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Karyotype and Chromosomal Distribution of C-band-Positive Constitutive Heterochromatin in *Opsariichthys uncirostris* (Cyprinidae, Pisces)

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Abstract

In the present study, we analysed the karyotype and chromosomal distribution of constitutive heterochromatin in *Opsariichthys uncirostris* "hasu" (Cyprinidae) using the C-banding method. *O. uncirostris* has the diploid chromosome number of 2n=78; the karyotype comprised four pairs of meta- and submetacentric chromosomes and 35 pairs of acrocentric chromosomes. The majority of the acrocentric chromosomes were very small. C-banding analysis revealed intensely stained C-bands in the centromeric regions of meta- and submetacentric chromosomes, and small C-bands were observed in the centromeric regions of the majority of acrocentric chromosomes. The very small chromosomes showed no B-chromosome-like features. Previous cytogenetic findings and our present results suggested that the karyotype of *O.uncirostris* may have differentiated involving centric fission events when compared to the karyotypic features of closely related species.

Key words: Karyotype; C-bands; Constitutive heterochromatin; Cyprinid fish

1. Introduction

The family Cyprinidae is the largest family of freshwater fishes and comprises about 4,200 species[1]. Cytogenetic studies have been carried out in about 650 of all Cyprinidae species. The cyprinids are characterized by the following karyotypic features. In general, 50% of the species studied have the chromosome number of 2n=50, and the karyotypes consist of many bi-armed chromosomes based on the findings of Arai[2]. In Cyprinidae, species with 2n=100 or near 2n=100, such as carps and crucian carps, are known to be of tetraploid origin[3]. Ojima et al.[4] reported unusual chromosome number in *Opsariichthys uncirostris*, which is commonly known as "hasu" in Japanese. Its chromosome number is 2n=78, and the karyotype is mostly comprised of acrocentric chromosomes; in addition, most of the chromosomes had very small sizes. Whether such very small chromosomes are B-chromosomes (or supernumerary chromosomes) are an interesting topic of study. Generally, B-chromosomes are C-band positive and show intraindividual variations. In Cyprinidae, such B-chromosomes have been reported in triploid crucian carps[5]. However, C-banding analysis *O. uncirostris* has not been carried out. Thus, the unusual karyotypic features of this species are interesting in terms of karyotypic differentiation among cyprinid fishes.

In the present study, we analyzed the karyotype and the chromosomal distribution of C-band-positive constitutive heterochromatin in *O. uncirostris* and discuss the process of karyotypic differentiation.

2. Materials and Methods

Three *O. uncirostris* specimens collected at Lake Biwa (Shiga Prefecture) were used for this study.

Air-dried slides containing the chromosomes from kidney tissues were prepared according to the direct method[6]. The slides were stained with 2% Giemsa diluted with 1/15 M phosphate buffer (pH 7.0) for 10 min, observed microscopically, destained with 70% alcohol, and analyzed for C-banding.

C-banding analysis was carried out according to the BSG (Balium/Saline/Giemsa) method reported by Sumner[7]. The slides were treated with 5% $Ba(OH)_2$ solution at 50 ° C for 60-120 s, incubated in 2× SSC (0.3 M NaCl + 0.03 M Na citrate) at 60 ° C for 1 h, and stained with the 2% Giemsa for 10-20 min as described above. Normal and C-band staining of the chromosomes in the same metaphase plate were analyzed.

For each specimen, the chromosome number was determined based on counts higher than 20 metaphases. Karyotype analysis was performed following the chromosome classification reported by Levan et al.[8]. For the calculation of fundamental number (FN), two were assigned to metacentric and submetacentric chromosomes and one was assigned to subtelocentric and acrocentric chromosomes.

The relative length of each chromosome (RLC) was calculated as the percentage of its length relative to the total length of all chromosomes. *O. platypus* collected at Muko River (Hyogo) was used for comparing the RLC with that of *O. uncirostris*. Metaphases comprising elongated chromosomes were selected for measurement.

