Bull. Natl. Res. Inst. Aquaculture, No. 24, 17-21 (1995)

Karyotypic Variation in Japanese Rosy Bittering, *Rhodeus ocellatus kurumeus* (Jordan and Thompson)

Kouichi Kawamura* and Kazumi Hosoya*

(Received December 9, 1994)

The karyotype of the endangered Japanese rosy bittering, *Rhodeus ocellatus kurumeus* was analyzed as one of the basic studies for preservation. The chromosome number was constantly 2n=48 in all materials. The karyotype of Yao population was composed of 18 metacentrics (M), 10 submetacentrics (SM) and 20 subtelocentrics (ST), while that of Yanagawa population 14 M, 14 SM and 20 ST. By the silver banding technique, nucleolar organizer regions (NORs) were recognized in one pair of the second largest ST regardless of localities. The karyotype of another subspecies *R. ocellatus ocellatus* from the Yodo River seems to be different from that of *R. ocellatus kurumeus* in the karyotype. There could also be a possibility considered, that *R. ocellatus ocellatus* from the Yodo River was not genetically pure, but hybrids between two subspecies of *R. ocellatus* or their offspring.

Key words: acrocentric, chromosome, karyotype, metacentric, nucleolar organizer region, *Rhodeus ocellatus kurumeus*, submetacentric, subtelocentric,

Introduction

Japanese rosy bittering, *Rhodeus ocellatus kurumeus* (Jordan and Thompson) is the endemic subspecies of *R. ocellatus* in Japan. This subspecies once inhabited low lands of western Honshu, Shikoku and Kyushu. During the World War II, another subspecies, *R. ocellatus ocellatus* (Kner) which originally inhabited Eastern part of Asian Continent, was accidentally introduced into Kanto District from China with contaminated in grass carp, *Ctenopharyngodon idellus* and silver carp, *Hypophthalmichthys molitrix* (Nakamura, 1955). The exotic subspecies had gradually expanded its distribution to all over Japan. *R. ocellatus ocellatus* is distinguishable by having a distinct white line along the anterior margin of the pelvic fins from *R. ocellatus kurumeus*. In the process of the dispersal of *R. ocellatus ocellatus*, the intermediate type, which has both of the external features in the two subspecies, was found to appear here and there in the areas which *R. ocellatus kurumeus* had originally inhabited (Nakamura, 1969). As one of the most commonest features found in the intermediate type, the white line is known (Nakamura, 1969). It was certified with electrophoresis that genetic introgression of *R. ocellatus ocellatus* to *R. ocellatus kurumeus* already occurred (Ueno, 1985). Through hybridization genetically pure local popula-

* National Research Institute of Aquaculture, Nansei, Mie 516-01, Japan (河村功一, 細谷和海：養殖研究所)

tions of *R. ocellatus kurumeus* have become very rare. Consequently they can be found only in some restricted areas of Fukuoka, Kagawa and Osaka Prefecture (Nagata, 1989). So, it is emergently necessary to preserve the endangered subspecies, *R. ocellatus kurumeus* (Hosoya and Maehata, 1994). And it seems very meaningful to study the biological aspects of this subspecies for preservation. The detailed karyological information of *R. ocellatus kurumeus* had not been obtained so far. So in this paper, we reported the karyotype of *R. ocellatus kurumeus* and tried to compare it with the other subspecies, *R. ocellatus ocellatus*.

Materials and Methods

Parental fish were collected from two localities in 1994: small ponds in Yao City, Osaka Prefecture and from the Futatsu River in Yanagawa City, Fukuoka Prefecture. Populations which we sampled materials from, had previously been confirmed on each genetic identity by isozymic analysis (Prof. Nagata, personal comm.). Chromosome preparation was made by using embryo cells, basically following the technique of Ueda *et al.* (1991). Our modifications were the following five points. (1) Fertilized eggs were kept in 25°C incubator for 12 hours, instead of 17–19°C for one day. (2) The culture medium was Leibovitz medium (L–15) with 5% fetal calf serum and 0.005% colchicine at pH 7.5. (3) Pieces of the tissues were suspended with 0.5% sodium citrate solution at 25°C for four to five minutes. (4) Before staining, slide glasses, on which cell suspension was dropped, were slightly dipped into 70% methanol to remove little waste on their surface. (5) The cells were stained with the 4% Giemsa's solution (Merck index No. 9204) diluted with phosphate buffer (pH 7.4). To some slide preparations, after Giemsa staining, Ag-NORs staining technique (Howell and Black, 1980) was applied to elucidate nucleolar organizer regions (NORs). The nomenclature of chromosomes followed Levan *et al.* (1964).

