Chromosome Science 3 : 101-103, 2000

Short Communications

Banding karyotype and 5S ribosomal DNA loci in the Japanese bitterling, *Rhodeus ocellatus* (Cyprinidae)

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Received November 6, 1999/Accepted March 23, 2000

Abstract. To obtain a detailed karyotype of *Rhodeus ocellatus* (2n=48), we employed bromodeoxyuridine (BrdU) replication banding method. All chromosome pairs were identified by R-banding patterns. Fluorescence *in situ* hybridization (FISH) using a ribosomal DNA probe exhibited that 5S rDNA was located at a centromeric region of one pair of acrocentric chromosomes. However, the silver-stained nucleolar organizer regions (Ag-NORs) located at a different acrocentric pair of the smallest one, demonstrating that the Ag-NOR loci did not show the 5S rDNA loci in this species. These results indicate a new karyotypic characteristics in this species. Moreover, the karyotypical difference between the present species and the related species of *Acheilognathus tabira* (2n=44) was briefly discussed.

Keywords: R-banding, Japanese bitterling, *Rhodeus ocellatus*, 5S rDNA, FISH

Introduction

The distribution of bitterlings (acheilognathine fishes) is restricted to eastern Asia. More than forty species of bitterlings have been recorded in this area and fifteen of them currently inhabit Japan. The phylogenetic systematics of bitterlings has been studied by many investigators (Matsubara, 1955; Nakamura, 1969; Miyaji *et al.*, 1976), but the morphological classification of these species is very difficult. Japanese bitterlings could be karyologically classified into three groups with 2n=48, 2n=46 and 2n=44 (Ojima *et al.*, 1973). The karyotype analysis is important for classification of species with the same chromosome number.

In general, the karyotypic analysis in fish species was performed mostly by the C-banding and the Ag-NOR staining techniques because of difficulty of G-banding in fish chromosomes. For this reason, studies of fish chromosomes have scarcely been advanced except for a few species such as zebrafish (Daga *et al.*, 1996).

In this study, we examined the R-banded karyotype of *Rhodeus ocellatus* by employing BrdU replication banding for identifying each pair of chromosomes. Localization of 5S ribosomal DNA and Ag-NORs was also analyzed by use of FISH and silver staining methods.

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Materials and methods

Fibroblast cells from fins of a male and a female *R. ocellatus* were cultured in Eagle's MEM supplemented with 10% FBS for 4 to 12 months at 24°C. The chromosome preparations were obtained using colcemid (0.1 μg/ml), hypotonic treatment (0.4% KCl) and a routine flame- or air-drying technique.

For R-band, BrdU replication banding technique of Dutrillaux *et al.* (1973) was used with some modifications (100 μg/ml BrdU, 8 h) and chromosomes were stained using the Fluorescence Plus Giemsa (FPG) technique (Perry and Wolff, 1974).

Chromosome slides were allowed to hybridize with a 5S rDNA sequence of *R. ocellatus* by FISH after treatment with RNase A (Nabeyama *et al.*, in preparation). The probe DNA was labeled with biotin 7-dATP using a nick translation kit (BRL) according to the protocol from the supplier. The denaturation of chromosomal DNA and probe DNA, hybridization, rinsing and signal detection were performed as described previously (Kubota *et al.*, 1993). The slides treated with fluorescein avidin and counterstained with propidium iodide were photographed under a microscope (Zeiss) equipped with epifluorescence optics (filter combination: Zeiss 08, exciter, 450-490 nm; barrier, 520 nm). Photo-

