Chromosome Studies in Pisces
Ⅶ. A Comparative Study of the Chromosomes in Six Species of the Gobiidae

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It has been shown that the cytological differences concern wholly genetic systems with which specialization, evolution and adaptation deal. Knowledge of the chromosomes of fish is still meagre and the basic date for understanding the taxonomy and evolution is quite incomplete in every respect, as referred to the list published by MAKINO (1956). It is thus desirable to complete an accurate survey of the chromosomes in this field, from both cytological and taxonomical viewpoints. In the former papers the author has dealt with the cytotaxonomical differences of fish which exist between related species (NOGUSA 1943, 1950, 1953 a, 1955 b). The present paper reports the chromosomes of six species of the Family Gobiidae, with special regard to the karyological relationship in closely related species. Previously, the chromosomes of this family have been reported in three species by the present author (NOGUSA 1950, 1955 a). The finding of the present study will furnish additional data to the above.

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Material and method

The following six species of the Gobiidae, *Gobius similis* (GILL), *Gobius abei* (JORDAN et SNYDER), *Chaenogobius urotaenia* (HILGENDORF), *Chaenogobius isaza* (TANAKA), *Boleophthalmus pectinirostris* (OSBECK) and *Periophthalmus cantonensis* (OSBECK) are animals which came under investigation in the present study.

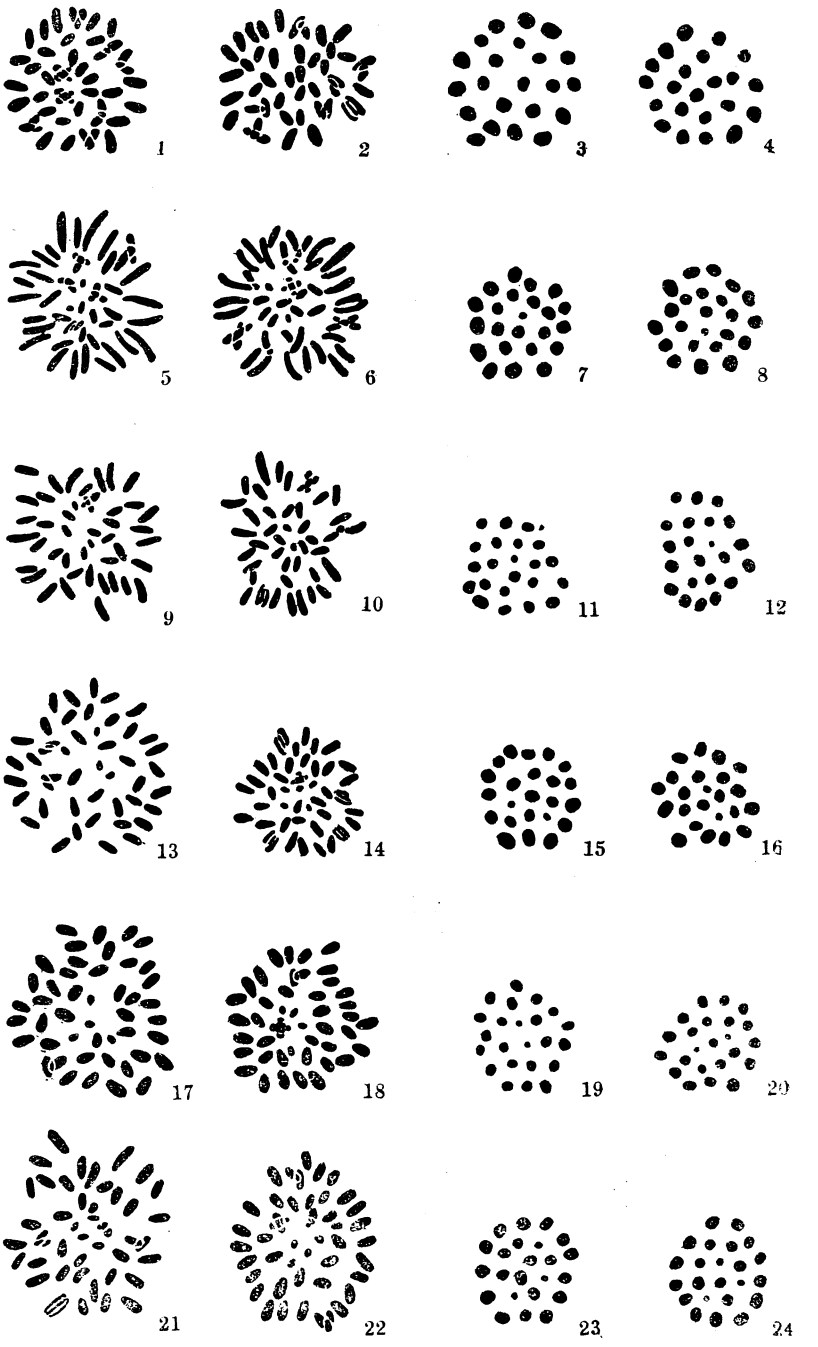
The male gonads were fixed in Champy's mixture containing 3.5 % potassium bichromate. Through the usual paraffin procedure, the testicular material was cut 4 to 5 micro thick and they were stained after the Heidenhain's iron-haematoxylin method with light green.

