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# Aberrant meioses in diploid and triploid progeny of gynogenetic diploids produced from eggs of natural tetraploid loach, *Misgurnus anguillicaudatus*

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A b s t r a c t . Gynogenetic diploids were produced from the eggs of natural tetraploid loach *Misgurnus anguillicaudatus* (Pisces: Cobitidae) without any manipulation for chromosome duplication. When eggs of a four-year-old diploid gynogenetic individual were fertilized with spermatozoa of specimens from normal diploid and natural tetraploid lines, viable diploid and triploid progeny were produced, respectively. Thus, egg nucleus of the diploid gynogen is haploid. In the gonads of diploid progeny, diploid (2n = 50) and tetraploid (4n = 100) mitotic metaphases were observed. The majority of oocytes (76%) showed regular 25 bivalents as in normal diploids, but the other 16% showed a few univalents. The remaining 8% exhibited about 50 bivalents, suggesting chromosome duplication by premeiotic endomitosis. In the testes, a few spermatocytes (6%) showed normal 25 bivalents, but 86% contained various number of univalents and the remaining 8% contained about 50 bivalents. No peaks of spermatozoa were identified in the testis by flow cytometry. In the triploid progeny, triploid (3n = 75) and hexaploid (6n = 150) mitotic metaphases were observed in both ovaries and testes. Most meiotic figures (about 90%) contained approximately 25 bivalents and 25 univalents in both sexes; the rest contained approximately 75 or more bivalents. Spermatozoa were not identified in the testis by flow cytometry. Thus, the diploid males between the diploid gynogens and common diploid, and both sexes of triploids between the diploid gynogens and tetraploid, show aberrant meioses such as frequent formation of univalents, but the diploid females seem to be less affected.

**Key words**: polyploid, gynogenesis, univalent

## Introduction

In the loach *Misgurnus anguillicaudatus* (Pisces: Cobitidae), most individuals in Japanese populations have 50 chromosomes (2n = 50), but tetraploid individuals with 100 chromosomes have been found in the specimens obtained from fish dealers (O j i m a & T a k a i 1979, A r a i et al. 1991a). The individuals with 100 chromosomes are considered genetically true tetraploids with four sets of homologous chromosomes, because viable gynogenetic and androgenetic diploids were produced using their gametes without treatment to double their chromosomes (A r a i et al. 1993, 1995). Although the actual origin of these tetraploids has not been identified and no tetraploid individuals were found in a total 1815 wild individuals collected from 33 locations in Japan (Z h a n g & A r a i 1999a), the same species from Hubei Province, China was reported to be tetraploid with 100 chromosomes (L i et

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al. 1983). Thus, the tetraploid specimens are likely to originate from the Asian continent and are genetically different from common diploid loach in Japan.

Triploid males produced by fertilizing eggs of common diploids with spermatozoa of tetraploids formed no spermatozoa and were absolutely sterile (Matsubara et al. 1995, Zhang & Arai 1996), but triploid females simultaneously laid large and small eggs, with triploid and haploid female pronucleus after fertilization, respectively (Matsubara et al. 1995). Multilocus DNA fingerprinting revealed that most triploid gynogens artificially induced from the large eggs of such triploids belonged to a clone genetically identical to their mother (Arai & Mukaino 1997). Cytogenetic studies demonstrated that the unreduced triploid eggs were developed from hexaploid oocytes generated by premeiotic endomitosis, whereas haploid eggs were produced from triploid oocytes with both bivalents and univalents in the first meiotic prophase, but eliminated unpaired univalents during later processes of meiosis (Zhang et al. 1998).

These atypical mechanisms of reproduction may be caused by the imbalance of genomes between diploid and tetraploid parents. To obtain further insight into the genetic incompatibility between normal diploid and natural tetraploid loach, diploid gynogens were artificially produced by inseminating eggs of tetraploids with UV-irradiated heterospecific spermatozoa. Thereby, the resultant diploid gynogens should have two genomes (sets of chromosomes) derived from the tetraploid loach.

In the present study, we fertilized the eggs of a mature diploid gynogen with spermatozoa of normal diploid and natural tetraploid lines, respectively, and then produced diploid progeny comprising one genome from diploid and one genome from tetraploid as well as triploid progeny comprising three genomes from tetraploid loach. We observed the survival potential of these two types of progeny as well as metaphase spreads of premeiotic cells and counted chromosomes to examine the occurrence of endomitosis during gametogenesis. We also observed chromosome configurations in the early meiotic stages of oocytes and spermatocytes to examine the abnormalities of meiotic chromosome behaviors in the diploid and triploid progeny.

