83. A Heteromorphic Chromosome of *Beryx splendens*, *Berycidae* (Pisces)^{*)}

By Yoshio OJIMA and Tsukasa KIKUNO

Department of Biology, Faculty of Science,
Kwansei Gakuin University, Nishinomiya

(Communicated by Sajiro MAKINO, M. J. A., Oct. 13, 1986)

In 1916 W. R. B. Robertson pointed out that a metacentric chromosome in one species, race or individual may correspond to two acrocentrics in another. Chromosomal changes involving the Robertsonian fusion, inversion, translocation and so on may take place in relation to species and the sexual differentiation.

The present article deals with the chromosomal features of *Beryx splendens*, in relation to the polymorphism between male and female. The Berycidae includes two genera, *Beryx* and *Centroberyx* with about 10 species in the world. Almost all the specimens referred to here were collected at the point of 300 meters deep in the offing of the Izu peninsular.

Materials and methods. The fins were rinsed five times with aseptic Ca^{2+} , Mg^{2+} free phosphate-buffered saline and cut into small fragments after being sterilized in sodium hypochloride (3.6%). The pieces were digested by stirring with a magnetic stirrer for one hour with 8 ml of 0.15% trypsin-EDTA solution in an MA-30 culture bottle. Afterward the separated cells were collected by the centrifugation at 1500 rpm for 10 min. The cells were cultured in a 60 mm tissue culture dish at 25°C. Under these conditions the cells grew and achieved confluency within two days. A trypsin-EDTA solution was used to detach the cells from the surface of the dish. Six hours before the harvest, 0.2 ml of $10\ \mu\text{M}$ TN-16 was added and then slides of the chromosome were prepared. The BSG method of Sumner (1972) was employed for C-banding analysis. The silver staining of NORs was carried out following Howell and Black (1980).

Results and remarks. As shown in Figs. 1 and 3 in the diploid state the male of *Beryx splendens* has 47 chromosomes comprising 5 metacentrics, including one big metacentric chromosome; 8 submetacentrics; and 34 acrocentrics. The big metacentric chromosome shows a pale C-band staining in the centromere region, but no peculiar NORs staining.

Figs. 2 and 4 show female karyotypes having 4 metacentrics, 8 submetacentrics, and 36 acrocentrics. The big metacentric chromosome present in the male is absent in the female.

In both sexes the biggest submetacentric chromosomes have strongly stained C-banding and the nucleolus organizer regions in a secondary constriction near each centromere (Figs. 1-4). A decrease of two elements of the A-type complement regularly resulted in an increase of one element of the M-type complement. Then the chromosome number differs between the male and the female. The NF value was 60 in the male and female. This indicated that the chromosomal polymorphism resulted from the Robertsonian fusion in relation to the

^{*)} This work was supported by a grant-in-aid of the Science Research Promotion Fund from the Japan Private School Promotion Foundation.

sexual differentiation.

The cytogenetic identification of the sex-chromosomes in teleosts is sometimes impossible, even though their existence has been proved by genetic methods. Generally speaking, teleosts seem to be sexually undifferentiated, and sexuality is characterized by such a diversity as the hermaphroditism, gonochorism, and unisexuality. 73 species of fishes with heteromorphic sex chromosomes have been presented in the world up to the present (Ojima, 1986). The heteromorphic complexes included all the types, such as XX-XY, XX-XO, $X_1X_1X_2X_2-X_1X_1X_2$, $X_1X_1X_2X_2-X_1X_2Y$, XX-XY₁Y₂ in the male, and ZW-ZZ, ZO-ZZ, and ZW_1W_2-ZZ in the female. Among these the common possibility for a sex-chromosome mechanism involving a multiple sex chromosome was provided through the karyological evidence that the male possesses one less acrocentric chromosome than the female

Figs. 1-2: Metaphase figures of male and female. The big arrow indicates a big metacentric chromosome present in the male and not in the female. The fine arrows indicate the biggest submetacentric chromosomes with a secondary constriction near the centromere.

and, in addition, generally a single large metacentric chromosome (Uyeno and Miller, 1971; 1972; Miller and Walters, 1973; Murofushi *et al.*, 1980; 1983; Bertollo *et al.*, 1983; Pezold, 1984). Further evidence has been provided by two chromosomal observations: (1) the existence of a single large Y chromosome at the spermatogonial prophase, and (2) except for bivalents, the existence of one trivalent in which X_1 and X_2 chromosomes are attached to the Y chromosome in an end to end association at diakinesis in meiosis (Uyeno and Miller, 1971, 1972; Murofushi *et al.*, 1980, 1983; Bertollo *et al.*, 1983; Pezold, 1984). Though the chromosomes of X_1 and X_2 have never been identified, this evidence showed that the metacentric Y chromosome has originated by a Robertsonian fusion between an acrocentric Y and one member of an acrocentric autosome pair. In the present case, the big heteromorphic metacentric of the male can be understood as the Y on the $X_1X_1X_2X_2-X_1X_2Y$ constitution. This should be confirmed by the appropriate examination of the sex-chromosome differentiation by the banding techniques, using not only somatic cells but germ cells as well.

Figs. 3-4: Karyotypes of male ($2n=47$, $NF=60$) and female ($2n=48$, $NF=60$). Conventional stain (upper row), C-banding stain (middle row), and Ag-NOR stain (lower row). Arrows show the intense stain of C-banding and NORs. The scales indicate 10 μm .

Acknowledgements. We are very indebted to Dr. Sajiro Makino, M. J. A., Professor Emeritus, Hokkaido University, and Dr. James R. Miller at the Central Research Division, Takeda Chemical Industry Co., Ltd., for improving and revising the manuscript.

References

- Bertollo, L. A. C. *et al.* (1983): *Cytologia*, **48**, 1–12.
Howell, W. M., and Black, D. A. (1980): *Experientia*, **36**, 1014–1015.
Miller, R. R., and Walters, V. (1973): *Chromosome Atlas*, **2**, 15.
Murofushi, M. *et al.* (1980): *Japan. J. Genetics*, **55**(2), 127–132.
—— (1983): *Proc. Japan Acad.*, **59B**, 58–61.
Ojima, Y. (1986): *Fish Chrom. Data Retrieval List*.
Pezold, F. (1984): *Copeia*, 1984(1), 235–238.
Robertson, W. R. B. (1916): *J. Morph.*, **27**, 179–331.
Sumner, A. T. (1972): *Exp. Cell Res.*, **75**, 304–306.
Uyeno, T., and Miller, R. R. (1971): *Nature (Lond.)*, **231**, 452–453.
—— (1972): *Experientia*, **28**, 223–225.