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Extensive karyotype variation in somatic and meiotic cells of the loach *Misgurnus anguillicaudatus* (Pisces: Cobitidae)

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A b s t r a c t. Extensive karyotype variation was found in both somatic and meiotic cells of the progeny in a laboratory-reared family of the loach *Misgurnus anguillicaudatus*. Only one of seven individuals examined showed the standard karyotype, 2n = 50 comprising 10 metacentrics, 4 submetacentrics and 36 telocentrics. However, the other six individuals exhibited hyperdiploid karyotypes due to the presence of additional telocentrics and micro-chromosomes, resulting in chromosome numbers 2n = 51 to 53, or 58 plus 0 to 5 micro-chromosomes within or between individuals. Such inter- and/or intra-individual variation in chromosome number was also observed in primary spermatocytes of three males with an increase of bivalents and univalents involving additional telocentrics, although micro-chromosomes were seldom paired. Accumulation of additional chromosomes was apparent in spermatogonia and spermatocytes with greater incidence than in somatic cells. Euploid and aneuploid loaches were not discriminated by the external morphology, suggesting the observed additional chromosomes to be genetically neutral.

Key words: karyotype, aneuploid, micro-chromosome, B-chromosome

Introduction

In Japanese populations of the loach *Misgurnus anguillicaudatus* (Pisces: Cobitidae), the normal diploid chromosome number is 2n = 50, karyotype comprising 10 metacentric (m), 4 submetacentric (st) and 36 telocentric (t) chromosomes in both sexes (O j i m a & T a k a i 1979, A r a i et al. 1991). Natural triploids with 75 chromosomes have been found in wild populations in Japan (A r a i et al. 1991, Z h a n g & A r a i 1999a). Natural tetraploid loaches with 100 chromosomes were also reported for specimens obtained from dealers (O j i m a & T a k a i 1979, A r a i et al. 1991) as well as those from wild populations in Hubei province, China (L i et al. 1983). Using diploid gametes of natural tetraploids, various cytotypes including gynogenetic diploids, androgenetic diploids, gynogenetic tetraploids, pentaploids and hexaploids were successfully produced by means of chromosome manipulation techniques (A r a i et al. 1993, 1995, 1999, A r a i 2001). Viable hyper-diploid and hyper-triploid aneuploid loaches have been generated by insemination of aneuploid sperm from the artificially induced triploids to haploid and diploid eggs, respectively (Z h a n g & A r a i 1999b, A r a i & I n a m o r i 1999). These results demonstrate that this loach species can tolerate polyploidy as well as aneuploidy to a certain extent and suggest that there may be karyotype variants with additional chromosomes awaiting discovery.

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In the present paper, we report the presence of extensive karyotype variation, as revealed by various numbers of Giemsa-stained additional chromosomes prepared from somatic and meiotic cells of the loach.

Materials and Methods

The specimens used in the present study were laboratory-reared progeny produced from a pair-cross between common diploids (2n x 2n) performed in July 1994. The female parent was collected from a stock population mainly including loaches from fish dealers in Mie prefecture, Japan; thus its origin is unknown. The male parent was caught in paddy fields, Sera town, Hiroshima prefecture, Japan. In 1997, two females and five males (4.7–7.6 cm in total length and 0.5–2.2 g in body weight) from the progeny of the pair were injected intraperitoneally with 0.2 ml of 0.02% colchicine dissolved in Ringer's solution (NaCl 7.5g, KCl 0.2g, $CaCl_2$ 0.4g, $MgCl_2$ 0.2g /L) three hours prior to sacrifice. Gill, spleen, and gonad (ovary or testis) tissues were removed and minced with forceps in Ca, Mg–free phosphate buffered saline (CMF–PBS) solution (Z h a n g & A r a i 1996). Following centrifugation at 1200 rpm (130*g*) for 5 min and decanting, the cells were treated in 0.8% trisodium citrate hypotonic solution for 40 min at room temperature. The cells were collected by centrifugation and then fixed three times with fresh chilled Carnoy's fixative (3 parts of ethanol : 1 part of glacial acetic acid). Air-dried slides were stained with Giemsa and observed under a microscope. Chromosomes were classified according to L e v a n et al. (1964).

