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Chromosomal Studies of Masculinized Hybrids in Bitterlings (Teleostei: Cypriniformes: Acheilognathinae)

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Abstract

The chromosome analysis of the masculinized hybrid between female *Tanakia limbata* and male *T. signifer* in bitterlings (Acheilognathinae) was done. It was presumed that they had intermediate karyotype between the parents, and formed sperms with heteroploidy resulting from the incomplete pairing of homologous chromosomes in meiosis. Due to the abundance of species and the ease of artificial fertilization, the study of the factor of the hybrid sterility in bitterlings would lead to the clarification of the mechanism about species differentiation and karyotype differentiation, and also to developing a new variety.

Keywords

Bitterling, Hybrid, Chromosome, Species Differentiation, Karyotype Evolution, Develop a New Variety

1. Introduction

Bitterlings are freshwater fish species ascribed to the subfamily Acheilognathinae (Cyprinidae), and are distributed throughout Eurasia, and more widely in East Asia. Three valid genera, *Acheilognathus*, *Rhodeus*, and *Tanakia* [1], grouping approximately 80 species/subspecies [2], have been recognized. It is known fact that all bitterlings are characterized by peculiar reproductive behavior which involves egg and sperm deposition in the mantle cavity of living freshwater bivalves.

Due to the abundance of species and the ease of artificial fertilization, many hybridization experiments in bitterlings were tried for the purpose of clarification on the phylogenetic relationships of bitterlings, the mechanism

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of species differentiation, and others. On the one hand [3]-[9] have reported some fertile hybrids, but on the other hand they have found that the sex ratio of bitterling hybrids was biased toward males. [10] observed similar phenomena and made mention of masculinization mechanism of bitterling hybrids. In any case, they had only limited information of chromosomes.

We have been studying hybridization experiments in bitterlings to make clear on the mechanisms of species differentiation and karyotype evolution, and to develop a new variety.

In the present report, the chromosome analysis of the masculinized hybrid between female *T. limbata* and male *T. signifer* in bitterlings was done.

2. Materials and Methods

2.1. F_1 Hybrids between Female *Tanakia limbata* and Male *T. signifer*

Thirty eggs from a female of *T. limbata* and sperms from a male of *T. signifer* were fertilized artificially. Hatchability was 83% (25 hatched embryos/30 eggs). Survival rate at about one year old after hatching was 32% (8 fishes/25 hatched embryos), and all these 8 hybrids had vivid colors just like a male.

Sperms were gotten from a F_1 hybrid. Chromosomal slides of this fish were made from kidney and testis cells. And also chromosomal slides of other three F_1 hybrids and their parents were obtained from kidney cells.

2.2. F_2 Hybrids between Female *Rhodeus ocellatus* and Male F_1 Hybrid

Thirty-five eggs from a female *R. o ocellatus* and sperms from a male F_1 hybrid (*T. limbata* ♀ × *T. signifer* ♂) were fertilized artificially. Fertilization rate was 57% (20 fertilized eggs/35 eggs used). Chromosomal slides of eight F_2 hybrids were obtained from gastrula cells. Hatching of remaining embryos has not been found.

2.3. Meiosis of *Rhdeus atremius* Fangi

Chromosomal slides of *R. a. atremius* were made from kidney and testis cells to compare with F_1 hybrid.

2.4. Chromosomal Slides

All of these chromosomal slides were made by direct air-drying method and chromosomes were stained with Giemsa. Karyotytpes of F_1 hybrids were analyzed from twenty metaphases in each individual.

3. Results and Discussion

Karyotypes of *T. limbata*, *T. signifer*, and *R. o. ocellatus*, as shown in **Figures 1-3** respectively, had $2n = 48$ including 8 metacentrics (M), 20 submetacentrics (SM) and 20 subtelocentrics (ST) or acrocentrics (A). Three karyotypes were quite similar and there was not distinct difference among them.