## Materials and Methods

Tetraploid individuals of loach were found among the specimens obtained from a fish dealer in Mie Prefecture, Japan (Arai et al. 1991a) and tetraploid line was made by mating between them (Arai et al. 1991b, 1993). Normal diploid individuals were caught in paddy field in Sera town, Hiroshima Prefecture, Japan. Diploid gynogens (G2n) were produced by inseminating eggs of a tetraploid female with UV-irradiated carp (*Cyprinus carpio*) spermatozoa in 1993 (Zhang & Arai 1996). One surviving diploid gynogen reached maturation in 1997. Using the methods previously reported (Arai et al. 1993), the eggs of the mature gynogen were fertilized with spermatozoa of two normal diploid males (A and B; G2n × 2nA and G2n × 2nB, respectively). The eggs were also fertilized with spermatozoa of a laboratory-reared tetraploid male (G2n × 4n) that was selected from the progeny of a natural tetraploid pair.

The ploidy status of all parental fish used in the crosses, as well as six- and eight-month-old progeny, was determined by DNA-content flow cytometry according to the method described by Zhang & Arai (1996). Nine-day-old progeny were minced finely with forceps in two drops of Ca, Mg-free phosphate buffered saline solution (CMF-PBS,

Zhang & Arai 1996) in 1.5 ml microcentrifuge tubes, and then all suspension pipetted with a 1-ml syringe equipped with a 22 gauge needle after adding 0.5 ml of CMF-PBS. The cell suspensions were centrifuged at 130g (1200 rpm) for 5 min, decanted, stained and DNA content was measured by flow cytometry. Chromosome preparation of eyed embryos and hatched progeny was performed according to Arai et al. (1991b).

At eight months of age, two females and four males (4.5–6.0 cm in total length) were taken from each group of G2n × 2nA and G2n × 4n crosses and sacrificed for chromosomal examination after confirmation of their ploidy by flow cytometry. The testes of two diploids and two triploids were assayed by flow cytometry (Zhang & Arai 1996) to detect the ploidy status of gonadal cells. The remaining individuals were used for chromosome observation in the gill, spleen and gonad from eight-month-old fish according to the previously described method (Zhang et al. 1998).

## Results

### Survival of the progeny

The gynogenetic individuals induced from eggs of tetraploids were confirmed to be diploid by flow cytometry. When a mature gynogenetic individual was crossed with two normal diploids (G2n × 2n) and one tetraploid (G2n × 4n), the mean egg diameter of this gynogenetic progeny (1.11±0.03 SD mm, n = 35) was not different from that of normal diploid one (1.11±0.06 SD mm, n = 49).

One diploid male (A) and a tetraploid male gave fair fertilization rates (Table 1), whereas the other diploid male (B) showed lower fertilization rate. A higher hatching rate was observed in G2n × 2nA, but a low rate of normal progeny (4.1%) was recorded because 82.7% (321/ total 388) of progeny were abnormal at hatching. At three weeks after fertilization, a small proportion of individuals survived (Table 1). G2n × 2nB cross showed poor rates of hatching and normal progeny(0.7%). Abnormal progeny comprised 74.2% (72/total 97) at hatching and few individuals survived at three weeks after fertilization. Comparatively, G2n × 4n cross showed lower hatching rate than G2n × 2nA cross did, but higher rate of normal progeny (7.4%). A small proportion of progeny were abnormal at hatching (Table 1), a small proportion survived to three weeks after fertilization.

### Ploidy status of the progeny

Of the 31 specimens from the G2n x 2nA cross, 29 were diploid and two were triploid (Table 2). On the contrary, all 18 specimens from the G2n x 4n cross were triploid.

**Table 1.** Developmental potential of the progeny of the diploid gynogen (G2n) produced from eggs of natural tetraploid loach when crossed with common diploid (G2n × 2n) and natural tetraploid (G2n × 4n). 1: Relative to no. of eggs used, 2: Relative to no. of hatching, 3: Relative to no. of eggs used; Recorded at three weeks after fertilization.

| Cross | No. of | Fertilization | | Hatching | | Normal progeny | | Survival | |
|---|---|---|---|---|---|---|---|---|---|
| | eggs | No. | (%)[1] | No. | (%)[1] | No. | (%)[2] | No. | (%)[3] |
| G2n × 2n A | 1652 | 1188 | 71.9 | 388 | 23.5 | 67 | 17.3 | 112 | 6.8 |
| G2n × 2nB | 3731 | 1836 | 49.2 | 97 | 2.6 | 25 | 25.8 | 21 | 0.6 |
| G2n × 4n | 1235 | 982 | 79.5 | 105 | 8.5 | 92 | 87.6 | 68 | 5.5 |

**Table 2.** Ploidy status of the progeny of the diploid gynogenetic individual (G2n) produced from eggs of natural tetraploid loach when crossed with common diploid (G2n × 2n A) and natural tetraploid (G2n × 4n) loaches. 1: Ploidy was determined by counting chromosome numbers (CHM), 2: Ploidy was determined by DNA -content flow cytometry (FCM).