3. Results and Discussion

O. uncirostris has the diploid chromosome number of 2n=78, and the karyotype consisted of twopairs of metacentric, two pairs of submetacentric, and 35 pairs of acrocentric chromosomes (Figure 1, 2). The FN was 86. These results were consistent with those reported by Ojima et al.[4] and Takai and Ojima[9]. No inter- and intra-individual variations in the chromosome number were observed. The chromosome sizes gradually decreased from the largest to the smallest chromosomes. The RLC was

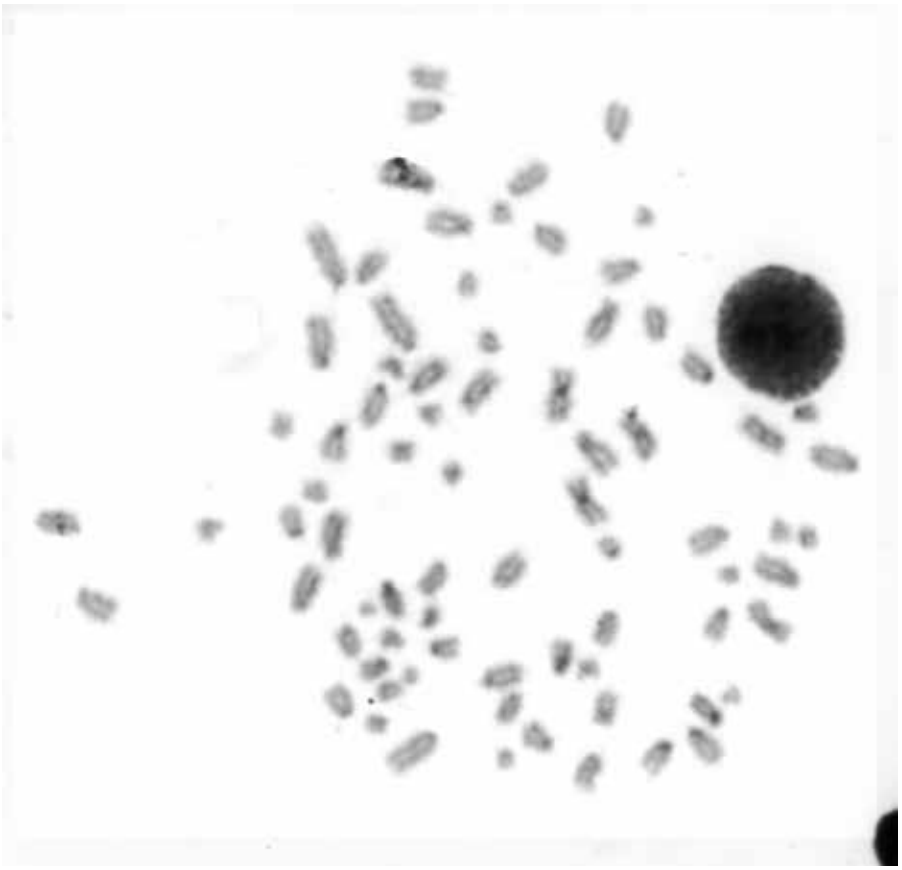

Figure 1. C-band staining metaphase chromosomes of *Opsariichthys uncirostris* “hasu” (Cyprinidae).

