Chromosome Studies in Pisces, VI. The X-Y Chromosomes Found in *Cottus pollux* Günther (Cottidae)

By

Shyunsaku Nogusa

(Zoological Laboratory, Hyogo College of Agriculture,
Sasayama, Hyogo Prefecture, Japan)

(With 13 Text-figures)

The cytological sex-determining mechanism of Pisces has been a matter of discussion during these thirty years by both cytologists and geneticists, from the viewpoint of genetics on the one hand, and on the other, from the viewpoint of the evolution of vertebrates. The present author seems to be the first to present definite evidence regarding the sex chromosomes in fish, having reported a clear-cut XY mechanism in a species of the Gobiidae, *Mogrunda obscura* (Temminck et Schlegel) (Nogusa 1955). Recently, additional evidence has been found by the author in *Cottus pollux* (Cottidae), contributing to the cytological demonstration of male heterogamety. The findings are reported in the following.

It is the author's pleasure to dedicate this article to the Jubilee Volume of Professor Tohru Uchida. The author wishes to express his sincere gratitude to Dr. Sajiro Makino, Professor at Hokkaido University, for revising the manuscript and for helpful criticism. Cordial thanks are also extended to Prof. T. Mori, Prof. K. Suzuki and Dr. O. Minouchi for their valuable advice during the course of the study.

Material and method

Cottus pollux Günther is one of the common fresh water teleosts belonging to the Cottidae. They are widely distributed through Japan. The specimens for this study were collected in the vicinity of Sasayama, Hyogo Prefecture at various times of the year. The fishes killed in November and January furnished favorable material for this study. The testicular tissues were taken out by vivisection and fixed in Champy's mixture. Subjected to the usual paraffin method, the sections were stained with iron-haematoxylin after Heidenhain, and partly with Feulgen's stain.

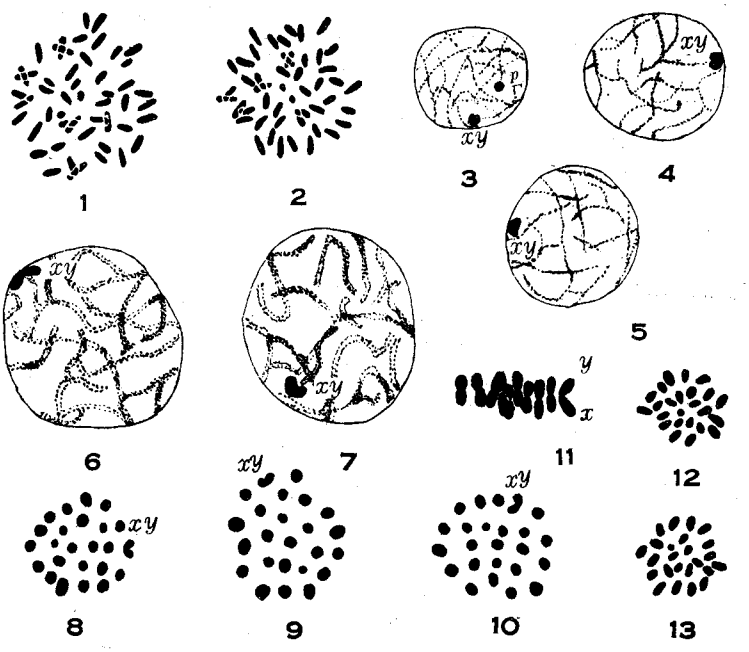
Observations

Growing stages of spermatocytes. Through all stages of the growth period, the primary spermatocytes show a definite chromatic body in each nucleus. Apparently it is easily distinguished from the nucleolar element on account of the intense affinity for haematoxylin and of the Feulgen positive reaction. Based on the staining reaction of these bodies, it is evident that the former is the hetero-

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 13, 1957 (Prof. T. Uchida Jubilee Volume).

pycnotic sex-element and the latter is the true nucleolus (plasmosome). The heteropycnotic XY is found free in the nucleus without associating with the true nucleolus. The nucleus at early leptotene shows the heterochromatic sex-element which lies always along the inner side of the nuclear membrane, in contrast to the true nucleolus lying in the interior of the nuclear vesicle (Fig. 3). At late leptotene, the sex-element is recognizable as a heart- or V-shaped body, showing a distinction into two components consisting of the X and Y elements in end-to-end connection (Figs. 4–5). The plasmosome nucleolus becomes invisible during the period through late leptotene to zygotene. The heteropycnotic XY bivalent is very striking in occurrence in the pachytene nucleus (Figs. 6–7). Through diplotene to diakinesis the XY-body is easily distinguished from the autosome bivalents by its peculiar configuration.