Karyotype of *Rhodeus ocellatus kurumeus*

Five materials from each of two localities, Yao and Yanagawa, were analyzed in their karyotypes. Counts on chromosome number in more than 5 cells in each sample always showed 2n=48, regardless of materials and localities (Fig. 1). Seven karyotypes were analyzed and almost stable in each locality. In Yao population, the karyotype was composed of 18 metacentrics (M), 10 submetacentrics (SM) and 20 subtelocentrics (ST) (Fig. 2). In Yanagawa population, it was composed of 14 M, 14 SM and 20 ST. Between the two populations, a minor difference could be observed in the number of M and SM. Regardless of localities and samples, satellites were found in one pair of the second largest ST and these could be characteristically well stained by Ag-NORs staining technique.

Ojima *et al.* (1973) reported 2n=48 chromosomes in *R. ocellatus smithii* (=*kurumeus*) from the Yabe River, Fukuoka Prefecture, together with *R. ocellatus ocellatus* from the Yodo River, Osaka Prefecture. They shared the same karyotype of 8 M, 20 SM and 20 ST, with satellites in one pair of the second largest ST. Though the chromosome number and information about NORs of *R. ocellatus ocellatus* at present study was almost coincident with Ojima *et al.* (1973), a karyotypic difference was found in the number of M and SM. It is due to subspecific distinction. Samples from the Yodo River in

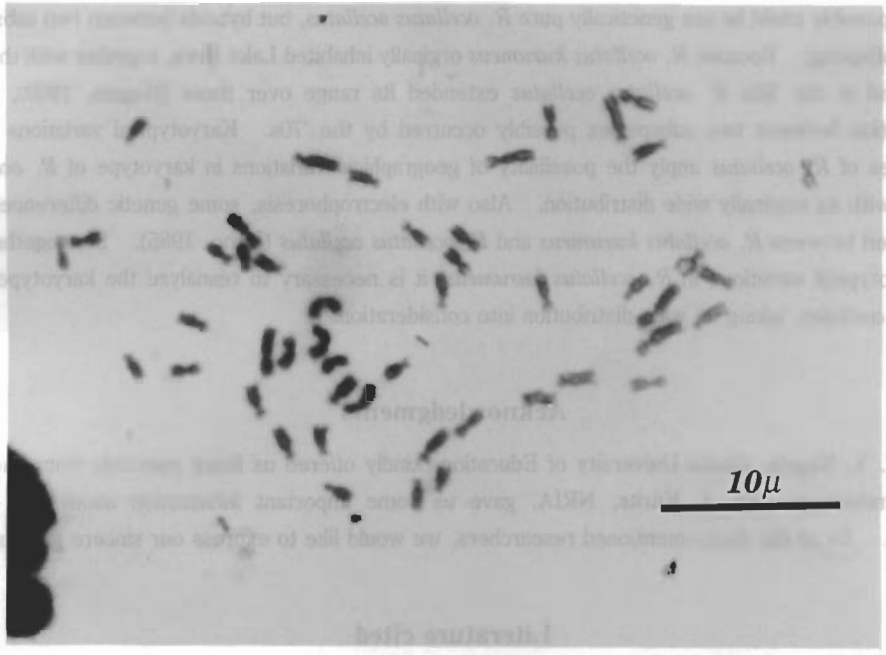

Fig. 1 Metaphase spread of Japanese rosy bittering, *Rhodeus ocellatus kurumeus* from Yao, Osaka.

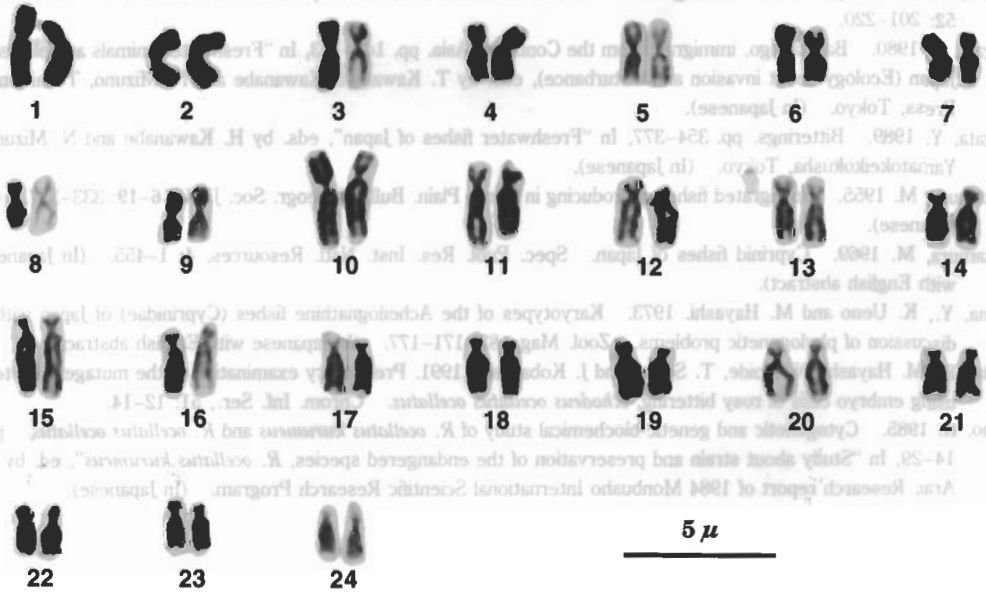

Fig. 2 Karyotype of Japanese rosy bittering, *Rhodeus ocellatus kurumeus*, derived from metaphase spread in Fig. 1. 2n=48 (1–9: metacentric, 10–14: submetacentric: 15–24: subtelocentric).

