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# Chromosomal Studies of the Hybrid between Female *Rhodeus ocellatus ocellatus* and Male *Rhodeus atremius fangi* in Bitterlings (Teleostei: Cypriniformes: Acheilognathinae)

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## Abstract

The chromosome analysis of the masculinized hybrid between female *Rhodeus ocellatus ocellatus* and male *R. atremius fangi* 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 develop a new variety. And also, it would also be important to make the hybrid various natures clear in environmental preservation.

## Keywords

Bitterling, Hybrid, Chromosome, Species Differentiation, Karyotype Evolution, Environmental Preservation

## 1. Introduction

Bitterlings are freshwater fish species ascribed to the subfamily Acheilognathinae (Cyprinidae), and are distributed throughout East Asia, and more widely in Eurasia. About 80 species/subspecies [1], have been recognized. It is a known fact that all bitterlings have a unique ecology in that they lay their eggs in freshwater bivalves.

Bitterlings illustrate the symbiosis between humans and nature, because the

habitats of them are closely related to human activities. They have decreased with a change in human activity. And, many kinds of them are endangered species. Preservation of such an environment as "Satoyama" is an important issue; a study of bitterlings would provide a good example to grasp the biodiversity and ecosystems of this environmentally sensitive area. One of the decrease causes of rare native species will be introduction of alien species of close relation. Genetic disturbance by reduction in habitat environment and hybrid formation is considered as bad influence.

Due to the abundance of species and easy doing of artificial fertilization, many cytogenetic experiments in bitterlings were tried for the purpose of clarification on the phylogenetic relationships of bitterlings, the mechanism of species differentiation, and others. And, many hybridization experiments have been tried equally. On the one hand [2]-[8] have reported some fertile hybrids, but on the other hand they have found that the sex ratio of bitterling hybrids was biased toward males. [9] 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. On the one hand there is fear of a decline of the procreative power of the native species and hereditary disturbance by hybrid formation. And, a hybrid study will give useful suggestion in environment preservation.

In the present report, the chromosome analysis of the masculinized hybrid between female *Rhodeus ocellatus ocellatus* and male *Rhdeus atremius fangi* in bitterlings was done.

## 2. Materials and Methods

### 2.1. $F_1$ Hybrids between Female *R. o. ocellatus* and Male *R. a. fangi*

*R. ocellatus ocellatus* and of *R. atremius fangi* were collected in Tochigi-prefecture, Japan and Fujian-province, China, respectively. Thirty eggs from a female of *R. o. ocellatus* and sperms from a male of *R. a. fangi* were fertilized in a plastic petri dish at 20C artificially. Then, fertilized eggs were kept at 20C.

Hatchability was 87% (26 hatched embryos/30 eggs). Obvious form abnormality wasn't found in the individuals. Survival rate at about one year old after hatching was 35% (9 fishes/26 hatched embryos), and all these 9 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 two $F_1$ hybrids and their parents were obtained from kidney cells.

### 2.2. $F_2$ Hybrids between Female *R. o. ocellatus* and Male $F_1$ Hybrid

Forty-eight eggs from a female *R. o. ocellatus* and sperms from a male $F_1$ hybrid

(*R. o. ocellatus* ♀ × *R. a. fangi* ♂) were fertilized artificially. Fertilization rate was 52% (25 fertilized eggs/48 eggs used). Chromosomal slides of fifteen $F_2$ hybrids were obtained from early- and late-gastrula cells. Hatching of remaining embryos has not been found.

In addition, chromosomal slides of four $F_2$ hybrids between female *R. a. fangi* and $F_1$ hybrid were obtained from late-gastrula cells.

### 2.3. Meiosis of *R. a. fangi*

Chromosomal slides of *R. a. fangi* 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. Each slide was observed by 600 times of optical microscope. Karyotytpes of $F_1$ hybrids were analyzed from twenty metaphases in each individual.

## 3. Results and Discussion

Karyotypes of *R. o. ocellatus*, as shown in Figure 1, had $2n = 48$ including 8 metacentrics (M), 20 submetacentrics (SM) and 20 subtelocentrics (ST) or acrocentrics (A). Karyotype of *R. a. fangi* had $2n = 46$ including 4 SM, 42 ST or A (Figure 2).