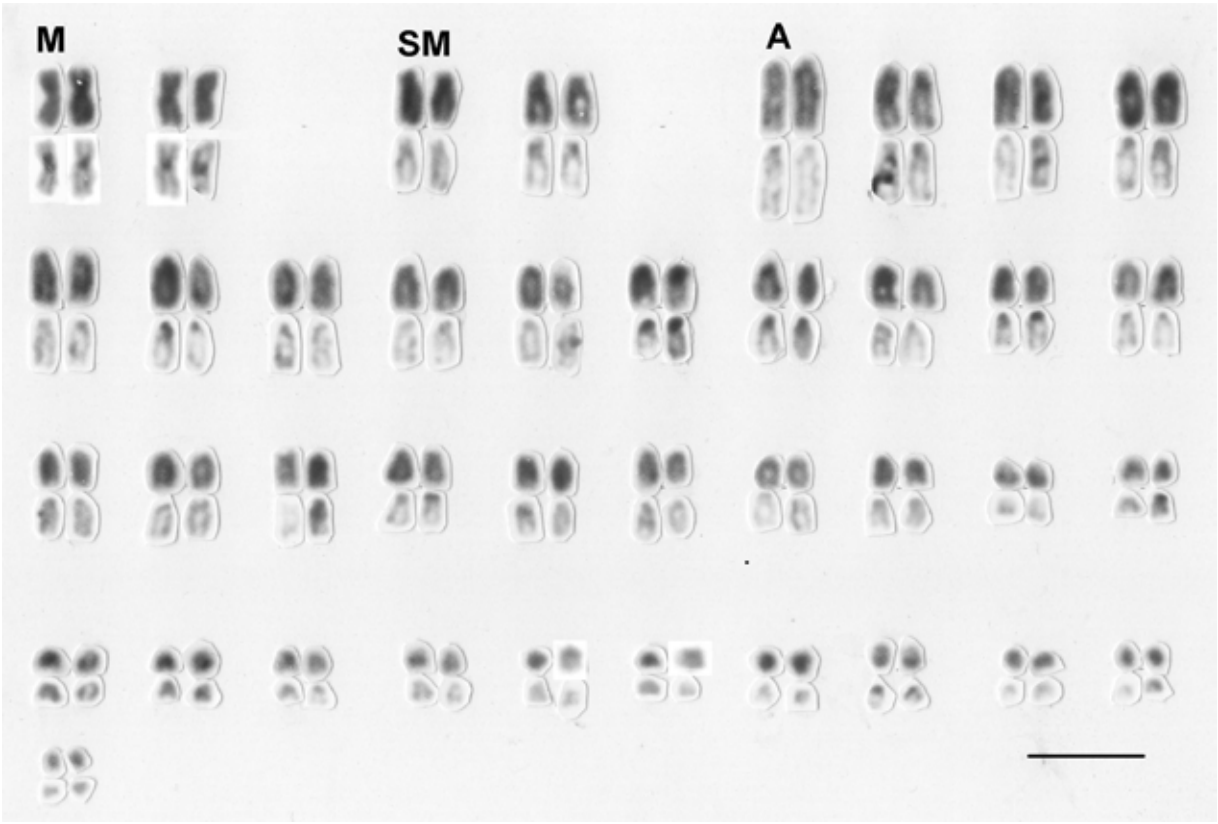

Figure 2. Conventional (upper row) and C-band (lower row) staining karyotypes of *Opsariichthys uncirostris* "hasu" (Cyprinidae). M: metacentric, SM: submetacentric, A: acrocentric. Bar indicates 5μm.

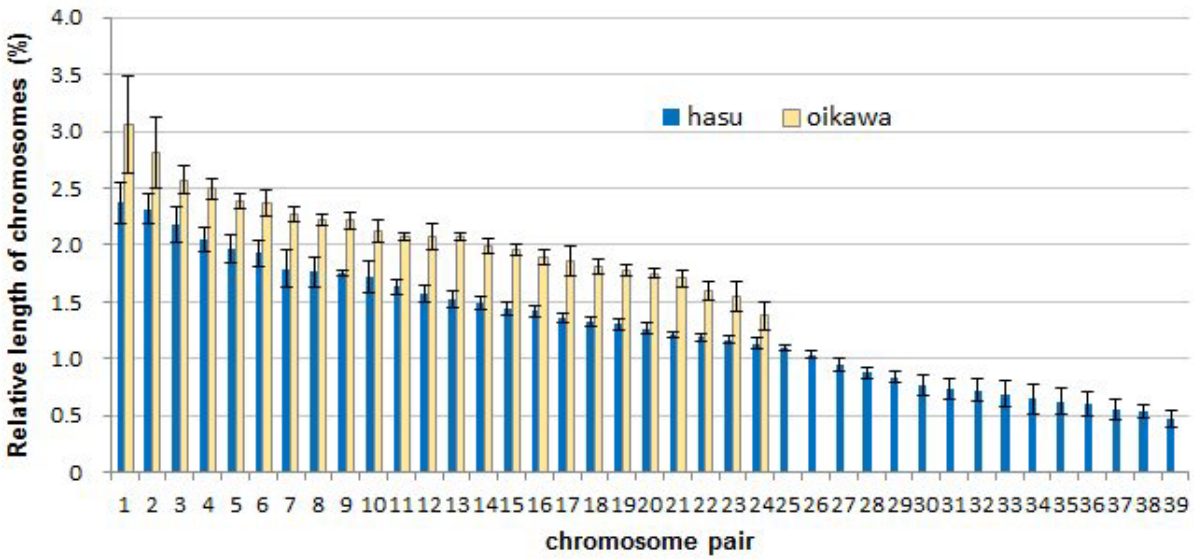

Figure 3. Relative length of the chromosomes of *Opsariichthys uncirostris* "hasu" and *O. platypus* "oikawa". Error bars show standard deviation.