Observations

(1) *Gobius similis*

This is one of the most common freshwater fishes in Japan. The material for study was obtained in Sasayama, Hyogo Prefecture, in April 1951.

Every metaphase spermatogonial plate constantly showed 44 chromosomes as the



Figs. 1–4. Chromosomes of *Gobius similis*. **1–2**, Spermatogonial metaphases. **3–4**, Primary spermatocyte metaphases. **Figs. 5–8.** Chromosomes of *Gobius abei*. **5–6**, Spermatogonial metaphases. **7–8**, Primary spermatocyte metaphases. **Figs. 9–12.** Chromosomes of *Chaenogobius urotaenia*. **9–10**, Spermatogonial metaphases. **11–12**, Primary spermatocyte metaphases. **Figs. 13–16.** Chromosomes of *Chaenogobius isaza*. **13–14**, Spermatogonial metaphases. **15–16**, Primary spermatocyte metaphases. **Figs. 17–20.** Chromosomes of *Periophthalmus cantonensis*. **17–18**, Spermetogonial metaphases. **19–20**, Primary spermatocyte metaphases. **Figs. 21–24.** Chromosomes of *Boleophthalumus pectinirostris*. **21–22**, Spermatogonial metaphases. **23–24**, Primary spermatocyte metaphases. All are camera-lucida drawings ; ×3800.

diploid number (Figs. 1—2). The chromosomes seem to be of a telomitic type, because all the composing elements appear in a form of short rod having a slight fluctuation in length, though it is difficult to say whether they are acrocentric or telocentric in nature. The chromosome counting in the primary spermatocyte metaphases gave 22 as the reduced number (Figs. 3—4).

(2) *Gobius abei*

Preferring brackish waters, this species abounds near the estuaries or ascends the rivers of middle and southern Japan. The specimens for study were secured in Oomuta, Kyushu, in April 1955 and May 1957.

The diploid number observed in the spermatogonium was clearly 46 (Figs. 5—6), being two more than that of the former species. The chromosomes appear to be of a simple telomitic type in morphology, since all elements assume a short rod-shaped appearance showing a slight variation in length, two of them being conspicuously smaller in striking contrast to others. The individual chromosomes of this species are extremely slender as compared with those of the other species. The haploid chromosome number of 23 was determined through observations of primary spermatocytes (Figs. 7—8). The metaphase complex contains a small bivalent distinctive among the others.

(3) *Chaenogobius urotaenia*

This is also one of very common freshwater fishes in Japan. The study was based on the material obtained in Koyama, Tottori Prefecture, in November 1954.

Careful examinations of several spermatogonial metaphases revealed that there were 44 chromosomes as the male diploid complex (Figs. 9—10). The chromosome complement consists of simple rod-shaped elements of various length, a single pair of which are extremely small in contrast of others. The corroborative evidence for this finding was furnished from the observations of meiotic chromosomes by the fact that the primary spermatocyte metaphase always shows 22, containing a small-sized bivalent (Figs. 11—12).

(4) *Chaenogobius isaza*

The isaza goby lives at the depth of 17 or 18 m. in Lake Biwa, Shiga Prefecture and in the rivers pouring into the lake. In spring the fish migrates toward the shore and spawns in May and June. The specimens for observations were obtained in Katada, Lake Biwa, in March and April 1955.

