Mitotic and Meiotic Analyses of the 'Large Race' of *Cobitis striata*, a Polyploid Spined Loach of Hybrid Origin

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Accepted April 25, 2003

SAITOH K. 2003. Mitotic and meiotic analyses of the 'large race' of *Cobitis striata*, a polyploid spined loach of hybrid origin. Folia biol. (Kraków) **51**(Suppl.): 101-105.

The large race of the *Cobitis striata* complex is a bisexual polyploid population. It is difficult to distinguish this polyploid loach morphologically from a sympatric diploid *C. striata*, the Biwa small race, indicating the close relationship between these two populations. The polyploid loach does have *striata*-specific satellite DNAs, but it also harbors *C. biwae*-related mtDNA. The large race has 2n=4x=98 chromosomes with 56 acrocentric chromosomes. Diploid *C. striata* has 2n=50 and *C. Biwae* 2n=48 chromosomes, suggesting the karyotype of polyploid *striata* as a combination of these two genomes. Meiotic figures showed a few quadrivalent formations besides many bivalents. Many of the quadrivalents were of metacentric chromosomes. Chiasmata were visible in some quadrivalent association. Quadrivalent formation with chiasmata indicates the presence of homologous segments capable of crossing-over between two genomes. Thus, the general polyploid model is also applicable to this case of polyploidy.

Key words: Lower teleostean fish, genome duplication, quadrivalent, chiasma, counterdiploidization, general polyploid model.

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Genome duplication played an important role in vertebrate evolution at least twice in early vertebrates before the splitting of lobe- and ray-finned fishes, and an additional duplication occurred in an early ray-finned fish (GATES *et al.* 1999). Studies of the recent occurrence of polyploidy will facilitate understanding the nature of genome duplication in vertebrate evolution.

Polyploidy is the complete genome duplication which occurs in a number of lower teleostean groups including cobitids. A Japanese *Cobitis* nominal species, *C. striata* contains a diploidtetraploid complex (UENO & OJIMA 1976). This diploid-tetraploid complex may provide good material for examination of the establishment and subsequent change in genome structure of polyploid populations. A previous report demonstrated the tetraploid (large race) is a bisexual allotetraploid of hybrid origin (SAITOH *et al.* 2000). Sexually reproducing allotetraploids should have chromosome sets of 2+2 component called amphidiploidy which ensures the proper segregation of genomes to gametes.

In this study, I analysed mitotic and meiotic chromosomes of the large race of the *striata* complex to test its amphidiploidy.

Material and Methods

Specimens and localities

Three males and three females of the large race were picked up from by-catch among fishery landings to Hamabun port from Lake Biwa, Japan. They were kept in an aquarium until sacrifice.

Chromosome preparation

Preparation of mitotic spreads from kidney and spleen tissues followed OJIMA and KURISHITA (1980). Preparation of male meiotic spreads were successful in early spring prior to the spawning season. Biopsied testis was minced with scissors and incubated for two hours at room temperature in Eagle's MEM (Nissui, Tokyo, Japan) containing colchicine (0.006 μ g/ml) (Nacalai, Kyoto, Japan) and collagenase S-1 (0.5 mg/ml) (Nitta-Gelatin, Osaka, Japan). Testis cells were then hypotonized for 20 min in 1% sodium-citrate, and then fixed in Carnoy solution. A modified airdrying method was applied to obtain meiotic spreads. When the fixative on the slide became the

thinnest spread, a drop of glacial acetate was applied onto the slide to facilitate cell spreading.

Chromosome spreads were once stained with 2% Giemsa (Merck, Germany), destained, and Cbanded according to SAITOH (1986) with brief modifications as follows. Ba(OH)₂ treatment was applied for 90-100 s for mitosis and for 80-90 s for meiosis. Sequencial staining procedure of routine Giemsa and C-banding made it easier to recognize both chromosomal gross morphology and banding pattern.

Results

Karyotype

Karyotype of the large race examined had 2n=4x=98 chromosomes with 42 bi-armed and 56 mono-armed elements (Fig. 1). Some of the bi-armed meta- or submetacentric chromosomes had large C-positive heterochromatin blocks at pericentromeric or in interstitial regions. Based on the C-banding pattern and centromeric position, it was easy to establish homologous pairs for bi-armed chromosomes. At least four combinations of quartet chromosomes were recognizable (Fig. 1, brack-eted).

Mono-armed acrocentric chromosomes had small C-heterochromatin blocks at each centromeric region only. Hence it was difficult to arrange these chromosomes in pairs or quartets, and the arrangement in the figure is tentative. Satellite elements were visible at the short arm of four middle-sized acrocentric chromosomes (Fig. 1, arrows), although it is not clear if these four elements constitute a quartet.

