

Karyotype and Constitutive Heterochromatin Distribution in a Monocentrid Fish, *Monocentris japonica* (Beryciformes)

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Abstract. The karyotype and constitutive heterochromatin distribution of a monocentrid fish (Beryciformes), *Monocentris japonica*, were investigated. The diploid chromosome number was 48, and the karyotype consisted of all acrocentric chromosomes. Large-sized C-banded constitutive heterochromatin was distributed in the centromeric regions of all chromosomes. On the basis of these results, problems of karyotypic variations and speciation were discussed.

Key words: karyotype, C-band, constitutive heterochromatin, monocentrid fish, Beryciformes

1. Introduction

The family Monocentridae (Beryciformes) comprises two genera, *Monocentris* and *Cleidopus*, and four species¹⁾. In Japan, only one species, *M. japonica* “matsukasauo”, is known²⁾.

Cytogenetical studies of *M. japonica* have been reported by Arai and Nagaiwa³⁾ and Murofushi⁴⁾. Arai and Nagaiwa reported that the chromosome number was $2n=48$ and the karyotype consisted of all acrocentric chromosomes. On the other hand, Murofushi reported the same chromosome number but a different karyotypic constitution consisting of two subtelocentric and 46 acrocentric chromosomes. This difference is an important problem related to specia-

tion. Chromosome banding making more detailed chromosome analysis possible has greatly contributed to recent advances in fish cytogenetics, but no banding study has been conducted in this species.

The present paper deals with a karyotypic study with analysis of C-banded constitutive heterochromatin of *M. japonica*.

2. Materials and methods

A female specimen of *Monocentris japonica* was captured in Kushimoto, Wakayama Prefecture, Japan, and used for this study.

Chromosome slides were prepared according to the direct method using kidney tissue⁵⁾. The specimen was injected intraperitoneally with colchicine at about

0.1 $\mu\text{g/g}$ (body weight). After five hours, kidney tissue was removed, placed on a petridish with a small amount of culture medium such as Eagle's MEM, and crushed well with tweezers with flat heads. About 4 ml of the medium was added and sufficient pipetting was done to obtain more liberated cells and tissue fragments were removed. The cells were treated with 0.068M KCl hypotonic solution for 20 min at 25°C and fixed with a 1:3 mixture of acetic acid and methanol three times. One drop of the cell suspension was spread on a clean slide and air-dried. The chromosome slides were stained with 2% Giemsa solution diluted with 1/15M phosphate buffered saline (pH 6.8) and observed microscopically.

After the chromosome slides were destained with 70% alcohol, C-banding was conducted according to a modification⁶⁾ of the BSG methods by Sumner⁷⁾. The slides are immersed in saturated (5%) $\text{Ba}(\text{OH})_2$

solution at 50°C for 60–90 s, dipped in 0.1N HCl for several seconds, rinsed well with distilled water, and air-dried. They are incubated in $2\times\text{SSC}$ (0.3M NaCl+0.03M trisodium citrate) solution at 60°C for 60 min, rinsed with distilled water, and stained with 4% Giemsa diluted with 1/15M phosphate buffer (pH 6.8) for about 10 min. The conventional and C-banding chromosomes on the same metaphase plate were analyzed.

3. Results

Figure 1 shows the conventional and C-band staining karyotypes of *Monocentris japonica*. The diploid chromosome number of this species was $2n=48$ and the karyotype consisted of all acrocentric chromosomes, the fundamental number being 48. The size of the chromosomes showed a gradual declining series.

In C-banding chromosomes, large- and similar-sized C-banded constitutive hete-

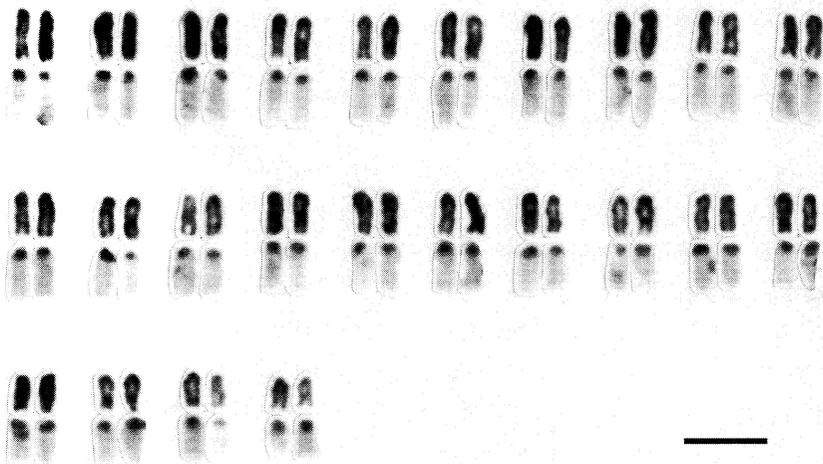


Figure 1. Conventional and C-band staining karyotypes of a monocentrid fish, *Monocentris japonica*. The chromosome number is $2n=48$ consisting of all acrocentric chromosomes. Bar scale indicates 5 μm .

rochromatin blocks were distributed in the centromeric regions of all chromosomes.