The diploid number observed in spermatogonial metaphase was given to be 46, two more than that of the former species (Figs. 13—14). The chromosomes are all of a simple rod-type ; they arrange in the metaphase plate with their inner ends pointing towards the centre of the equatorial plate. This species is characterized by having two pairs of small-sized chromosomes in its complex. The primary spermatocyte metaphase shows 23 bivalents, of which two are strikingly small in size (Figs. 15—16).

(5) *Periophthalmus cantonensis*

This fish ranges in distribution along the coast of the southern part of Japan, hopping about on the mud-flats in ebb-tide. In winter they remain dormant deep in mud.

The present material was collected in Oomuta, Kyushu, in April 1955 and May 1957.

The diploid number of chromosomes was found to be 46 in the spermatogonium, and the haploid number to be 23 in the primary spermatocyte (Figs. 17—20). The chromosome complex consists of rod-shaped elements with a variation of length. It is a noticeable fact that this species is characterized by showing three pairs of remarkably small-sized chromosomes.

(6) *Boleophthalmus pectinirostris*

This species is abundant in Ariake Bay, Kyushu, in Japan. The material on which the study was carried out came from Yanagawa, Kyushu, in Apirl 1955 and May 1957.

The chromosome complex of this species is nearly similar to that of the previous species. In the spermatogonial metaphase there are observable 46 distinct chromosomes arranging radially in the equatorial plate (Figs. 21—22). The chromosomes are all rod-shaped in appearance. They show a gradual diminutoin in length, two pairs being extremely small. The metaphase plate of the primary spermatocyte contains 23 bivalent chromosomes (Figs. 23—24).

Comparision of the chromosomes in the closely related species

The chromosome study of the Family Gobiidae has been carried out by the present author (NOGUSA 1950, 1955 a) in *Tridentiger obscurus*, *Acanthogobius flavimanus* and *Mogurnda obscura*. *T. obscurus* and *A. flavimanus* were found as 44 in diploid, all elements being of a telomitic type with gradual difference of length. Both species have two pairs of the small chromosomes in their complements. It was pointed out that the small chromosomes exhibit a size variation by species ; the small-sized chromosomes of *A. flavimanus* are slightly larger than these of *T. obscurus*. It is a striking contrast that the chromosomes of *M. obscura* are considerably different from the above two species, showing 62 in $2n$.

The chromosome numbers and chromosomal formulae of six species of the Gobiidae under consideration are as shown in Table 1.

Table 1

Species	Chrom. number		Chrom. formula
	$2n$	n	
Gobius similis	44	22	44R's
Gobius abei	46	23	44R's+2m's
Chaenogobius isaza	46	23	42R's+4m's
Chaenogobius urotaenia	44	22	42R's+2m's
Boleophthalmus pectinirostris	46	23	40R's+6m's
Periophathalmus cantonensis	46	23	42R's+4m's

R : rod-shaped chromosome. m : small-sized chromosome.

By reference to the karyograms of these six species it is apparent that the karyotypes of these speies are closely related with each other, though there is a variation of the chromosome number from 44 to 46. Further, every one is characterized by possessing

a definite number of the particularly small-sized chromosome which is tentatively referred to as m-chromosome.

The chromosomes of *Gobius abei* shows 46 in diploid, consisting of 44 simple rod-shaped chromosomes and two small-sized m-chromosomes, whereas in *Gobius similis* the diploid number is 44, containing no element corresponding to the m-chromosome. As the result the chromosome number of *G. similis* is two less than that of *G. abei*. Thus, the numerical and morphological relationship of chromosomes between the two species of *Gobius* may be explained on the basis of the presence and absence of the m-chromosomes.

The similar relationship is found to occur between *Chaenogobius urotaenia* and *Chaenogobius isaza*. If the smallest pair of *C. isaza* would disappear, the induced number of chromosomes becomes 44 in total, that is the number for *C. urotaenia*.

Boleophthalmus pectinirostris possesses three pairs of m-chromosomes, while the related species, *Periophthalmus cantonensis*, has two pairs of such elements. The only difference between the two forms is that of the number of the m-chromosomes. It is then evident that these two species are akin to each other in both taxonomical and karyological relationships.