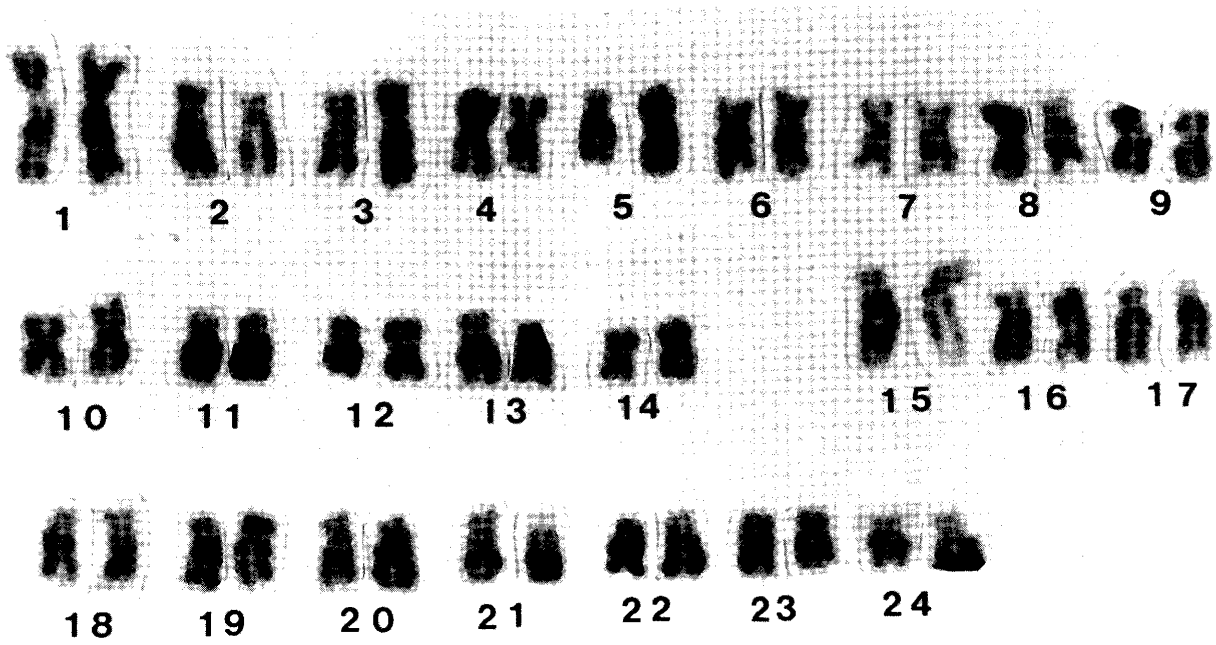

Figure 1. An R-banded karyotype of *Rhodeus ocellatus*.