The karyotype of $F_1$ hybrid, shown in Figure 3, had 47 chromosomes including 4 M, 12 SM and 31 ST or A, 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


Figure 1. Karyotypes of *R. o. ocellatus.*

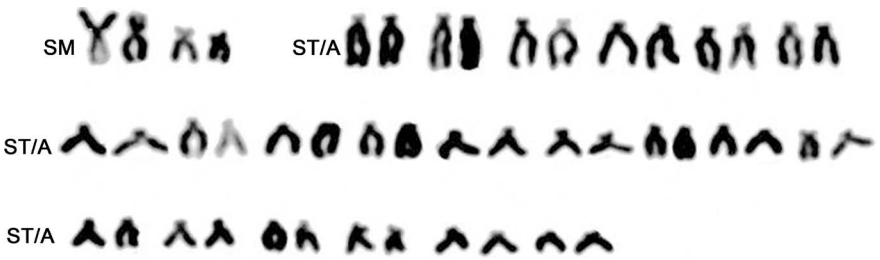


Figure 2. Karyotype of *R. a. fangi.*


**Figure 3.** Karyotype of $F_1$ hybrid (*R. o. ocellatus* ♀ × *R. a. fangi* ♂).

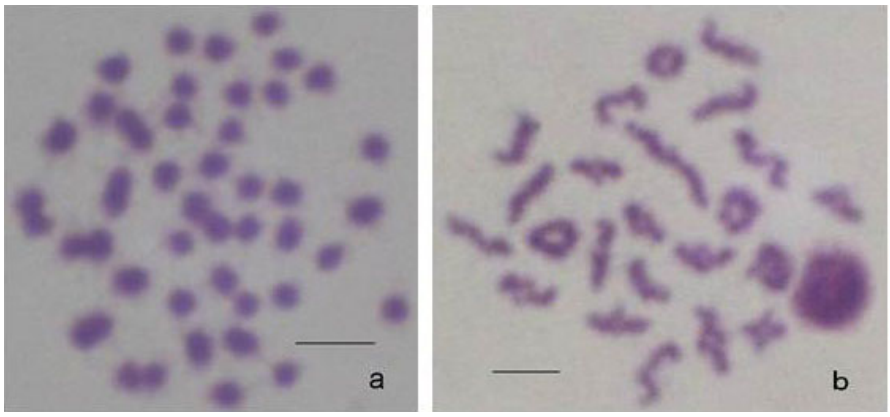


**Figure 4.** Two metaphase figures of $F_1$ hybrid (a) and *R. a. fangi* (b) at the first cleavage of meiosis. They were stained with Giemsa. The scales indicate 5 micrometer.

between the parents. And, unusual metaphase chromosomal figures at the first cleavage of meiosis were observed (**Figure 4(a)**) compared to a normal figure observed in *R. a. fangi* (**Figure 4(b)**). *R. a. fangi* ($2n$ = 46, **Figure 2**) had 23 bivalent chromosomes in meiosis (**Figure 4(b)**). And in the metaphase figure of $F_1$ hybrids, many univalent chromosomes were found besides some bivalent chromosomes (**Figure 4(a)**). And then, the chromosomal number (bivalents + 2 × univalents) in each metaphase was 47. So, the omission of the chromosome was not recognized in that stage.

Metaphase figures from 9 embryos (at early-gastrula stage) and 6 embryos (at late-gastrula stage) in $F_2$ hybrid (*R. o. ocellatus* ♀ × $F_1$ ♂) were observed (**Figure 5(a)**). Besides, metaphase figures from 4 embryos (at late-gastrula stage) in $F_2$ hybrid (*R. a. fangi* ♀ × $F_1$ ♂) were observed.

The distribution of the chromosomal numbers in $F_2$ hybrid is shown in **Table 1**. Clear form abnormality wasn't admitted in most metaphase figures. The embryo showed wide distribution, and modes were observed in each embryo. And, the mode varied from individual to individual. In some metaphase figures, the structural chromosomal aberration was found (**Figure 5(b)**). 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.

