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Chromosome Analysis in Three Species of Fishes Belonging to Family Gobiidae

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So far as we are aware, cytological studies of 10 species of Gobiidae have been carried out (Nogusa 1960, Kaur and Srivastava 1965, Subrahmanyam 1969), but most of the data suffer some limitation because of the techniques employed by earlier workers were older as compared to the present one. Among the three species of the present study, chromosomes from the testes of *G. giuris* were studied before by Kaur and Srivastava (1965) but we have extended the study in more detail. The present paper deals with the diploid number, morphology and metrical studies of somatic chromosomes in both sexes of *B. glaucus* and in males of *G. giuris* and *G. rubicundus*, as well as the study of male germinal chromosomes in the first two species mentioned above.

Materials and methods

Between 4 and 6 hours after the injection of 0.1% colchicine solution at the rate of 2 ml/100 gm of body weight, the kindney of both sexes of *B. glaucus* and only of males of *G. giuris* and *G. rubicundus* were fixed in aceto-alcohol for the study of the somatic chromosomes and testes of *B. glaucus* and *G. giuris* for the meiotic chromosomes. Cytological preparations were made according to colchicine-sodium citrate-acetic alcohol-Giemsa-air-drying technique, the details of which as well as the method of scoring of metrical data, arm ratio and centromeric index etc. have been described elsewhere (Manna and Prasad 1973). Morphological types of chromosomes were assigned from the arm ratio as suggested by Levan *et al.* (1964).

Results

1. *Boleophthalmus glaucus* (Day)

Somatic chromosomes: Materials were obtained from 2 out of 4 males and 2 out of 5 females since they survived upto the fixation time after the colchicine injection. The chromosome number in the kidney varied between 44 and 48 in males and 44 and 46 in females but a distinct frequency peak at 46 chromosomes was found which represented the diploid number. In males 73 out of 82 metaphases (89.02%) and in females 70 out of 76 metaphases (92.11%) had the diploid constitution. Only one plate in male had the hyperdiploid number of 48 chro-

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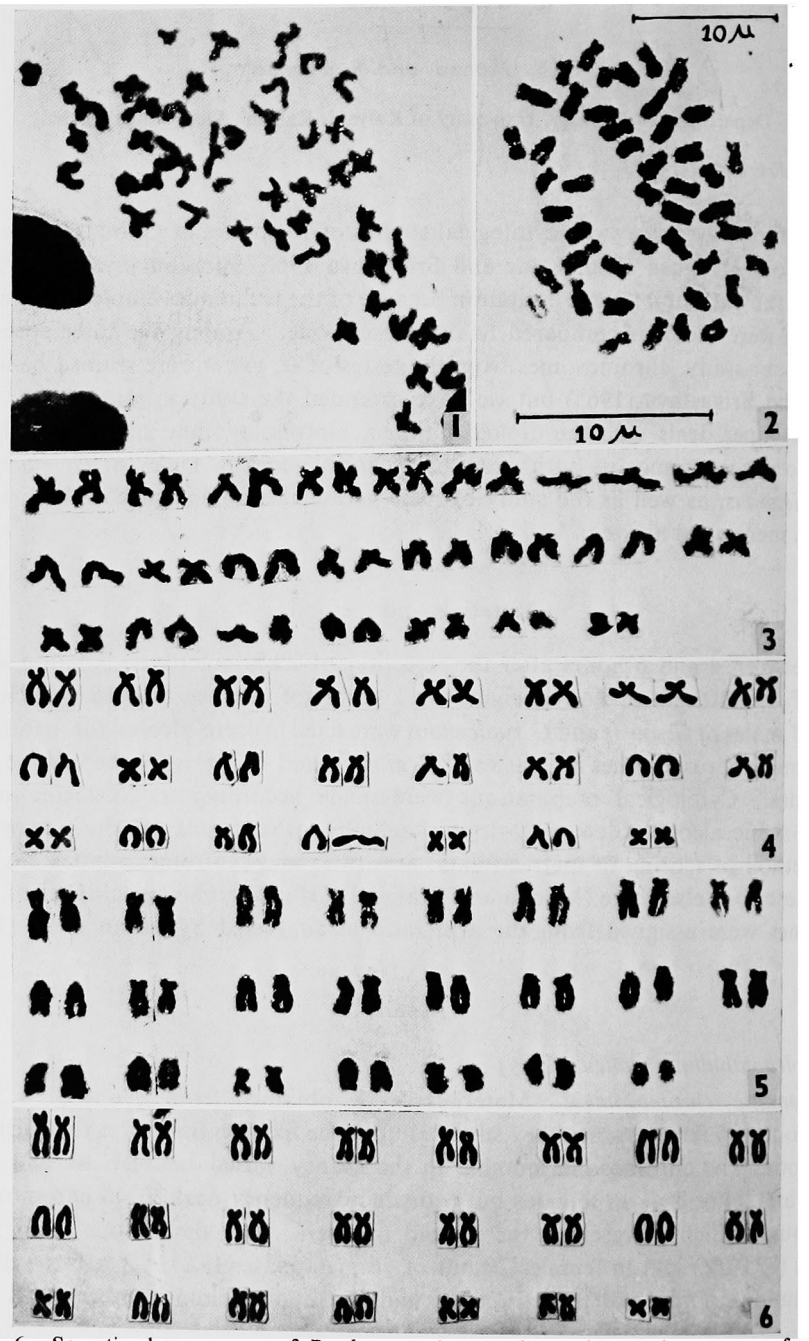
mosomes but none found in female. Among the 6 subdiploid metaphases in males, 2 had 44 and 4 had 45 chromosomes and in females out of 4, 1 had 44 and 3 had 45 chromosomes. Besides the aneuploid cells, 2 each in male and female had the