| Cross | Age | Ploidy determination | No. of sample | Ploidy | |
|---|---|---|---|---|---|
| | | | | Diploid (2n) | Triploid (3n) |
| G2n × 2n A | Eyed embryo | CHM[1] | 9 | 9 | 0 |
| | Hatched progeny | CHM | 5 | 5 | 0 |
| | 9-day-old progeny | FCM[2] | 2 | 2 | 0 |
| | 6-month-old fish | FCM | 9 | 7 | 2 |
| | 8-month-old fish | FCM | 6 | 6 | 0 |
| | Total | | 31 | 29 | 2 |
| G2n × 4n | Eyed embryo | CHM | 5 | 0 | 5 |
| | Hatched progeny | CHM | 2 | 0 | 2 |
| | 9-day-old progeny | FCM | 2 | 0 | 2 |
| | 6-month-old fish | FCM | 3 | 0 | 3 |
| | 9-month-old fish | FCM | 6 | 0 | 6 |
| | Total | | 18 | 0 | 18 |

## Chromosomes of gonadal cells

In two diploid females derived from the G2n × 2nA cross (Table 3), all 56 examined cells from the gills and spleen showed the diploid chromosome number (2n = 50) (Fig. 1a), whereas in their ovaries 6.5% of the metaphase spreads were tetraploid (4n = 100) (Fig. 1b).

All 92 somatic cells of the two diploid males were diploid (Table 3). In the testes, however, 13% were polyploid (including tetraploid, hexaploid and octaploid) metaphase spreads, although the other cells were diploid (Table 3).

Somatic cells in the gills and spleen of two 8-month-old triploid (G2n × 4n) females showed only triploid metaphase figures (Table 3, Fig. 1c). In contrast to the triploidy of somatic tissues, the ovaries of one female contained about 17% hexaploid cells (Fig. 1d). In

**Table 3.** Ploidy status of somatic (gill / spleen) and gonadal (ovary / testis) cells in the diploid (G2n × 2n A) and triploid (G2n × 4n) progeny of the diploid gynogenetic individual produced from eggs of natural tetraploid loach.

| Cross | Sex | Fish | Gill/spleen | | | Ovary/testis | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | # | 2n | 3n | Sum | 2n | 3n | 4n | 6n | 8n | 9n | 12n | Sum |
| G2n × 2n A | Female | 1 | 24 | 0 | 24 | 15 | 0 | 2 | 0 | 0 | 0 | 0 | 17 |
| | | 2 | 32 | 0 | 32 | 28 | 0 | 1 | 0 | 0 | 0 | 0 | 29 |
| | | Sum | 56 | 0 | 56 | 43 | 0 | 3 | 0 | 0 | 0 | 0 | 46 |
| | Male | 1 | 47 | 0 | 47 | 63 | 0 | 9 | 1 | 4 | 0 | 0 | 77 |
| | | 2 | 45 | 0 | 45 | 124 | 0 | 11 | 2 | 1 | 0 | 0 | 138 |
| | | Sum | 92 | 0 | 92 | 187 | 0 | 20 | 3 | 5 | 0 | 0 | 215 |
| G2n × 4n | Female | 1 | 0 | 28 | 28 | 0 | 25 | 0 | 5 | 0 | 0 | 0 | 30 |
| | | 2 | 0 | 7 | 7 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 8 |
| | | Sum | 0 | 35 | 35 | 0 | 33 | 0 | 5 | 0 | 0 | 0 | 38 |
| | Male | 1 | 0 | 31 | 31 | 0 | 62 | 0 | 12 | 0 | 2 | 2 | 78 |
| | | 2 | 0 | 21 | 21 | 0 | 36 | 0 | 5 | 0 | 0 | 2 | 43 |
| | | Sum | 0 | 52 | 52 | 0 | 98 | 0 | 17 | 0 | 2 | 4 | 121 |

the ovaries of the other female, only 8 triploid cells were observed because of few readable metaphase spreads, and no hexaploid cells were found.

Testes of the triploid males contained 14% hexaploid cells, and 1.7% 9n and 3.3% 12n cells (Table 3). In the testes, 19% were polyploid.

### DNA content of testicular cells

Flow cytometrical analysis of DNA content of the testes of normal diploids revealed one prominent peak of 1C spermatozoa, one low peak of 2C somatic cells and secondary