4. Discussion

Two different karyotypes in *Monocentris japonica* have been reported by Arai and Nagaiwa³⁾ and Murofushi⁴⁾ as described above. This difference is an important problem related to the speciation. The result in this study was identical with that of Arai and Nagaiwa. The specimens used by Arai and Nagaiwa and Murofushi were obtained from different areas, Chiba and Shizuoka, respectively.

A karyotype consisting of 48 acrocentric chromosomes (48A karyotype) is the ancestral form in fish and many fish groups at the order and family levels⁸⁾. It is well known that fish chromosomes have been diversified from the 48A karyotype involving pericentric inversion, Robertsonian rearrangement, tandem fusion, and so on⁹⁾. The karyotype presented in this study was a representative 48A karyotype. The karyotypic difference may have resulted from a karyotypic change involving pericentric inversion. Therefore, it is likely that the 48A karyotype shown in this study and by Arai and Nagaiwa is the original karyotype of this species and that the karyotype shown by Murofushi is a derived karyotype. Further interesting problems are whether the karyotype shown by Murofushi distributes in only a limited area and whether the karyotypic difference shows a variation at the species or subspecies levels. Further investigation in various areas in Japan is needed to solve this problem.

Chromosomal regions differentially stained by C-banding techniques, called C-

bands, show localized sites of constitutive heterochromatin. In fish, C-bands are observed mostly in centromeric regions, and sometimes in telomeric and interstitial regions^{10, 11}. Although C-banded heterochromatin distribution on fish chromosomes shows a great variation among chromosomes and species, C-bands of many species are distributed uniformly as small dot-like forms in centromeric regions and their stainability is often weak.

A feature of C-banded heterochromatin distribution shown in this study was that large-sized C-banded heterochromatin were distributed on the centromeric regions of all chromosomes. This feature may be a result of the accumulation of heterochromatin. Thus, the result of C-banding reveals that the karyotype of this species has evolved involving chromosomal changes in the heterochromatic regions though looks like the ancestral type involving no chromosomal change in conventional staining. It is hoped that more interesting results in C-banding and other banding studies in the fish of Monocentridae and related groups will be found.

References

- 1) Nelson, J. S. : Fishes of the World, 3rd Edition. John Wiley & Sons, Inc., New York (1994).
- 2) Masuda, H., Amaoka, K., Araga, C., Uyeno T. and Yoshino T. (eds.) : The Fishes of the Japanese Archipelago. Tokai University Press, Japan (1984).
- 3) Arai, R. and Nagaiwa, K. : Chromosomes of two species of Beryciform fishes from Japan. *Bull. Natn. Sci. Mus. Ser.*, **A2**, 199-203 (1976).
- 4) Murofushi, M. : Karyotypes of two Beryciform fishes. *Rep. Mishima Res. Inst. Sci. Liv. Nihon Univ.*, **5**, 17-20 (1982).

- 5) Takai, A. and Ojima, Y. : Comparative studies of karyotypes and distributions of nucleolus organizer regions in pomacentrid fish. 2. *Cytobios*, **65**, 199-205 (1991).
- 6) Takai, A. and Ojima, Y. : Characteristics of chromosomes and electrophoretic patterns of muscle protein and lactate dehydrogenase in fish of genus *Carassius* (Cypriniformes). *Cytologia*, **55**, 255-263 (1990).
- 7) Sumner, A. T. : A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.*, **75**, 304-306 (1972).
- 8) Ohno, S. : Protochordata, Cyclostomata, and Pisces. In *Animal Cytogenetics Vol. 4, Chordata 1*, pp.1-92, Borntreager, Berlin (1974).
- 9) Ojima, Y. : Fish Cytogenetics. In *Chromosomes in Evolution of Eukaryotic Groups, Vol. 1* (A.K. Sharma and A. Sharma eds.), pp.111-145. CRC Press, Florida (1983).
- 10) Takai, A. and Ojima, Y. : Chromosomal distribution of C-banded heterochromatin in cyprinid fishes. *Proc. Japan Acad.*, **64B**, 49-52 (1988).
- 11) Takai, A. and Ojima, Y. : Constitutive heterochromatin distribution in the chromosomes of pomacentrid fishes. *Cytologia*, **64**, 87-91 (1999).

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(和文要旨)

マツカサウオ *Monocentris japonica* (Beryciformes) における核型と構成ヘテロクロマチンの分布
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キンメダイ目 (Beryciformes) のマツカサウオ *Monocentris japonica* の核型と構成ヘテロクロマチンの分布を研究した。染色体数は $2n=48$ で核型はすべて端部型染色体で構成されていた。C-band 分染により示された構成ヘテロクロマチンはすべての染色体の動原体部に分布し、そのサイズは大型であった。これらの結果を基に、マツカサウオの染色体変異と種分化の問題を議論した。