The primary and secondary spermatocytes. The chromosomes of the first



Figs. 1-13. Chromosomes of *Cottus pollux*. ×3800. 1–2, spermatogonial metaphases, 48 chromosomes in each. 3, nucleus at early leptotene stage, indicating the heteropycnotic nucleolus (XY element). p, plasmosome. 4–5, nuclei at late leptotene stage. 6–7, the same at pachytene. 8–10, primary spermatocyte metaphases, 23 bivalents and a XY bivalent in each. 11, side view of the first division, showing XY complex. 12–13, secondary spermatocyte metaphases. 24, dyads in each.

meiotic metaphase plate are observable with great clearness. Every primary spermatocyte contains at metaphase 24 distinct chromosomes in haploid. Closer examination has revealed that the haploid complex consists of 23 autosomal bivalents and a heteromorphic bivalent provided with the X- and Y-element (Figs. 8–10). The latter always occupies a peripheral position in the equatorial arrangement. The structural configuration of the XY-complex is better observed in the side view of the spindle than in the polar view (Fig. 11). In the side view the autosomal bivalents are all dumbell-shaped, lying vertical to the equatorial plate, while the XY-complex is distinctly characterized by an open V-shaped form. The X-element conjugates with the Y by means of an end-to-end attachment. It is one of the larger sized chromosomes. The size difference existing between the X- and Y-element is very small; the X-element seems to be slightly, but visibly, larger than the Y. As a result of the segregation of the X and Y in the first division, there are produced two kinds of secondary spermatocytes as regards the distribution of the X and Y. Every metaphase of the secondary spermatocytes always shows 24 chromosomes (Figs. 12–13). The author has failed to identify the X- and Y-element complement, on account of the slight size-difference between the X and Y.

The spermatogonium. The resting nucleus of the spermatogonium generally shows a single plasmosome nucleolus of small size. Careful counting of several metaphase plates of the spermatogonia gave 48 as the diploid number in every case, furnishing corroborative evidence for the haploid number of 24 (Figs. 1–2). The diploid group consists of elements of a simple rod-type with slight variations in length, one pair of which are extremely small. There is no evidence for the occurrence of a metacentric V-shaped element. Judging from the data obtained from the observations in the meiotic phases, it is apparent that the spermatogonial complement includes the X- and Y-element. The identification of the X- and Y-chromosome, however, seems to be practically difficult, due to the prevalence of the chromosomes of similar size, and to the slight size difference between the X and Y.

Remarks

On reviewing the literature, it is evident that a considerable number of papers has been published on the chromosomes of various forms of teleost fishes (see Makino's list, 1956). It has been emphasized by Makino (1934), Wickbon (1943) and White (1954) that the sex chromosomes of Pisces as well as Amphibia are in state of the lowest differentiation in evolution. According to their view, the sex-chromosomes of the lower vertebrates show no morphological differentiation from the autosomes.

Clear-cut evidence for the XY-mechanism has been demonstrated by the present author (Nogusa 1955) in the male of the gobiid fish, *Mogrunda obscura*. That paper seems to be the first report to offer cytological evidence of male heterogamety in fishes. The XY chromosome were clearly observable during

the growth period and at the metaphase of the primary spermatocyte on account of their morphologically differential characters. Recently, cytological male heterogamety has been reported by Yoshida (1956) to occur in the tree frog, *Hyla arborea japonica*. Using his improved squash technique, he demonstrated the sex-chromosomes of an XY-type. These are evidence indicating that the sex-chromosomes of lower vertebrates are not always morphologically indistinguishable from the autosomes. The findings of the present study offer additional evidence for the above view. It is noticeable that the difference in size between the X- and Y-element is very slight, and therefore, they are distinguishable only in the side view of the first meiotic division. It is then probable that the sex-chromosomes of the present species show lower morphological differentiation than those of *Mogrunda obscura*.

Summary

The XY sex-determining mechanism was established in male germ cells of *Cottus pollux*, a species of the Cottidae. The diploid number was 48 in the spermatogonium and the haploid number of 24 was found in both the primary and secondary spermatocytes. The karyotype of this species consists of chromosomes of simple rod type with gradual difference of length. During the growth period, the X- and Y-elements were traced each as heteropycnotic bodies in the primary spermatocytes. The sex-chromosomes are represented by two rod-shaped ones of larger-sized elements, and slightly differ in length. The XY-complex is best demonstrable in the side view of the first meiotic spindle.

Literature

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