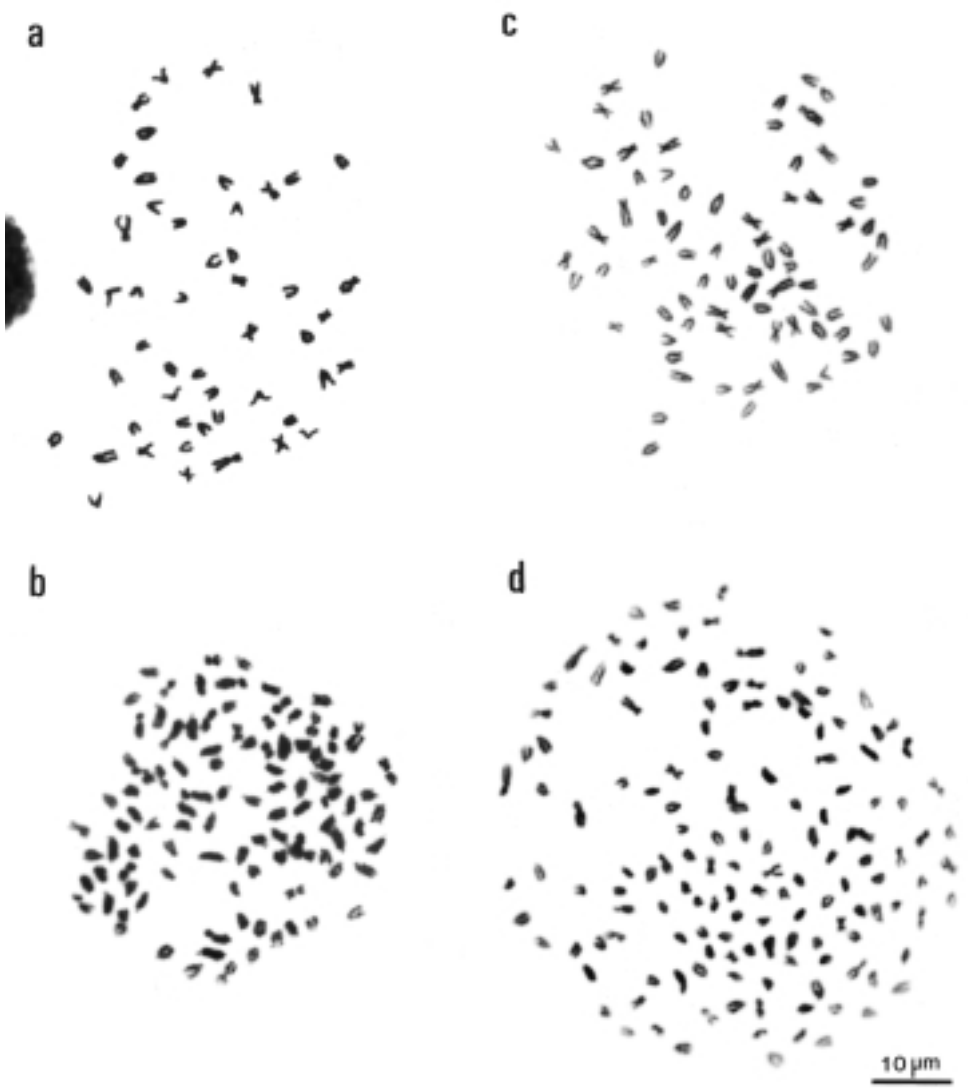


**Fig. 1.** Mitotic metaphases prepared from diploid (G2n × 2n) and triploid (G2n × 4n) progeny. (a) diploid cell (2n = 50) from gill of the diploid progeny, (b) tetraploid cell (4n = 100) from ovary of the diploid progeny, (c) triploid cell (3n = 75) from gill of the triploid progeny, (d) hexaploid cell (6n = 150) from ovary of the triploid progeny.