Results

Individuals analyzed in the present study showed no difference in external appearance from other wild caught diploids.

The chromosome numbers of somatic cells from gill tissues are summarized in Table 1. In the gill of the fish #1, all 15 metaphase spreads observed had a diploid number of chromosomes, 2n=50 with standard karyotype, 10 m, 4 sm and 36 t (Fig. 1a and b). No mitotic metaphase spread was detected from the gill of fish #2. In the gills of the remaining five loaches (#3 to 7), cells with chromosomes additional to the normal complement were observed (Table 1). As shown in Fig. 1c-f, some additional chromosomes were apparently much smaller than the smallest element of the standard chromosome set, being identified as

Table 1. Variation in numbers of additional telocentrics and micro-chromosomes in metaphases from gills of the loach.

Fish	Sex	0[a]				1					2		3		Total no. of cells
#		0[b]	1	2	3	0	1	2	3	4	0	1	0	3	
1	M	15	0	0	0	0	0	0	0	0	0	0	0	0	15
2	M	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	F	24	4	3	3	5	1	1	1	0	0	0	0	0	42
4	F	1	0	1	1	0	0	0	0	1	0	0	0	1	5
5	M	7	1	0	2	0	0	0	0	0	0	1	0	0	11
6	M	28	8	1	0	4	0	0	0	1	1	0	1	0	44
7	M	3	1	0	0	1	0	0	0	0	0	0	0	0	5
Total no. of cells		78	14	5	6	10	1	1	1	2	1	1	1	1	122

[a]: The number of additional telocentrics, [b]: The number of micro-chromosomes.