Meiotic configuration

In male meiotic spreads, various number of quadrivalents with none, one, two, three, four and seven were visible as well as many bivalents (Table 1, Fig. 2). Among well-spread 16 meiotic cells, there was only one cell without quadrivalent. Meiotic cells were refractory for swelling, and some cell spreads contained overlapped chromosome pairs. The number of quadrivalents shown in Table 1 is, therefore, of those which were clearly recognizable, and their count may be an underestimation to some extent.

Table 1

Frequency of quadrivalent count in the male meiotic spreads

Count	0	1	2	3	4	5	6	7
Number of cells	1	5	5	3	1	0	0	1

Among 32 quadrivalents being observed totally, most of them were of metacentric chromosomes. Chiasmata appeared in ten of 30 metacentric quadrivalents (Table 2, Fig. 3). Some metacentric quadrivalents indicated a ring-like configuration with



Fig.1. Routine Giemsa stained (upper rows) and C-banded (lower rows) karyotype of the large race. Braces show chromosomal quartets. Arrows show satellited chromosomes.



Fig. 2. Routine Giemsa stained (A) and C-banded (B) male meiosis at diakinesis or metaphase-I of the large race with a submetacentric and three metacentric quadrivalents (arrowheads). Metacentric quadrivalents show large heterochromatin blocks around possible centromere.



Fig. 3. Samples of quadrivalent found in male meiosis of the large race showing configurations observed with routine Giemsa staining and C-banding, presumable synapsis, and diplotene models (top to bottom). Chromosomes in the synaptic diagrams are arranged side by side two pairs of homologoues, which pairs are homeologous with each other. Left three configurations are of metacentric quartet, and others are those in which acrocentric chromosomes are involved. A, ring-shaped quadrivalent without chiasma; B, C, quadrivalent with a chiasma which results in ring-shape (B) or dumbbell-shape (C) according to position of crossing-over in relation to chromatids with specific terminal associations; D, rod-shaped quadrivalent of four acrocentric chromosomes with two chiasmata (one is between two homologues and the other is between two homeologues); E, quadrivalent of two aerocentric chromosomes with a chiasma between acrocentrics.

four terminal associations (Fig. 3A). Two of such associations are attributable to connection between two homologues at one arm, while the rest between two homeologues at the opposite arm. Quadrivalent with four homeologous terminal associations can also be postulated, but it is unlikely because of the possible preferential association between homologues or closest arms at diplotene. Other metacentric quadrivalents indicated chiasma(ta). Chiasmatic metacentric quadrivalents were in many cases ring-like shaped with a cruciformed arm (Fig. 3B), while some quadrivalents showed a dumbbell-shape (Fig. 3C). This difference in shape of quadrivalents is due to the position of putative crossing-over relative to arms with specific terminal associations. If a crossing-over

Table 2

0	1	2	3	4	5	Total count of the types
4	8	2	1	0	1	20
9	4	3	0	0	0	10
16	0	0	00	0	0	0
15	1	0	0	0	0	1
16	0	0	0	0	0	0
15	1	0	0	0	0	1
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Number of cells which have particular types of quadrivalent

4MT, metacentric quadrivalent with terminal associations only (Fig.3A); 4MC, metacentric quadrivalent with chiasma(ta) (Fig.3B, C); 4AT, acrocentric quadrivalent with terminal associations only; 4AC, acrocentric quadrivalent with chiasmata (Fig.3D); 2M2AT, quadrivalent of two meta- and two acrocentrics with terminal associations only; 2M2AC, quadrivalent of two meta- and two acrocentrics with terminal associations only; 3E)

occurred between chromatids of arms terminally associating with each other, a ring-like shape appears. On the other hand, crossing-over between chromatids making terminal associations with those of the other partner arms results in a dumbbell-shaped configuration. Hence it was difficult to distinguish from the shape of quadrivalents between homologous and homeologous crossing-overs. A few metacentric quadrivalents with chiasmata showed shrinkage so that precise identification of configuration was difficult.

Only two quartets in which acrocentric chromosomes took part were recognizable. One of them was acrocentric quadrivalent showing two chiasmata among consecutive three arms (Fig. 3D). One of these chiasmata is clearly an indication of homeologous crossing-over, although identification of homologous or homeologous crossing-over was difficult because of the same reason as for metacentrics. The other quartet indicated synapsis of two acrocentric and two metacentric chromosomes (Fig. 3E). This quadrivalent showed a chiasma at the long arm of acrocentric association.