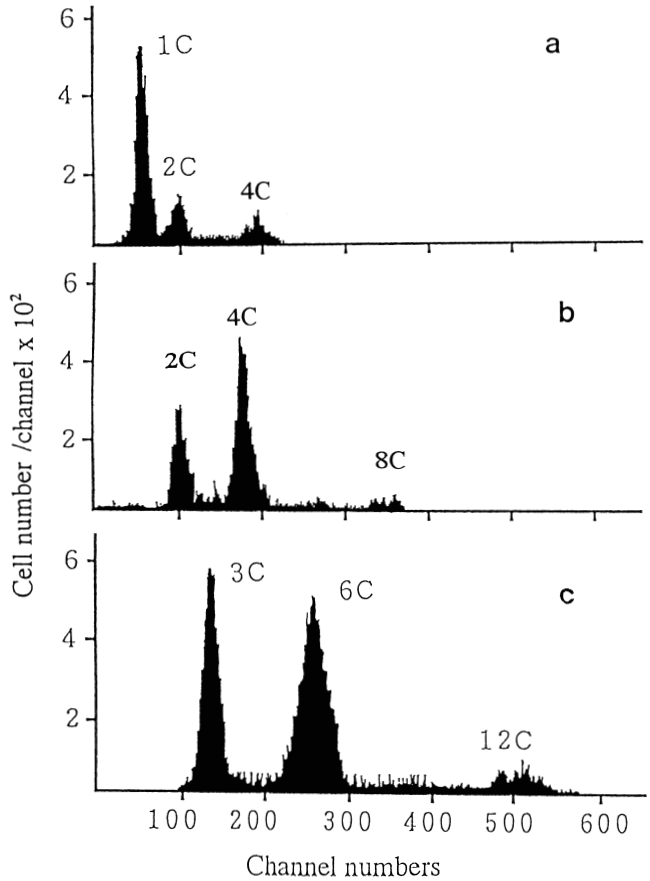


**Fig. 2.** Flow cytometric histograms in testes of the progeny of diploid gynogen produced from eggs of tetraploid. (a) normal diploid showing 1C spermatozoa, interphase 2C and DNA replicated 4C cells, (b) diploid progeny from G2n x 2nA cross showing 2C, 4C and a small 8C ranges of cells but no peak representing spermatozoa and spermatids, (c) triploid progeny from G2n x 4n cross showing 3C, 6C and a small 12C ranges of cells but no peak corresponding to spermatozoa and spermatids. The 2C and 4C peaks in diploid progeny and the 3C and 6C peaks in triploid progeny are markedly higher than the corresponding 2C and 4C peaks of normal diploid.

spermatocytes, and one low 4C peak of DNA replicated somatic cells and primary spermatocytes (Fig. 2a). Nevertheless, the testes of diploid progeny derived from the G2n × 2nA cross exhibited two distinct peaks of 2C and 4C cells (Fig. 2b). Both peaks were markedly higher than the corresponding peaks of normal diploids. A low peak in the 8C range, representing the G2 phase of tetraploid cells, was also detected. However, no peak representing spermatozoa was recognized (Fig. 2b). Similarly, triploid males derived from the G2n × 4n cross contained unusually high peaks in the 3C and 6C ranges and one low peak in the 12C range, respectively (Fig. 2c). No peak of lower ploidy level representing spermatozoa was detected.

## M e i o s e s

In the ovaries of eight-month-old diploids from the G2n x 2nA cross, 37 pachytene oocytes were observed and they were categorized into two groups according to their sizes (Table 4):

small oocytes represented 91.9% of which 75.7% contained only 25 bivalents (Fig. 3a), whereas the remaining 16.2% of small oocytes showed bivalents fewer than 25 and four, six, or infrequently 20 univalents (Fig. 3b,c); large oocytes represented 8.1% and had 50 or close to 50 bivalents (Fig. 3d).

In the two diploid male progeny from G2n × 2nA cross (Table 4), spermatocytes were observed and were categorized into two types. 91.7% were small spermatocytes of which 5.6% showed regular 25 bivalents (Fig. 3e); whereas the other 86.1% spermatocytes contained 21 to 24 bivalents and 2 to 8 unpaired univalents (Fig. 3f-h). These univalents generally congregated in one side of the cell and some of them showed delayed condensation (Fig. 3g,h). Large spermatocytes represented 8.3% and the numbers of chromosome elements were difficult to count exactly. Two showed approximately 50 bivalents and the third one showed about 50 bivalents and a few univalents (Fig. 3i).

Ovaries of triploid progeny from the G2n x 4n cross also had small and large pachytene oocytes (Table 4). The small oocytes represented 91.4% and had about 25 thick elements, probably bivalents, and about 25 thin elements, probably univalents (Fig. 4a). The five large oocytes (8.6%) had approximately 75 to no fewer than 60 countable bivalents and a few thin chromosome elements (Fig. 4b). Neither large nor small oocytes beyond the pachytene stage were observed.

In triploid males, 92.3% of the spermatocytes showed about 25 bivalents and about 25 univalents at meiosis I (Table 4, Fig. 4c). In the remaining spermatocytes (7.7%), 6.7% showed approximately 75 bivalents (Fig. 4d), but the eighth cell gave more than 100 bivalents.

**Table 4.** Variation in numbers of bivalent and univalent chromosomes observed in the diploid (G2n × 2n A) and triploid (G2n × 4n) progeny of the diploid gynogenetic individual produced from eggs of natural tetraploid loach. 1: The numbers of bivalents in these large oocytes / spermatocytes were approximate numbers because of some overlaps.

| Progeny | Sex | No. of | No. of | Small oocytes / spermatocytes | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (Cross) | | fish | meiosis | 25 II+ 0 I | 24 II+ 2 I | 23 II+ 4 I | 22 II+ 6 I | 21 II+ 8 I | 15 II+ 20 I | 25 II+ 25 I | Sum |
| Diploid (G2n × 2n A) | F | 2 | 37 | 28 | 0 | 2 | 3 | 0 | 1 | 0 | 34 |
| | M | 2 | 36 | 2 | 2 | 8 | 15 | 6 | 0 | 0 | 33 |
| Triploid (G2n × 4n) | F | 2 | 58 | 0 | 0 | 0 | 0 | 0 | 0 | 53 | 53 |
| | M | 2 | 104 | 0 | 0 | 0 | 0 | 0 | 0 | 96 | 96 |

| Progeny | Sex | No. of | No. of | Large oocytes / spermatocytes | | | |
|---|---|---|---|---|---|---|---|
| (Cross) | fish | meiosis | | 50 II | 60 -75 II[1] | 100 II[1] | Sum |
| Diploid (G2n × 2n A) | F | 2 | 37 | 3 | 0 | 0 | 3 |
| | M | 2 | 36 | 3 | 0 | 0 | 3 |
| Triploid (G2n × 4n) | F | 2 | 58 | 0 | 5 | 0 | 5 |
| | M | 2 | 104 | 0 | 7 | 1 | 8 |