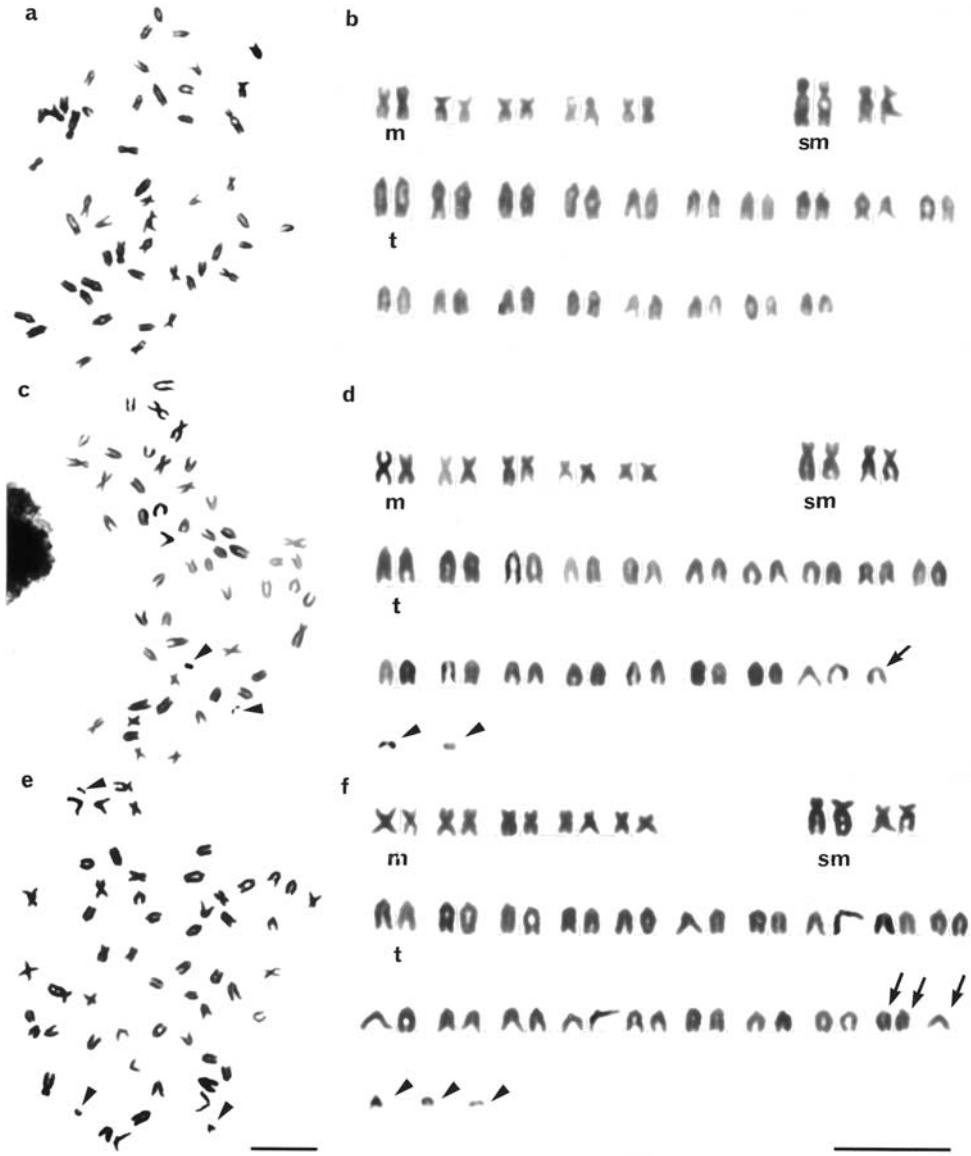

Fig. 1. Metaphase chromosomes (a, c, e) and corresponding karyotypes (b, d, f) of the loaches *Misgurnus anguillicaudatus*. a, b: a diploid cell form the gill of fish #1 with 2n = 50 chromosomes, c, d: a hyper-diploid cell from the gill of fish #3 with 51 chromosomes and two micro-chromosomes, e, f: a hyper-diploid cell from the gill of fish #4 with 53 chromosomes and 3 micro-chromosomes. m: metacentric, sm: submetacentric, t: telocentric. Arrows denote additional telocentics. Arrows denote micro-chromosome. Scale indicates 10 μm.

micro-chromosomes. But, other additional chromosomes were normal in size. As shown in Fig. 1c and d, the cell with 51 normal-sized chromosomes from #3 individual had the complete set of diploid elements (2n=50) plus one additional t and two micro-chromosomes. The other cell from different individual (#4) comprised 53 normal-sized chromosomes (diploid set plus three t) and three micro-chromosomes (Fig. 1e,f).

Intra- and inter-individual variation in numbers of additional t were observed, but the cell with 50 chromosomes was the most frequent (Table 1). In the five individuals (#3 to 7), the cells with both types of additional chromosomes were 41% of total cells observed (44/107) and those with only micro-chromosomes were 30% (32/107). Total number of additional chromosomes and micro-chromosomes in the five loaches was 85 and 60, respectively. Thus, the frequency of all the additional chromosomes and micro-chromosomes was 0.79 and 0.56 per cell, respectively.

Mitotic metaphases in gonad preparations were observed from all individuals (Table 2). In fish #1 only one out of 26 cells contained two micro-chromosomes, whereas other cells had 50 chromosomes. All the 30 metaphases detected from gonad of fish #2 had diploid number of chromosomes (Table 2). In the other five individuals (#3 to 7), two types of additional chromosomes were observed in 59% of cells observed (41/69) and the average frequency of additional chromosomes per cell was 1.25 (86/69). Micro-chromosomes were detected in 55% of cells (38/69) and the frequency was 0.88 per cell (61/69).

Meiotic metaphases were detected in only three males, #5–7, but not in females (Table 3, Fig.2). In the meiotic figures of these three males, various numbers of bivalents and univalents were observed, indicating the presence of additional chromosomes. In cells with 26 and 27 bivalents (Fig. 2b,d), some additional t appeared homologous, being able to form bivalents. Micro-chromosomes, however, did not pair in most of the metaphase I cells, and they seemed to be univalents (Fig. 2a,b). In a few cases, two micro-chromosomes seemed to associate together like a bivalent (Fig. 2c). Additional bivalents and univalents involving t

Table 2. Variation in numbers of additional telocentrics and micro-chromosomes in metaphases from gonads in the loach.