2.37±0.18 in the largest chromosome and 0.47±0.07 in the smallest chromosome (n=3). On the other hand, the RLC of *O. platypus* was 3.06±0.13 in the largest chromosome and 1.38±0.13 in the smallest chromosome (n=3). In *O. uncirostris*, the RLC values of 18 pairs of chromosomes were smaller than those of the smallest chromosomes of *O. platypus* (Figure 3).

In C-banding analysis, the C-bands were observed only in the centromeric regions in the majority of the chromosomes. Intensely stained C-bands were observed in the centromeric regions of bi-armed chromosomes, and small C-bands were observed in the centromeric regions of many acrocentric chromosomes (Figure 2).

As stated above, *O. uncirostris* has a distinct karyotype compared to other cyprinid species. One interesting problem in such a karyotype is the process of karyotypic differentiation. In the closely related species *O. platypus* in Japan, the diploid chromosomes are 2n=48, and FN is 88 4, 9). The karyotype of *O. platypus* shows a typical karyotypic feature in Cyprinidae. The chromosome number of *O. uncirostris* is about 1.6 times higher than those of *O. platypus*; however, the DNA values between the two species (3.0pg in *O. uncirostris* and 2.9pg in *O. platypus*) were almost equal[10]. In addition, the FN of *O. uncirostris* is very similar with that of *O. platypus*. The present results revealed that the very small acrocentric chromosomes of *O. uncirostris* had C-bands in the centromeric regions. Previous studies reported no variations in the chromosome number of this species. Takai and Ojima[9] reported that *O. uncirostris* has nucleolus organizer regions (NORs) in one pair of very small chromosomes. The above results suggested that the karyotype of *O. uncirostris* has been differentiated by chromosome rearrangements, which increased the number of chromosomes; in addition, all the small chromosomes of *O. uncirostris* are not B-chromosomes.

Centric fission is well known to serve as the mechanism responsible for the increased number of chromosomes in karyotypic differentiation. The occurrences of centric fission events increase the number of mono-armed chromosomes and reduce the number of bi-armed chromosomes; however, the FN value is not changed. However, centric fission events are rare. Recently, Takai and Izutsu[11] reported chromosome evolution involving centric fission events in pomacanthid fishes. Based on cytogenetic data of *O. uncirostris*, it is suggested that the karyotype differentiation of *O. uncirostris* involved fission events. In *O. uncirostris*, the sizes of C-bands in acrocentric chromosomes were smaller than those in the bi-armed chromosomes, which could be attributed to the division of C-banded regions during centric fission events.

The chromosome numbers of *O. uncirostris* subspecies and *O. bidens* in China and Korea were reported to be in the range 2n=74-78; furthermore, the *O. platypus* in China was reported to have 2n=78[2]. Chromosomal studies in the genus *Opsariichthys* are very interesting. Further studies involving detailed analysis using several banding techniques can elucidate chromosome evolution and the phylogenetic relationships within the genus *Opsariichthys* and the family Cyprinidae.

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コイ科魚類 *Opsariichthys uncirostris*（ハス）の核型と C- バンドで示される構成ヘテロクロマチンの染色体分布

高井　明徳

本研究において、コイ科魚類 *Opsariichthys uncirostris*（ハス）の核型と C- バンドで示される構成ヘテロクロマチンの染色体分布を分析した。ハスの染色体数 2n=78 で、核型は 4 対の中部型および次中部型染色体および 35 対の端部型染色体で構成され、端部型染色体の多くは微小な染色体であった。濃染する C- バンドは中部型および次中部型染色体のセントロメア部に認められ、多くの端部型染色体で小さなサイズの C- バンドがセントロメア部に認められた。微小な染色体は B 染色体の特徴を示さなかった。先行研究および今回の研究は近縁種との比較においてハスの核型は動原体開裂により分化したことを示唆した。