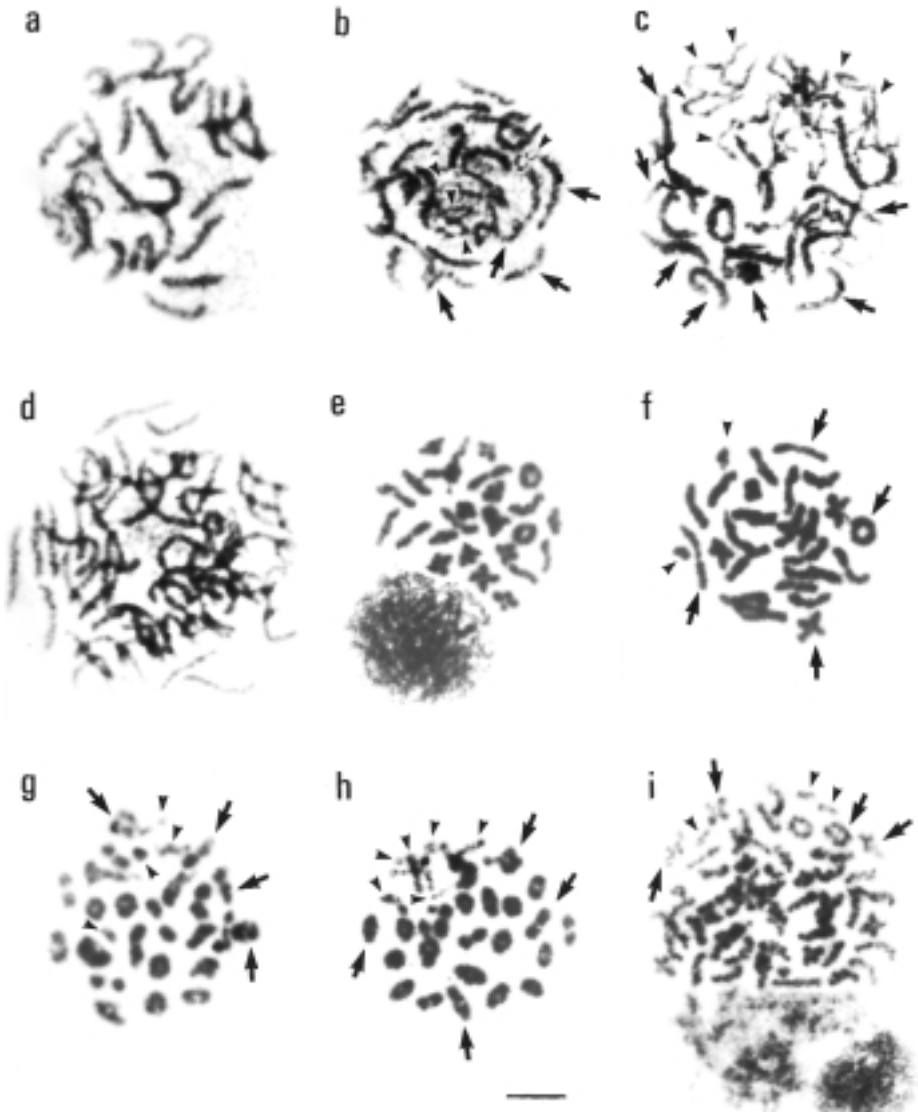


**Fig. 3.** Meiotic chromosome configurations in diploid progeny (G2n × 2nA). Typical bivalents and univalents indicated by arrows and arrowheads, respectively, (a) 25 bivalents in a pachytene stage oocytes, (b) 23 bivalents and 4 univalents in a pachytene stage oocyte, (c) 15 bivalents and 20 univalents in a pachytene stage oocyte, (d) 50 bivalents in a large pachytene stage oocyte, (e) 25 bivalents in a spermatocyte, (f) a spermatocyte showing 24 bivalents and 2 univalents, (g) a spermatocyte showing 23 bivalents and 4 univalents, (h) a spermatocyte showing 22 bivalents and 6 univalents, (i) a spermatocyte of diploid showing approximately 50 bivalents and a few univalents. Some of the univalents in (g) and (h) showed delayed condensation compared with the bivalents.