Discussion

Co-existence of *C. striata* satellite DNA and *C. biwae* specific mitochondrial (mt) DNA (SAITOH *et al.* 2000) and allozyme characteristics (SUZAWA & TANIGUCHI 1990) indicate allopolyploidy of the large race of *C. striata* originated from hybridization between diploid *C. striata* male and *C. biwae* female. Morphological resemblance between the large race and a diploid *C. striata* (Biwa small race) (SAITOH & AIZAWA 1987) is supporting evidence.

Karyotype characteristics is also supporting evidence of allopolyploidy, but the situation is complicated. The large race has 98 chromosomes with 28 pairs of acrocentric chromosomes. On the other hand, diploid *C. striata* generally has 50 chromosomes with 17 pairs of acrocentrics, and diploid *C. biwae*, 48 with 3-5 acrocentric pairs (TAKAHASI & OKA 1976; UENO & OJIMA 1976; SAITOH *et al.* 1984; UENO *et al.* 1980). The chromosome number is simply the addition of two diploid chromosome sets, but acrocentric chromosomes are too many compared with the extant diploid genomes.

If the present tetraploid genome is simply a combination of two diploid chromosome sets, the parental genomes should have at least six more acrocentric pairs in total. Since not only diploid C. striata but also related species have 17 pairs of acrocentrics (UENO et al. 1985), it is less likely that the ancestral C. striata had more than 17 pairs of acrocentric chromosomes. Thus most of the additional acrocentrics may be attributable to the C. biwae genome. There is no diploid C. biwae population reported which has more than five pairs of acrocentric chromosomes. C. biwae populations specifically have a small number of acrocentrics. This specificity is a derived character, because the general trend of karyotype evolution indicates an increase of metacentric chromosomes and decreasing acrocentrics (IMAI & MARUYAMA 1978). Recent pericentric inversions in diploid population(s) leading to the extant C. biwae may explain the excess number of acrocentrics of the large race which harbors an ancient C. biwae genome. The mtDNA haplotype found in the large race is not closely related to that of C. biwae from sympatry but is close to a haplotype from a distant locality (SAITOH et al. 2000). The ancestral diploid C. biwae population which transmitted its genome to the large race might be extinct or have replaced its mtDNA by introgression.

Karyotype distinction between diploid *C. striata* and *C. biwae* (UENO & OJIMA 1976) and severe sterility upon artificial hybridization between them (MINAMORI 1951) indicate structural genomic diversity between these two species. One might expect that an allopolyploid consisting of such diverged genomes should be an amphidiploid which produces gametes of diploid hybrid genome only.

Existence of quadrivalents with chiasma, however, questions the amphidiploidy. Chiasma is a cytological evidence of crossing-over. Capability for crossing-over maintains the homology between chromosomes. Formation of quadrivalent with chiasma between homeologues, then, makes the chromosomal segment homologous over time. Observation of a case in which chiasmata present on three consecutive arms indicating homeologous crossing-over. Presence of homeologous chiasma indicates the allopolyploid large race is not a complete amphidiploid. Morphologically similar metacentric quartets also suggest the presence of tetrasomic chromosomes.

Diploidization was emphasized for evolutionary changes of genomes after autotetraploidization (ALLENDORF & THORGAARD 1984). This concept extended to allopolyploidy so that quadrivalent should not appear (FERRIS 1984). The present study questions such a simple picture in which presence or absence of tetrasomy is the indication of the modes of genome duplication. Allotetraploidy originates from viable hybrids. Genomes in viable hybrids should be from related species so that chromosomal segments from hybridizing species still keep homology to some extent. Quadrivalent of two acro- and two metacentric chromosomes indicates that even morphologically differentiated homeologues have a segment capable of synapsis.

Partial homology capable of crossing-over makes it possible for homeologues to undergo counterdiploidization. This process is defined as evolution of a quartet of homologues or homologous segments from two pairs of homeologues encountered in a polyploid genome after hybridization. If this process works also between homeologous acroand metacentric chromosomes, an excess number of acrocentrics may be explainable, although an increase of acrocentric chromosomes by counterdiploidization is less likely, because fixation to which type of chromosomes might have randomly occurred. Because of the counter-diploidization, the general polyploid model (WU et al. 1999) is applicable also to polyploids which have a hybrid origin. Distinction between auto- and allotetraploidy is not clear as has been postulated.

Acknowledgements

The author express his cordial thanks to Yuzuru SUZAWA, Tadao KITAGAWA and Katsutoshi ARAI

for discussion. Special thanks should go to two anonymous reviewers for improvement of the manuscript.

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