Figs. 1–6. Somatic chromosomes of *B. glaucus*. 1, metaphase with 46 chromosomes from the kidney of male. 2, female. 3 and 4, caryotype of male. 5–6, caryotype of female.

polyploid constitution.

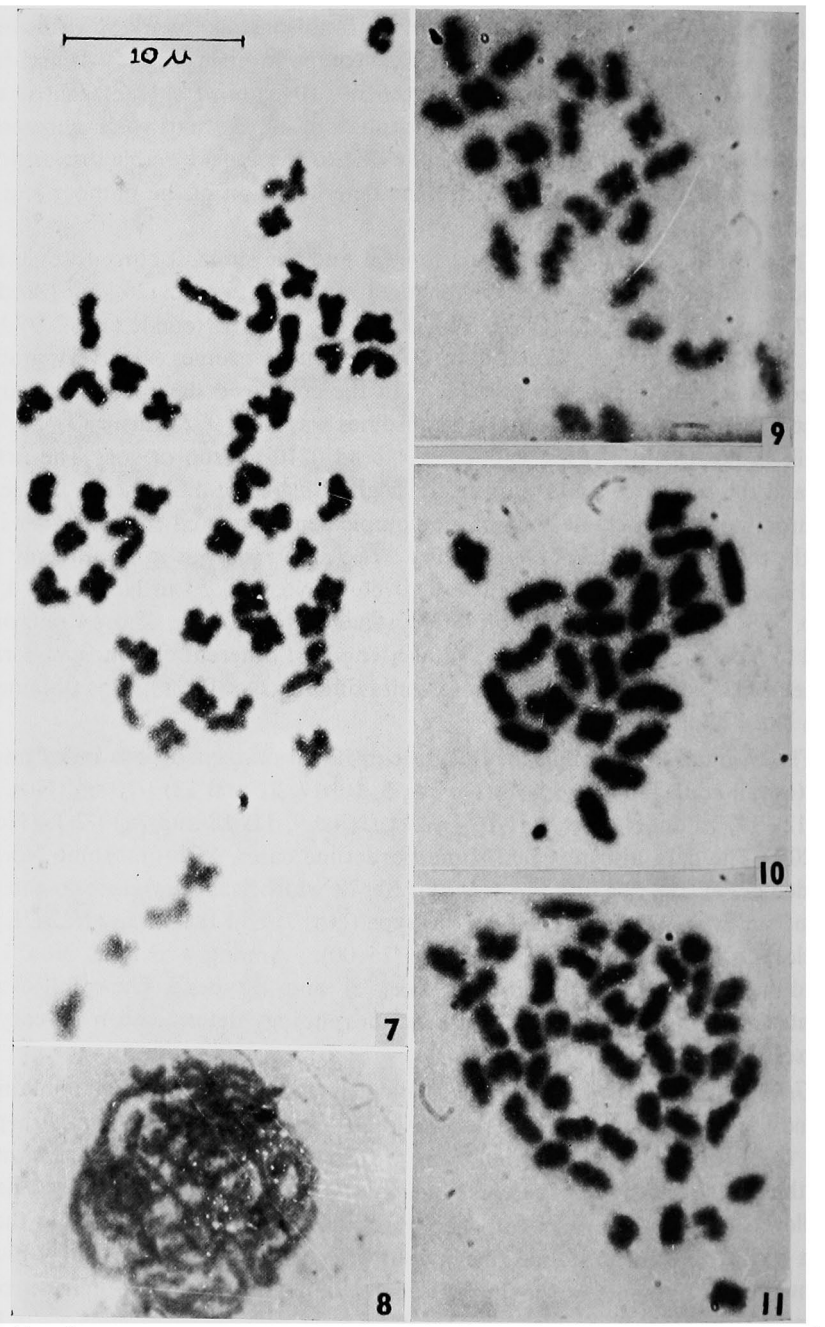
The diploid metaphase complements of male (Fig. 1) and female (Fig. 2) revealed 46 chromosomes of different shape and sizes. Although some unfavourable disposition might show some pair as heteromorphic or otherwise (Figs. 3–6), a careful analysis of several plates did not reveal any chromosome as "marker" or as heteromorphic sex element. An analysis of the caryotype in male (Figs. 3–4) and female (Figs. 5–6) would reveal 6 pairs of metacentric, 10 pairs of submetacentric and 7 pairs of rod-like chromosomes. The morphology of the particular chromosome was sometimes variable from plate to plate due to the unfavourable disposition for which several plates were compared for the determination of the number and form referred to above.