## Discussion

Viable gynogenetic diploid (G2n) individuals produced from eggs of a tetraploid individual female in 1993 reached maturation in 1996. A large number of mature eggs were obtained from one of the diploid gynogens. Viable diploid and triploid progeny were produced from

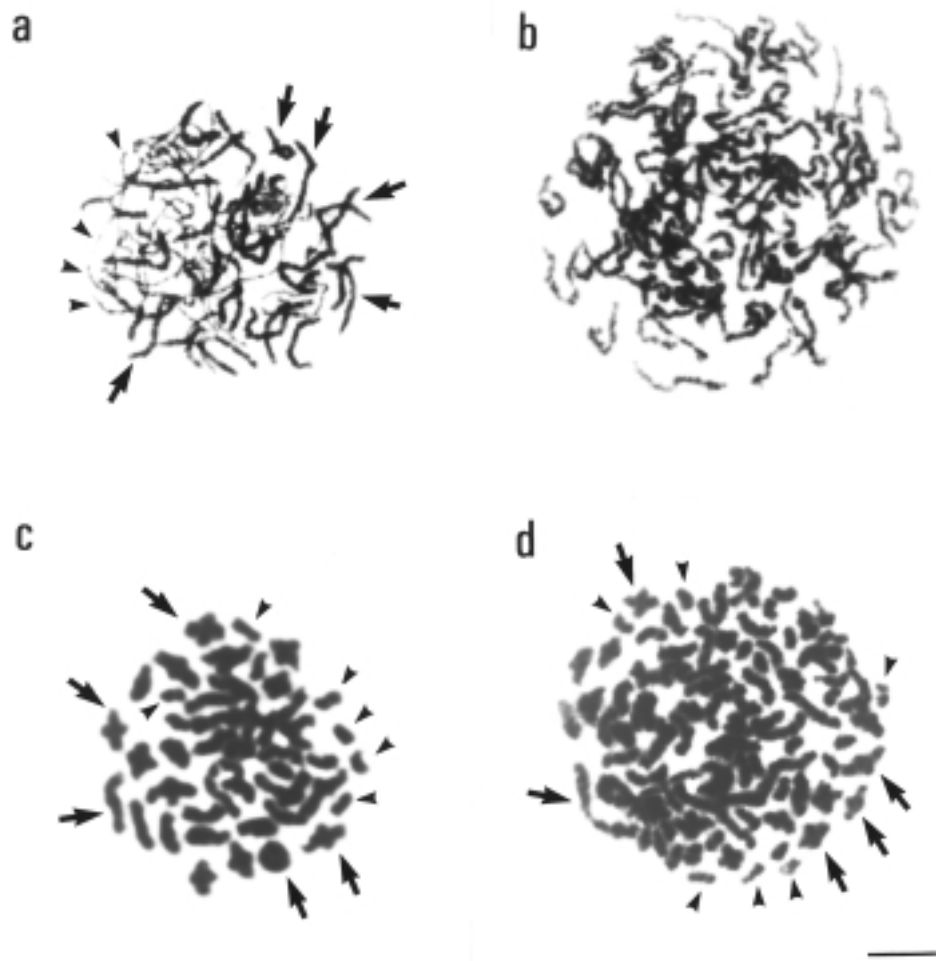


**Fig. 4.** Meiotic chromosomes configurations in triploid progeny (G2n × 4n). Arrows and arrowheads show typical bivalents and univalents, respectively. (a) approximately 25 bivalents and 25 univalents in pachytene stage oocyte of triploid, (b) a large oocyte showing at least 60 bivalents and a few univalents, (c) approximately 25 bivalent and 25 univalent chromosomes in a meiosis I spermatocyte of triploid, (d) a large spermatocyte showing approximately 75 bivalents.

these eggs by fertilization with spermatozoa of diploid (2n) and tetraploid (4n) males, respectively. Although the hatching and normal rates obtained in these crosses (Table 1) were lower than those normally observed in the crosses by common diploid pairs (42 to 68 % in hatching and 42 to 68% in normal rates; S u w a et al. 1994), our results indicated that the gynogenetic diploid (G2n) laid functional haploid eggs. However, low viability of the progeny can be explained by aneuploid eggs spawned by the G2n and subsequent death due to aneuploid embryos. On the other hand, the haploidy of the eggs provided additional evidence for the hypothesis of genetically true tetraploidy of the original grandparent (A r a i et al. 1993, 1995), because the gynogens derived from eggs of tetraploids were not only viable, but also able to form haploid eggs by means of normal meiosis. The small number of

triploid individuals detected in the G2n × 2nA cross might have been caused by spontaneous retention of the second polar body. Similar phenomena have been reported in other fish species (Cuellar & Uyeno 1972, Thorgaard & Gall 1979, Thompson et al. 1981, Ueda et al. 1984, Cherfas et al. 1991, 1995, Flajšhans et al. 1993).