The karyotype of F_1 hybrid, shown in **Figure 4**, had 48 chromosomes and the same chromosomal constitution (8M + 20SM + 20ST/A) of their parents, in all metaphases observed. The distinction on the karyotype among them and the chromosomal aberration were not recognized, and it was estimated that F_1 hybrid had the intermediate karyotype between the parents. And, unusual metaphase chromosomal figures at the first cleavage of meiosis were observed (**Figure 5a**) compared to a normal figure observed in *R. a. fangi* (**Figure 5b**). *R. a. fangi* ($2n = 46$, **Figure 5c**) had 23 bivalent chromosomes in meiosis (**Figure 5b**). And in the metaphase figure of F_1 hybrids, many univalent chromosomes were found besides some bivalent chromosomes (**Figure 5a**). And then, the chromosomal number (bivalents + 2 × univalents) in each metaphase was 48. So, the omission of the chromosome was not recognized in that stage.

Metaphase figures from 8 embryos in F_2 hybrid (*R. o. ocellatus* ♀ × F_1 ♂) were observed (**Figure 6**). The distribution of the chromosomal numbers in F_2 hybrid is shown in **Table 1**. All embryos had wide distribution and the mode varied from individual to individual. In some metaphase figures, the structural chromosomal aberration was found (**Figure 6b**). It is presumed that the variation of the mode was due to the difference of the chromosomal number in each sperm of F_1, probably resulting from the incomplete pairing of homologous chromosomes in meiosis. In some lethal hybrids of salmon, trout and char, similar phenomena, *i.e.* the wide distribution of chromosomal numbers and the occurrence of the structural chromosomal aberration, have been observed [11]-[13]. It has been speculated that those phenomena were due to the abnormal cell division during early

Figure 1. Karyotypes of *T. limbata*.

Figure 2. Karyotype of *T. signifer*.

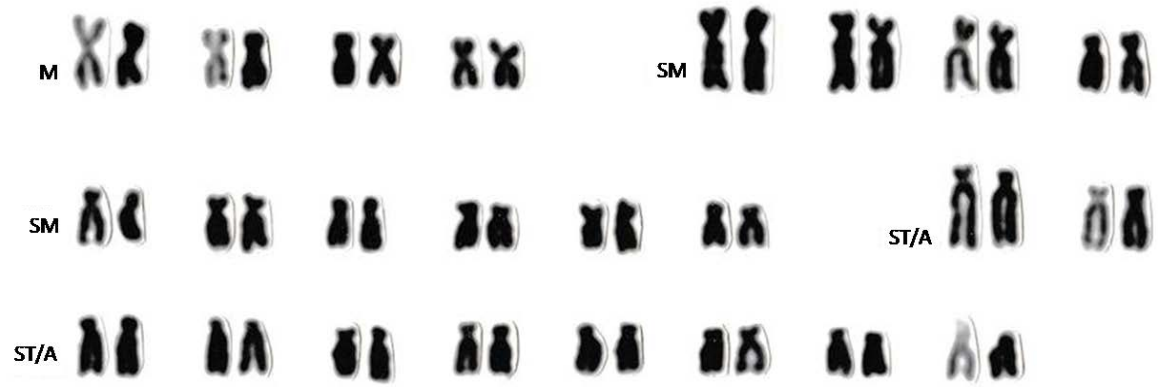

Figure 3. Karyotype of *R. o. ocellatus*.

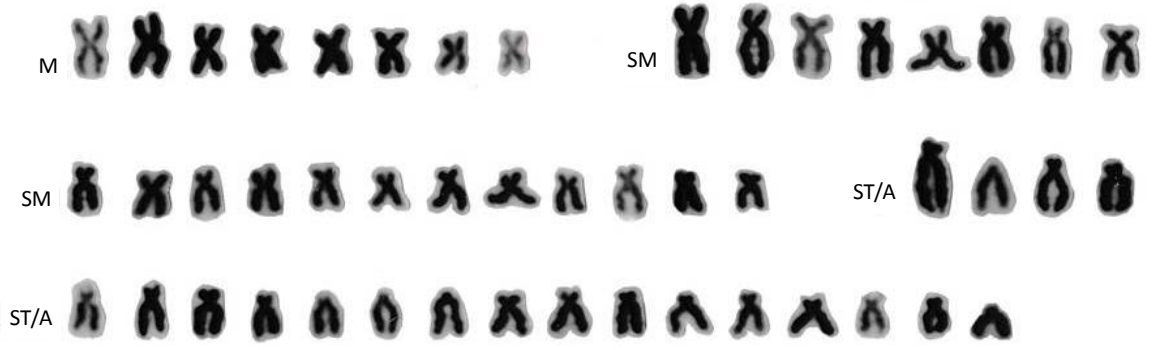

Figure 4. Karyotype of F_1 hybrid (*T. limbata* ♀ × *T. signifer* ♂).