Besides, a similar result to this study was reported in another bitterling hybrid [10]. In this study, chromosome analyses were separated and performed in the

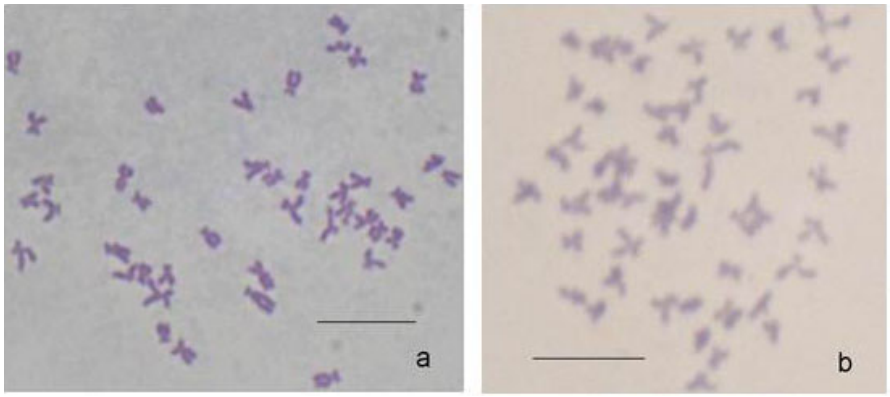


**Figure 5.** Two metaphase figures of $F_2$ hybrid. Some obvious structural chromosomal aberrations were observed (b). They were stained with Giemsa. The scales indicate 10 micrometer.

**Table 1.** Distribution of chromosomal numbers in $F_2$ hybrid.

| Embryo | | Chromosomal numbers | | | | | | | | | | | | | | | | | | | | | | | | | | | | | SCA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| nos. | Stage | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | |
| *R. o. ocellatus* ♀ × $F_1$ (*R. o. ocellatus* ♀ × *R. a. fangi* ♂) ♂ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 | | | | | | | | | | | | 1 | | | 2 | 12 | 2 | | | | | | | | | | | | | | |
| 2 | | | | | | 2 | 12 | | | | | | | | | | | | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | 1 | 1 | 7 | 1 | | | | | | | | | | | | | | | |
| 4 | Early- | | | | | | | | | | | | | | | | 2 | 7 | | | | | | | | | | | | | |
| 5 | gastrula | | | | | | | 1 | 4 | | | | | | | | | | | | | | | | | | | | | | |
| 6 | | | | | | | | 1 | 1 | 3 | | | | | | | | | | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | 2 | 7 | 2 | | | | | | | | | | | | | | | | | |
| 8 | | | | | | 1 | 1 | | 4 | 6 | | | | | | | | | | | | | | | | | | | | | |
| 9 | | | | | | | 11 | | | | | | | | | | | | | | | | | | | | | | | | |
| *R. o. ocellatus* ♀ × $F_1$ (*R. o. ocellatus* ♀ × *R. a. fangi* ♂) ♂ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 10 | | | | | | | | | | | | | | | | | | | | | 1 | | | | 3 | 1 | 7 | 2 | | | * |
| 11 | | | 1 | | 2 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | |
| 12 | Late- | | | | | | | | | | | | | | 1 | | 1 | | | | | | | | | | | | | | |
| 13 | gastrula | | | | | | 1 | | 2 | | | | | | | 1 | | 1 | | | | | | | | | | | | | | * |
| 14 | | | | | | | | | | | | | | | | | 1 | 1 | | | | | | | | | | | | | * |
| 15 | | | | | 1 | | | 1 | 1 | | | | | | | | | | | | | | | | | | | | | | |
| *R. a. fangi* ♀ × $F_1$ (*R. o. ocellatus* ♀ × *R. a. fangi* ♂) ♂ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 16 | | | | | | | | | | | | | | | | | | 1 | 1 | | | | | | 1 | 1 | | | 1 | 3 | |
| 17 | Late- | | | | 6 | | | | | | | | | | | | | | | | | | | | | | | | 1 | | |
| 18 | gastrula | | | | 7 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 19 | | | | | | | | | | | | | | | | | | | | | | | | 2 | | | 1 | 8 | | | * |

*: Some cells had structural chromosomal aberrations (SCA).

early-gastrula and the late-gastrula. And, indefiniteness of the mode and the tendency for which a structural chromosomal aberration appearance is conspicuous were seen in the late-gastrula. Cell division seems developed mechanically until early-gastrula period. Various genes begin to function after a blastula period. So, after gastrula period genetic manifested harmonization is disordered, and cell division and a morphogenesis will be warped. As a result, hatching of remaining embryos of $F_2$ wouldn't be found.

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 develop a new variety. On the other hand, introduction of foreign related species is regarded as a problem in environment preservation. Genetic disturbance by reduction in habitat environment and hybrid formation is considered as bad influence. A hybrid study will be to give useful suggestion in environment preservation.

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