14. Cytogenetical Studies on Fishes. V

Multiple Sex Chromosome Mechanism (XX-Y) found in Two Dragonet Fishes^{†)}

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Dragonet fishes inhabit generally in the sand and mud-bed of the shallow water region in the sea. The male and female of the dragonet fishes are well differentiated by the length of the spine and soft ray. Those of the male are usually longer than those of the female. The external organ is also clearly different in each sex. Although it has been considered that the sex-chromosomes are not differentiated in fishes, but recently the sex-differentiation has been revealed by finding of the sex-chromosomes in some fish species. An example of the XX-Y mechanism has been found by us to occur in a filefish (Murofushi *et al.* 1980). Very similar case of the multiple sex-chromosome mechanism has been found to occur in two dragonet fish species, *Callionymus beniteguri* and *C. ornatipinnis*. In the present paper the karyotypes from somatic cells of male and female specimens and male germ cells in these two dragonet fish species are to be reported with special reference to the differentiation of the sex-chromosomes in fishes in general.

Material and methods. Nineteen specimens including 10 males and 9 females of *Callionymus beniteguri*, and seventeen specimens containing 11 males and 6 females of *C. ornatipinnis* were provided as materials for the present study. The former specimens were obtained from the near coast of Hirao and Yanai of the Yamaguchi Prefecture, and from Mito and Shimoda of the Shizuoka Prefecture in a period ranging from October, 1978 to September, 1981, while the latter ones were collected from the near coast of Hirao and Yanai of the Yamaguchi Prefecture, ranging from May, 1978 to July, 1980. The total body-length of the specimens used ranged from 105 mm to 200 mm in *C. beniteguri*, while 127 mm to 195 mm in *C. ornatipinnis*. The male and female specimens were identified by their external morphology and sexual organs. The chromosome observations were

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carried out by our routine technique described previously (Murofushi and Yosida, 1979).

Results and discussion. The diploid chromosome-number of the female specimens of these two dragonet species was thirty-eight ($2n=38$), all elements being subtelocentric in morphology (Fig. 1, A and C). In contrast, the diploid chromosome-number of the male ones was thirty-seven ($2n=37$), among which one large metacentric chromosome was conspicuous in their karyotypes (Fig. 1, B and D). The metacentric element occurring only in the male specimens seemed to be the Y chromosome. If these species were characterized by a usual XY-mechanism, the chromosome-number of the female should be the same as that of the male. However, the odd chromosome-number ($2n=37$) was always present in the males, one chromosome being less in number in the male than in the female specimens. Based on the above-mentioned chromosome-complement, the following suggestion is to be possible that, in these two species, the male has the X_1X_2-Y , while the female has the $X_1X_2-X_1X_2$ in the sex-determination mechanism.

Fig. 1. Karyotypes of two dragonet fishes, *Callionymus beniteguri* and *C. ornatipinnis*. A and B: *C. beniteguri*. A, female ($2n=38$) with $X_1X_2-X_1X_2$. B, male ($2n=37$) with X_1X_2-Y . C and D: *C. ornatipinnis*. C, female ($2n=38$) with $X_1X_2-X_1X_2$. D, male ($2n=37$) with X_1X_2-Y .

To confirm the above findings the secondary spermatocytes were investigated in the male specimens of these two species. There were two cell types in them, one of them being $n=19$, whereas the other had $n=18$. In the former cells, one metacentric chromosome was always present (Fig. 2, B and D), whereas in the latter, no such element was found (Fig. 2, A and C). The above findings have shown that the sex-chromosome mechanism of these two species is male heterogametic with an X_1X_2 -Y mechanism. The metacentric Y chromosome in the multiple sex-chromosome mechanism occurring in these fishes should have been derived from the Robertsonian fusion between an ordinarily acrocentric Y and one member of an autosome pair. Thus, the following conclusion may be possible that these two dragonet fish species have the X_1X_2 -Y mechanism in the male, while the X_1X_2 - X_1X_2 in the female.

It has been well accepted for a long time that the sex-chromosome has not been differentiated in fishes, while recently some species, such as *Allodontichthys hubbsi* (Uyeno and Miller 1971), *Megupsilon aporus* (Miller and Walters 1973), and *Stephanolepis cirrhifer* (Murofushi *et al.* 1980) have been reported to carry a multiple sex-chromosome mechanism such as an XX-Y. Murofushi *et al.* (1980) have suggested

Fig. 2. Secondary spermatocytes in two dragonet fishes. A and B: *Callionymus beniteguri*. A, $n=19$ without any metacentric element. B, $n=18$ with a metacentric Y. C and D: *C. ornatipinnis*. C, $n=19$ without any metacentric element. D, $n=18$ with a metacentric Y. X_1X_2 chromosomes are not identified in the secondary spermatocytes.

that the origin of the multiple sex-chromosome in *S. cirrhifer* would be induced by the fusion of the sex-chromosome to an autosome. In the present species here dealt with, it was found that the multiple sex-chromosomes was also resulted from the Robertsonian fusion between the Y chromosome and an autosome. Although the sex-chromosome could not be morphologically differentiated in most species of the fishes, it would be differentiated genetically, because when the autosome is translocated to the Y the sex chromosome is easily identified from the autosome as a multiple sex chromosome system. These findings suggest that the Robertsonian fusion between the Y chromosome and an autosome is to be one of the mechanisms in morphological differentiation of the sex-chromosome in fishes in general.

Summary. The chromosome-numbers of females in two dragonet fish species, *Callionymus beniteguri* and *C. ornatipinnis*, are thirty-eight ($2n=38$), all being subtelocentric in morphology. In the males of these two species, however, the chromosome number is thirty-seven ($2n=37$), among which one chromosome is of large metacentric in morphology. Two haploid chromosome numbers, $n=18$ and 19, are found to occur in the secondary spermatocytes. In the latter with $n=18$, one large metacentric chromosome is always present in any cell. The metacentric element observed in somatic and germ cells is suggested to be the Y chromosome. Thus, the sex-chromosome mechanism in these two species is inferred to be a multiple system, being an X_1X_2 - X_1X_2 mechanism in the female, and an X_1X_2 -Y in the male.

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References

- Miller, R. R., and Walters, V.: Chromosome Atlas, **2**, 15 (1973).
Murofushi, M., and Yosida, T. H.: Japan. J. Genet., **54**, 191-195 (1979).
Murofushi, M., Oikawa, S., Nishikawa, S., and Yosida, T. H.: *ibid.*, **55**, 127-132 (1980).
Uyeno, T., and Miller, R. R.: Nature (Lond.), **231**, 452-453 (1971).