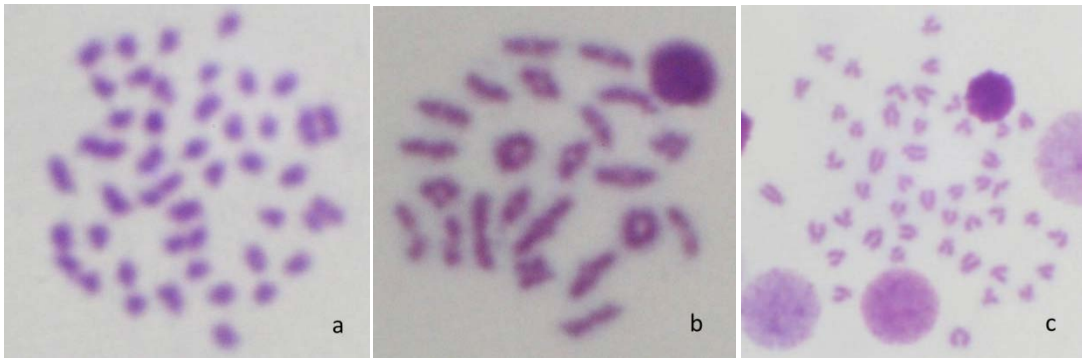

Figure 5. Two metaphase figures of F_1 hybrid (a) and *R. a. fangi* (b) at the first cleavage of meiosis, and a metaphase figure from a kidney cell of *R. a. fangi* (c).

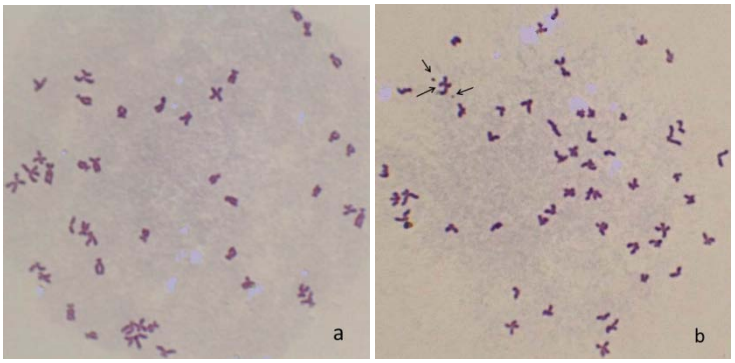

Figure 6. Two metaphase figures of F_2 hybrid. Arrows indicate obvious structural chromosomal aberrations.

Table 1. Distribution of chromosomal numbers in F_2 hybrid.

Embryo nos.	Chromosomal numbers																												
	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59
1							2	1		1	2		2	1															
2	1	1					2		4	5	11	10	7	3	3	2													
3							1												1	1	2	4	1	1					
4								1			2	1	1		8	9		1											
5								1										1	1	1		5	4	4	2	2			
6						2									2	1	1		3	1	5	5	3	2		1			
7										1												1	2	2	2	1	2		1
8			2	1		1			1			2	5	3	3	2	1	2	3	1									

development, resulting from the chromosomal discord between the parents. The present case can be interpreted in the similar way.

When we classify species, the important point is whether or not they can form hybrid. The species differentiation results from an accumulation of more than one reproductive isolation, and the evolution of the hybrid sterility may be the final important factor for the determination of species differentiation. The factor of the species differentiation may be concerned with that of the hybrid sterility. The study of the factor of the hybrid sterility in bitterlings would lead to the clarification of the mechanism about species differentiation and karyotype differentiation, and also to developing a new variety. The investigation at the gene-level is required to clarify the mechanism in addition to the chromosome-level.

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