In normal diploid loach, tetraploid metaphase spreads were not observed in the ovaries, but in the testes (Zhang et al. 1998). However, in the gonads of both male and female diploid progeny derived from the G2n × 2nA cross, a small number of tetraploid metaphase spreads were detected. These tetraploid metaphases observed in the gonads suggested the occurrence of premeiotic endomitosis several mitotic divisions before meiosis. A small number of oocytes as well as spermatocytes showed approximately 50 bivalents and were likely to originate from tetraploid oogonia and spermatogonia formed by means of premeiotic endomitosis, as in the case of triploid females produced by crosses between common diploid females and natural tetraploid males (Zhang et al. 1998). At present, the fate of these meiotic cells with 50 bivalents in the gametogenesis is inconclusive. If these 50 bivalents proceed to further meiotic events normally, then unreduced diploid gametes may be generated in the diploid (G2n × 2n) progeny. However, an alternative possibility is that active synchronous division of two or more adjacent spermatogonia may often resemble a metaphase of the single polyploid cell; this may explain the high rates of polyploid cells especially in the testis, since the frequencies of cells with polyploid chromosome numbers or 50 bivalents were higher in the testis than those in the ovary.

In diploid females from the G2n × 2nA cross, the pachytene oocytes normally had 25 bivalents, but a small number of the oocytes had bivalents fewer than 25 as well as four, six, or infrequently 20 univalents. In contrast to female meioses, chromosome pairings in spermatocytes of diploid (G2n × 2nA) progeny were more severely affected; most spermatocytes contained various numbers of univalents. Only a few spermatocytes formed 25 bivalents. Hybridization between different species gave rise to sterility and an increase in the number of univalent was reported in the meioses of hybrids (Nygren et al. 1972, Muller 1979, Okumoto 1980, Shimizu et al. 1997). Even hybridization between geographically different populations sometimes alters meiotic mode and results in formation of more univalents in the offspring than in their parents (Sumida 1994). On the other hand, hybridization often causes sterility of heterozygous sex in the $F_1$ offspring as proposed by Haldane (1922) and discussed by Coyne (1985). Therefore, the occurrence of univalents in both males and females of the diploid progeny, and the severe abnormality in the meioses of males of the diploid progeny, may suggest considerable genetic differentiation between naturally occurring diploid and tetraploid loaches. This finding may suggest a differentiation of the two species. The higher frequency of univalent formation in the diploid males suggests more abnormalities of meioses and subsequent expression of the sterility than those in the females. This was supported by the flow cytometrical results of testes of diploid individuals from the G2n × 2nA cross, which showed the absence of the peak representing spermatozoa. Considering the relatively lower incidence of univalents in the diploid female progeny, the meioses were assumed to be less affected in the females. Thus, females are more likely to proceed normal meiosis and to form mature gametes than the males do. However, at present, it is premature to conclude fertility of the diploid female progeny because we have not yet conducted breeding experiments.

In triploid progeny, most meioses showed formation of equal numbers of 25 bivalents and 25 univalents both in female and male. However, a small number of hexaploid metaphases were observed in the gonads and a few meioses exhibited approximately

75 bivalents. This suggested the synapses between sister chromosomes should be doubled by the premeiotic endomitosis. Thus, chromosomes in triploid females from the G2n × 4n cross behaved in the same way as reported in the triploid females produced by hybridization between normal diploid females and natural tetraploid males (Z h a n g et al. 1998). Large and small pachytene oocytes exhibited no difference between these two kinds of triploids, 2n × 4n (one genome from diploid, two genomes from tetraploid) and G2n × 4n (all three genomes from tetraploid). Similar meiotic configurations have been observed in early pachytene stage oocytes of triploid female loach from artificial introduction (Z h a n g & A r a i 1999b). However, in the testes of the triploids from the G2n × 4n cross in the present study, a large number of meiotic figures were observed to contain both bivalents and univalents. This observation was different from that in triploid males produced from diploid × tetraploid crosses that showed no meiotic figures (Z h a n g et al. 1998), but was similar to the case of artificially induced triploid males of this species (Z h a n g & A r a i 1999b). The differences of meioses among these three biotypes of triploids may be interpreted by their differences in genetic compositions. The triploids from crosses of diploid female x tetraploid male contained chromosomes from both diploid and tetraploid (Z h a n g et al. 1998), whereas the artificially induced triploids contained chromosomes only from diploids (Z h a n g & A r a i 1999b), and the triploids in the present study had chromosomes from only tetraploids. These results may also suggest considerable genetic differentiation of species between common diploids and natural tetraploids in the loach.

The formation of univalents in triploids usually results in aneuploid gametogenesis (A l l e n et al. 1986, B e n f e y et al. 1986, U e d a et al. 1987, Z h a n g & A r a i 1999b, A r a i & I n a m o r i 1999) owing to the odd number of chromosome sets. Thus, both the male and female triploids derived from the G2n × 4n cross are likely to exhibit reduced capacity in reproduction. However, there is a possibility of the infrequent occurrence of small number of unreduced triploid gametes in the triploid progeny, because a small number of hexaploid mitotic metaphases (showing 150 chromosomes) and meiotic figures (showing about 75 bivalents) were observed in the triploid progeny. This suggests involvement of premeiotic endomitosis leading to unreduced gamete formation.

A c k n o w l e d g m e n t s

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