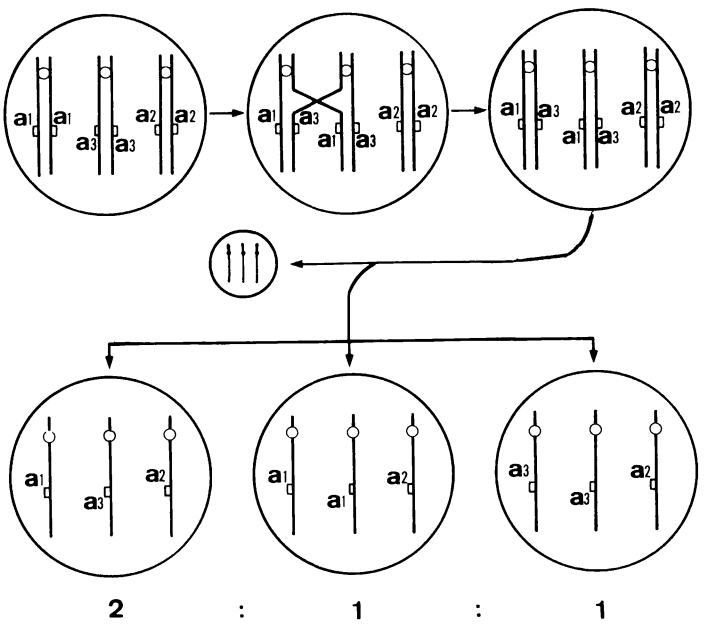


Fig. 5. Diagram illustrating the production of three types of the gametes and their ratio (below) through the recombination of three homologous chromosomes, in triploid ginbuna during meiosis prophase I.

triploid ginbuna did not undergo the first meiotic division. Kojima et al. (1984) observed cytologically the behavior of chromosomes during meiosis in triploid ginbuna, and concluded that pairs of homologue-like chromosomes may have undergone synapsis at the zygotene stage.

In the present study, electron microscope examinations of the primary oocytes of ginbuna have clearly shown characteristic synaptonemal complexes.

Polymorphisms of the monomeric enzyme PGM are known from many fish species. In the common carp, three phenotypes were found (Brody et al. 1979). In the crucian carp however, there have been few reports of PGM phenotypes. In this study, the slowest band, which had no variations and was stained intensely by MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide], was found in both gengoroubuna (2n) and ginbuna (3n). On the faster side, gengoroubuna had two bands, while ginbuna had three. It appeared, therefore, that both species had two PGM loci, the slower one being PGM-B and the faster one PGM-A.

It is suggested that the mother had three homologous chromosomes carrying three alleles (a1, a2 and a3) on the PGM-A locus, and that recombination of a1 and a3 occurred between their respective chromosomes at prophase I of meiosis. Thus three types of gametes, PGM-A (a1, a2 and a3), PGM-A (2a1, a2), PGM-A (a2, 2a3), would result following

the second meiotic division. In this way, only two bands of PGM-(2A1, A2) or PGM-(A2, 2A3) would occur (Fig. 5). Conceivably, this could be the reason why some of the juveniles possessed only two bands of PGM-A isozymes.

It is concluded therefore, that synapsis and recombination occurs between at least some homologous chromosomes in triploid ginbuna.

Monaco et al. (1984) classified gynogenesis of fish into two types: ameiotic and meiotic. In the ameiotic type, such as *Poecilia formosa*, synapsis, bivalent formation and reduction in chromosome number were not observed (Monaco et al. 1984). Conversely, in the meiotic type, such as *Poeciliopsis* (Cimino, 1972), premeiotic endoreduplication occurs, the chromosomes synapse and cross over, and two successional divisions occur. According to our study, ginbuna is classified neither as ameiotic nor meiotic, because the first meiotic division is suppressed after synapsis and recombination occurs between some homologous chromosomes.

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## 3倍体性雌性発生ギンブナの配偶子形成における相同染色体の対合と乗換え

張　峰・尾城　隆・隆島史夫

天然雌性発生3倍体性ギンブナの再生産機構を解明するために，その卵形成過程の電顕および光顕観察を行うとともに，ゲル電気泳動法により母子間のアイソザイムパターンの変異を分析した．その結果，(1) ギンブナ卵形成過程の第1減数分裂の前期に，相同染色体間の対合を示すシナプトネマ構造が観察され，(2) 母子間のPGMアイソザイムパターンに少数例ながら明らかな相違が認められた．従って，ギンブナの相同染色体間において，少なくとも部分的には対合と乗換えが起こるものと推察された．

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