the '70s possibly could be not genetically pure *R. ocellatus ocellatus*, but hybrids between two subspecies or their offspring. Because *R. ocellatus kurumeus* orginally inhabited Lake Biwa, together with the Yodo River, and in the '60s *R. ocellatus ocellatus* extended its range over there (Nagata, 1980), so the hybridization between two subspecies possibly occurred by the '70s. Karyotypical variations in two subspecies of *R. ocellatus* imply the possibility of geographical variations in karyotype of *R. ocellatus*, coupled with its originally wide distribution. Also with electrophoresis, some genetic differences were ascertained between *R. ocellatus kurumeus* and *R. ocellatus ocellatus* (Ueno, 1985). So, together with the karyotypical variations in *R. ocellatus kurumeus*, it is necessary to reanalyze the karyotype of *R. ocellatus ocellatus*, taking its wide distribution into consideration.

Acknowledgments

Prof. Y. Nagata, Osaka University of Education, kindly offered us living materials from Yao City, Osaka Prefecture. Mr. J. Kurita, NRIA, gave us some important information about cell culture mediums. To all the above-mentioned researchers, we would like to express our sincere gratitude.

Literature cited

Hosoya, K. and M. Maehata. 1994. Circumstances in endangered Japanese freshwater fishes with notes on the protection. Bull. Natl. Res. Inst. Aquaculture, 23: 17–25.

Howell, W. M. and D. A. Black. 1980. Controlled silverstaining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia, 36: 1014–1015.

Levan, A., K. Fredga and A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. Hereditas, 52: 201–220.

Nagata, Y. 1980. Baratanago, immigrant from the Continent Asia. pp. 147–153, In "Freshwater animals and plants of Japan (Ecology about invasion and disturbance), eds. by T. Kawai, H. Kawanabe and N. Mizuno, Tokai Univ. Press, Tokyo. (In Japanese).

Nagata, Y. 1989. Bitterings. pp. 354–377, In "Freshwater fishes of Japan", eds. by H. Kawanabe and N. Mizuno, Yamatokeikokusha, Tokyo. (In Japanese).

Nakamura, M. 1955. Immigrated fishes reproducing in Kanto Plain. Bull. Biogeogr. Soc. JPN, **16–19**: 333–337. (In Japanese).

Nakamura, M. 1969. Cyprinid fishes of Japan. Spec. Publ. Res. Inst. Natl. Resources, **4**: 1–455. (In Japanese with English abstract).

Ojima, Y., K. Ueno and M. Hayashi. 1973. Karyotypes of the Acheilognathine fishes (Cyprinidae) of Japan with a discussion of phylogenetic problems. Zool. Mag., 82: 171–177. (In Japanese with English abstract).

Ueda, T., M. Hayashi, N. Koide, T. Sofuni and J. Kobayashi. 1991. Preliminary examination of the mutagenicity test using embryo cells of rosy bittering, *Rhodeus ocellatus ocellatus*. Chrom. Inf. Ser., **51**: 12–14.

Ueno, K. 1985. Cytogenetic and genetic-biochemical study of *R. ocellatus kurumeus* and *R. ocellatus ocellatus*. pp. 14–29, In "Study about strain and preservation of the endangered species, *R. ocellatus kurumeus*", ed. by R. Arai, Research report of 1984 Monbusho International Scientific Research Program. (In Japanese).

ニッポンバラタナゴの核型変異

河村　功一・細谷　和海

希少種ニッポンバラタナゴの核型分析を行った。大阪八尾産と福岡柳川産について調べた結果，大阪八尾産は染色体数48本で中部着糸型18本，次中部着糸型10本，次端部着糸型20本であり，一方，福岡柳川産は中部着糸型14本，次中部着糸型14本，次端部着糸型10本であった。仁形成体の存在については，産地に関係なく第２次端部着糸型の１ペアにその存在が認められた。すでに報告されている大阪府淀川産のタイリクバラタナゴとでは染色体数は同じであるものの，核型構成に若干の違いが見られた。しかし，この報告は1970年代に採集された個体に基づくことより，純系のタイリクバラタナゴではなくニッポンバラタナゴとの雑種を用いた可能性も考えられた。