Fish	Sex	0[a]						1			2		8		Total no. of cells
#		0[b]	1	2	3	4	5	0	1	3	0	1	0	1	
1	M	25	0	1	0	0	0	0	0	0	0	0	0	0	26
2	M	30	0	0	0	0	0	0	0	0	0	0	0	0	30
3	F	5	2	1	0	1	1	1	0	0	0	0	0	0	11
4	F	6	1	0	0	0	0	0	0	0	0	0	0	0	7
5	M	10	13	5	2	0	0	0	1	1	0	1	0	0	33
6	M	4	3	2	1	0	0	0	0	0	0	1	1	1	13
7	M	3	1	0	0	0	0	0	0	0	1	0	0	0	5
Total no. of cells		83	20	9	3	1	1	1	1	1	1	2	1	1	125

[a]: The number of additional telocentrics, [b]: The number of micro-chromosomes.

Table 3. Variation in numbers of bivalens (II), univalents (I) and micro-chromosomes in MI of three male loches.

Fish	25II[a]					25II[a]+1I[b]			26II		26II+1I		26II+2I		27II		Total no. of cells
#	0[c]	1	2	3	4	0	1	2	0	1	0	1	0	3	0	3	
5	6	7	7	8	1	3	2	0	0	1	0	1	1	1	0	0	38
6	1	0	0	0	0	1	0	1	0	0	0	1	0	0	1	3	8
7	5	3	2	0	1	0	0	0	1	1	0	0	0	0	0	0	13
Total	12	10	9	8	2	4	2	1	1	2	0	2	1	1	1	3	59

[a]: Bivalent, [b]: Univalent, [c]: The number of micro-chromosomes.