It has been shown by OGUMA (1930) that the reduction of the chromosome number in certain species of dragonflies is due to an absence of a pair of the minute chromosome as referred to as the m-chromosome. Several such instances have been reported to occur in some other animals showing that gradual size-reduction of the m-chromosome and their disappearance occur between related species ; the examples are demonstrated in dragonflies by OGUMA (1930), OGUMA and ASANA (1932) and KICHIJO (1939), in grasshoppers by MOMMA (1943), in lizards by OGUMA (1934) and NOGUSA (1953 b), and in murine rodents by MAKINO (1941, 1942, 1943). It is then apparent that the disappearance of the m-chromosome deals with the change of chromosome number from species to species in animals.

Summary

The chromosome of six species of the Gobiidae were investigated in male germ cells through spermatogenesis, with special regard to the chromosomal relationship in closely related species. The chromosome numbers of the species under study and chromosomal formulae are summarized in Table 1.

The spermatogonial complements of the studied species contain small-sized chromosomes as referred to as m-chromosomes, with the exception of *Gobius similis*. Each species is characterized by possessing a definite number of such m-chromosomes.

The reduction of the chromosome number which occur beween *Gobius similis* and *Gobius abei*, and between *Chaenogobius urotaenia* and *Chaenogobius isaza* seems to be due to the disappearance of the m-chromosomes.

Literature cited

KICHIJO, H, 1939 : Chromosomes of *Tachopteryx pryeri* and *Gomphus hakiensis* (Odonata :Aeschnidae). Jap. Jour. Genet., 15.

MAKINO, S. 1941 : Studies on the murine chromosomes, I, Cytological investigations of mice, included in the genus *Mus*. Jour. Fac. Sci. Hokkaido Imp. Univ., Ser. VI, Zool. 7.

————, 1942 : Studies on the murine chromosomes, II. Morphological comparision of the chromosomes between the wild form and the domesticated variety of *Rattus norvegicus* (BERKENHOUT). Jour. Fac. Sci. Hokkaido Imp. Univ., Ser. VI, Zool. 8.

————, 1943 : Studies on the murine chromosomes, III. A comparative study of chromosomes in five species of *Rattus*. Jour. Fac. Sci. Hokkaido Imp. Univ., Ser. VI, Zool. 9.

———— , 1956 : A review of the chromosome numbers in animals. Tokyo.

MOMMA, E. 1943 : A karyogram study on 18 species of Japanese Acrididae (Orthoptera). Jour. Fac. Sci. Hokkaido Imp. Univ., Ser. VI, Zool. 9.

NOGUSA, S. 1943 : On the chromosomes of four species of the Cyprinidae (Pisces). Jap. Jour. Genet., 19.

————, 1950 : Chromosome studies in Pisces, I. On the chromosomes of two species of the Gobiidae. Jap. Jour. Genet., 25.

————, 1951 : Chromosomes studies in Pisces, III. The chromosomes of four species of the Cyprinidae. La Kromosomo, 11.

————, 1953 a : List of the chromosome complement in the Cyprinidae. Sci. Rep. Hyogo Univ. Agri., Ser. Nat. Sci. 1.

————, 1953 b : A study of the chromosomes of some lizards, with special remarks on the m-chromosomes. Cytologia, 18.

————, 1955 a : Chromosome studies in Pisces, IV. The chromosomes of *Mogrunda obscura*, with evidence of male heterogamety. Cytologia, 20.

————, 1955 b : Chromosome studies in Pisces, V. Variation of the chromosome number in *Acheilognathus rhombea* due to multiple-chromosome formation. Annot. Zool. Jap., 28.

OGUMA, K. 1930 : A comparative study of the spermatocyte chromosome in allied species of the dragonfly. Jour. Fac. Sci. Hokkaido Imp. Univ., Ser. VI, Zool. 1.

————, 1934 : Studies on Sauropsid chromosomes. II. The cytological evidence proving female heterogamety in the lizard (*Lacerta vivipara*). Arch. Biol., 45.

OGUMA, K. and J. J. ASANA, 1932 : Additional data to our knowledge on the dragonfly chromosome, with a note on occurrence of X–Y chromosome in the ant-lion (Neuroptera). Jour. Fac. Sci. Hokkido Imp. Univ., Ser. VI, Zool. 1.