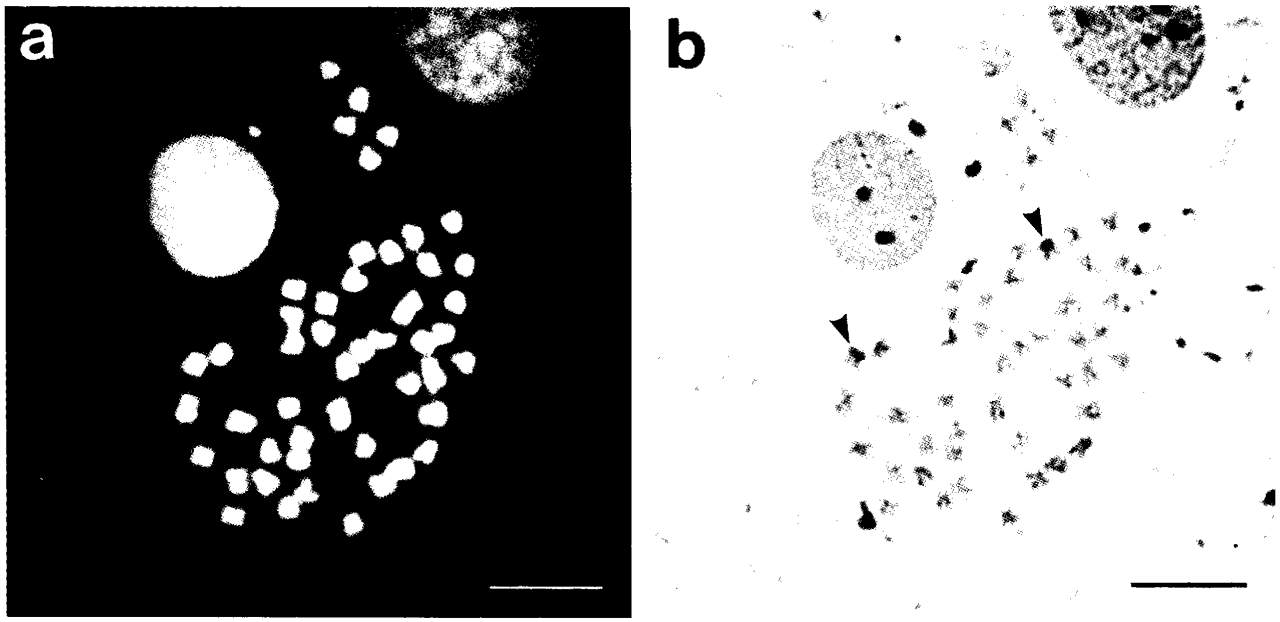

Figure 2. FISH pattern with 5S rDNA probe in metaphase chromosomes of *Rhodeus ocellatus* (a), and Ag-NOR staining of the same metaphase (b). Arrowheads indicate the NORs. Bar=10 μm.

graphs were taken on Ektachrome (ISO 400 color-slide film (Kodak).

After microscopic examination, the slides were used for Ag-NOR staining (Howell and Black, 1980).

Results and discussion

We obtained R-banded metaphases from both male and female cultured cells of *R. ocellatus* (2n=48) by the BrdU replication banding method. Each homologous pair was determined by the banding patterns and arranged according to the size and centromeric positions by reported by Takai and Ojima (1988) with a slight modification. No sex difference in the karyotypes was noted. A representative karyotype is shown in Fig. 1.

Although the present method produced R-banding patterns in *R. ocellatus* chromosomes, it may be necessary to examine the condition for treatment of BrdU to

be optimum for obtaining more fine banding patterns in the present species. Ueda and Naoi (1999) described the B-bands with G-band-like banding patterns in *R. ocellatus* by the 4Na-EDTA-Giemsa staining (Takayama *et al.*, 1981), and obtained clear and reproducible bands in elongated chromosomes. However, the difference in appearance of the bands in metaphase chromosomes of cold-blooded vertebrates seemed to be the weak compartmentalization of the DNA base composition (Medrano *et al.*, 1988). The poor banding patterns of *R. ocellatus* chromosomes here studied may be the similar situation as they proposed. In our previous studies on salamanders, Hynobiidae, no R-bands were obtained in *Onychodactylus japonicus* with the same banding method here employed but clear and reproducible R-bands were produced in *Hynobius* and *Salamandrella* species (Kohno *et al.*, 1991)

FISH with a 5S rDNA probe exhibited two distinct signals on an acrocentric pair of *R. ocellatus* metaphases (Fig. 2a). However, when the same spreads were silver-stained after the FISH method, Ag-NORs appeared on the secondary constriction of the smallest acrocentric pair (Fig. 2b) in conformity with the previous report (Takai and Ojima, 1988). Thus, the present result showed that Ag-NOR bearing chromosomes were different from chromosomes carrying 5S rDNA, indicating a new karyotypic characteristics in this species.

In the related species, *Acheilognathus tabira* (2n=44), 5S rDNA was found in two pairs of acrocentric chromosomes (Inafuku *et al.*, 2000), and Ag-NORs were located at the position of 5S rDNA in one of these two pairs. Although the reason why the difference in the number of chromosomes that carried 5S rDNA and NORs in these two species is not clear, evolution could be marked by the loss and acquisition of 5S rDNA and NORs.

The differences in chromosome number between *R. ocellatus* (2n=48) and *A. tabira* (2n=44) could be a result from Robartsonian rearrangements, fusion or fission. Two large metacentric pairs found in *A. tabira* appeared to be resulted from Robertsonian fusion of four acro- and/or telocentric pairs in *R. ocellatus*. To understand the karyotype evolution in Japanese bitterling species, it is necessary to clarify each chromosome rearrangement that caused the karyotype change. Application of BrdU replication banding and FISH techniques has provided great potential for identifying minor and/or major rearrangements in the chromosomes of Japanese bitterlings. The accumulation of cytogenetic and molecular genetic data should clarify the phylogenetic relationships of Japanese bitterlings as discussed by Ojima *et al.* (1973).

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