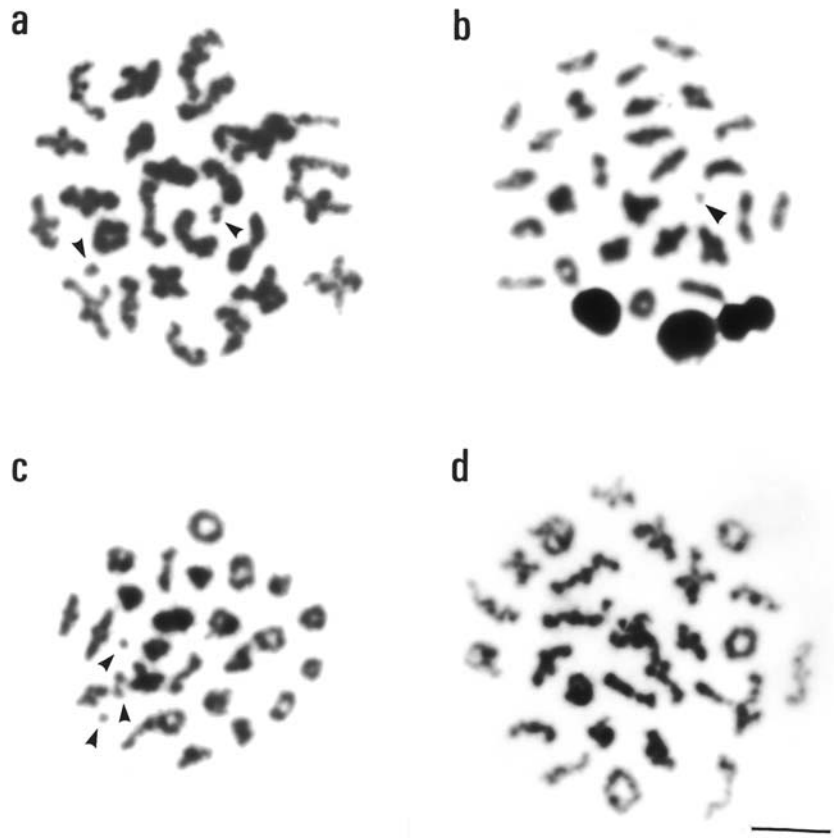

Fig. 2. Meiotic metaphase figures showing various numbers of bivalents and univalents as well as micro-chromosomes. a: a spermatocyte with 25 bivalents and 2 micro-chromosome, b: a spermatocyte with 26 bivalents and 1 micro-chromosome, c: a spermatocyte with 25 bivalents and 4 micro-chromosomes, the two of which paired, d: a spermatocyte with 27 bivalents. Arrows indicate micro-chromosomes. Scale indicates 10 μm.

and micro-chromosomes were observed in 80% of the cells (47/59). Micro-chromosomes were observed in 68% of the cells observed (40/59). The average frequency of all the additional chromosomes was 2.1 per cell (123/59) and the frequency of micro-chromosomes was 1.4 per cell (80/59).

Discussion

In the present study, extensive karyotype variation was observed in the number of additional t and micro-chromosomes. According to J o n e s & R e e s (1982), B-chromosomes can easily be distinguished from normal chromosomes by 1) their smaller sizes than the smallest karyotype member, 2) inter- and intra-individual numerical variation, 3) no obvious morphological effect on the phenotypes , 4) heterochromatic (C-band positive) , 5) higher frequency in meiotic cells, and 6) no pairing during meiosis. The micro-chromosomes in the present study satisfied all the above-mentioned criteria, except for the positive C-banding that was not studied here. Thus, the micro-chromosomes are considered B-chromosomes.

Comparing the numbers of micro-chromosomes between gill and gonadal tissues, there is apparent increase from 0.56 per cell in the gills to 0.88 per cell in the gonadal cells, and to 1.4 per cell in the meiotic metaphases. This observation suggests that the micro-chromosomes can be accumulated in meiotic cells as well as in premeiotic gonial cells.

The number of the additional t varied within and between individuals and their frequencies increased from somatic cells to gonadal tissues. They were not micro-chromosomes, judging from their sizes. Such additional chromosomes have been reported in several fish species (Oliveira et al. 1988, Fenocchio & Bertollo 1990; Valcarcel et al. 1993, Pandey & Lakra 1997). The present observation that the meiotic spermatocytes showed 26 and 27 bivalents, suggests that the additional t should pair and form bivalents. Considering the meiotic behavior of typical B-chromosomes that do not pair (Jones & Rees 1982), the additional t are not considered B-chromosomes.

Although Robertsonian polymorphism has been reported in the loach (Ojima & Takai 1979), the occurrence of additional t is not possible to explain by this system. Thus, the cells containing additional t were considered aneuploid and the present loach specimens were viable mosaic individuals comprising both eu-diploid cells and hyper-diploid cells. In the loach, viable aneuploids including a few additional chromosomes were produced by fertilization with aneuploid gametes from triploids (Zhang & Arai 1999b, Arai & Inamori 1999). This suggests the possibility that such additional chromosomes might be derived from aneuploid gametes of infrequently occurring triploids, produced by spontaneous retention of the polar body extrusion. Non-disjunction of chromosomes during meiotic divisions may result in trisomy including such additional chromosomes. Natural trisomy has been reported in brook trout *Salvelinus fontinalis* providing cytogenetic analysis to map a certain enzyme locus on a certain chromosome (Davisson et al. 1972). Extensive karyotype variation in the present specimens may be explained by the involvement of aneuploid gametes due to spontaneously occurring triploid and/or trisomy. Such aberrant cytological event may have occurred more frequently than we have predicted in wild populations of this loach species.

The male parent of the present specimens was collected from the Sera population in which additional chromosomes have never been detected, but the origin of female parent was unknown because it was selected from a stock population including loaches obtained mainly from fish dealers. Thus, little is known about the origins and significance of the additional chromosomes observed in this study. Further detailed study is necessary to detect such polymorphism as well as to unveil the origin and the nature of additional chromosomes in the loach.

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