The mean length in micra of the longest and the shortest chromosome in the haploid set of mitotic metaphase complement ranged between 3.26 ± 0.61 and 1.41 ± 0.17 micron in male, 2.71 ± 0.27 and 1.27 ± 0.10 in female and 2.99 ± 0.55 and 1.34 ± 0.15 in the combined data. As the chromosomes were very gradually seriated, no size-grouping was possible. In the combined data the maximum difference between the two adjacent chromosomes was only 0.26 micron (Nos. 1 and 2) while in most of the cases, it did not exceed 0.10 micron or so. The first few pairs and the last three pairs showed the higher degree of differences. In general, the chromosomes in female metaphase complement appeared to be smaller in size than the corresponding ones of the male. The difference was not uniformly maintained and it was 0.55 micron in No. 1, 0.66 in No. 2, 0.54 in No. 3 and 4, 0.51 in No. 5, 0.47 in No. 6 and so on. The minimum difference of 0.14 micron was found in No. 23. The relative percentage length of different chromosomes ranged between 6.32% and 2.83%. The maximum difference of 0.55% was obtained between No. 1 and 2.

The chromosome formula of this species based on centromeric index and arm ratio (Levan *et al.* 1964) was 6 m (Nos. 1, 5, 10, 17, 21 and 23)+10 sm (Nos. 2—4, 6, 8, 12—14, 16 and 19)+1 st (No. 7)+4 t (Nos. 9, 11, 18 and 22)+2 T (Nos. 15 and 20). The data also revealed some borderline cases. Chromosome No. 1 was regarded as 'm' type having the value of 37.79 which was very close to the upper limit of 'sm' type (37.50). The only 'st' type (No. 7) had the value of 22.71 which was close to the lower limit of 'sm' type (25.00). Among 4 't' type, Nos. 11 and 22 had the values on the borderline between 'st' and 't' types. The result of metrical data was in broad agreement with the morphology determined from caryotype analyses.

Germinal chromosomes: Spermatogonial metaphase complement contained 46 chromosomes (Fig. 7) similar to that of kidney cell. There was a growth period from the early primary spermatocyte prophase. Pachytene nucleus contained long thread-like entangled bivalents (Fig. 8) and their individual identity was hard to follow. No sex chromatin could be traced during the prophase I or at the later stages by the differential staining behaviour or heteromorphic nature of a bivalent. The number of bivalents in the first spermatocyte nucleus was determined from the diakinesis stage (Fig. 9) and it was 23. They were more clear at metaphase I (Fig. 10) when the bivalents attained the maximum condensation. Almost all the biva-

lents had one chiasma each at different regions which gave rise to rod- or cross-shaped bivalents. Terminalization of chiasmata between diakinesis and metaphase I was not very palpable. Some sporadic instances of polyploid metaphase



Figs. 7–11. Germinal chromosomes in males of *B. glaucus*. 7, spermatogonial metaphase. 8, pachytene. 9, diakinesis. 10, metaphase I. 11, polyploid metaphase I.

I were encountered (Fig. 11) but no multivalent structure contained in them.

2. *Glossogobius giuris* (Ham.)

Somatic chromosomes : Out of 5 males only 3 but none of the females survived upto the fixation time. Earlier to the present investigation, the diploid number and the morphology of the chromosomes from the testes were carried out by Kaur and Srivastava (1965). Our findings were in agreement with them. However, these authors did not make any metrical study or determine the centromeric index of different chromosomes. In the present study in 91 metaphases of the kidney, the chromosome number varied between 44 and 47 chromosomes but the definite frequency peak at 46 was the diploid number. The frequency of diploid metaphases was 78.02% (71 in 91) while the number of subdiploid and hyperdiploid complements was 14 and 1 respectively. Five plates had polyploid constitution. Among the 14 subdiploid complements 7 each had 44 and 45 chromosomes while the hyperdiploid ones had 47 chromosomes.

The diploid metaphase complement (Fig. 12) in the kindney of males consisted of 46 gradually seriated acrocentric chromosomes. It was interesting to note that unlike *B. glaucus* none of the chromosomes was morphologically submetacentric or metacentric type. An analysis of the caryotypes (Figs. 13–14) indicated 23 pairs of gradually seriated acrocentric chromosomes. The grouping of chromosomes on the basis of their morphology or sizes was impracticable. No pair of chromosome was heteromorphic in nature which could be regarded as the sex chromosome.

The mean length in micra of longest and the shortest chromosomes in the metaphase complement of males ranged between 2.35 ± 0.35 and 1.30 ± 0.17 micra. The chromosomes were gradually seriated and the maximum difference between two adjacent chromosomes was only 0.21 microns (Nos. 22 and 23) while in some cases no difference was observed (Nos. 15 and 16).

On the basis of the arm ratio and centromeric index, the chromosome formula for this species was 2t + 21T. Among the haploid set of 23 chromosomes only No. 11 and 13 had a very small second arm and in some plates its identity was lost. The data did not show any borderline case.

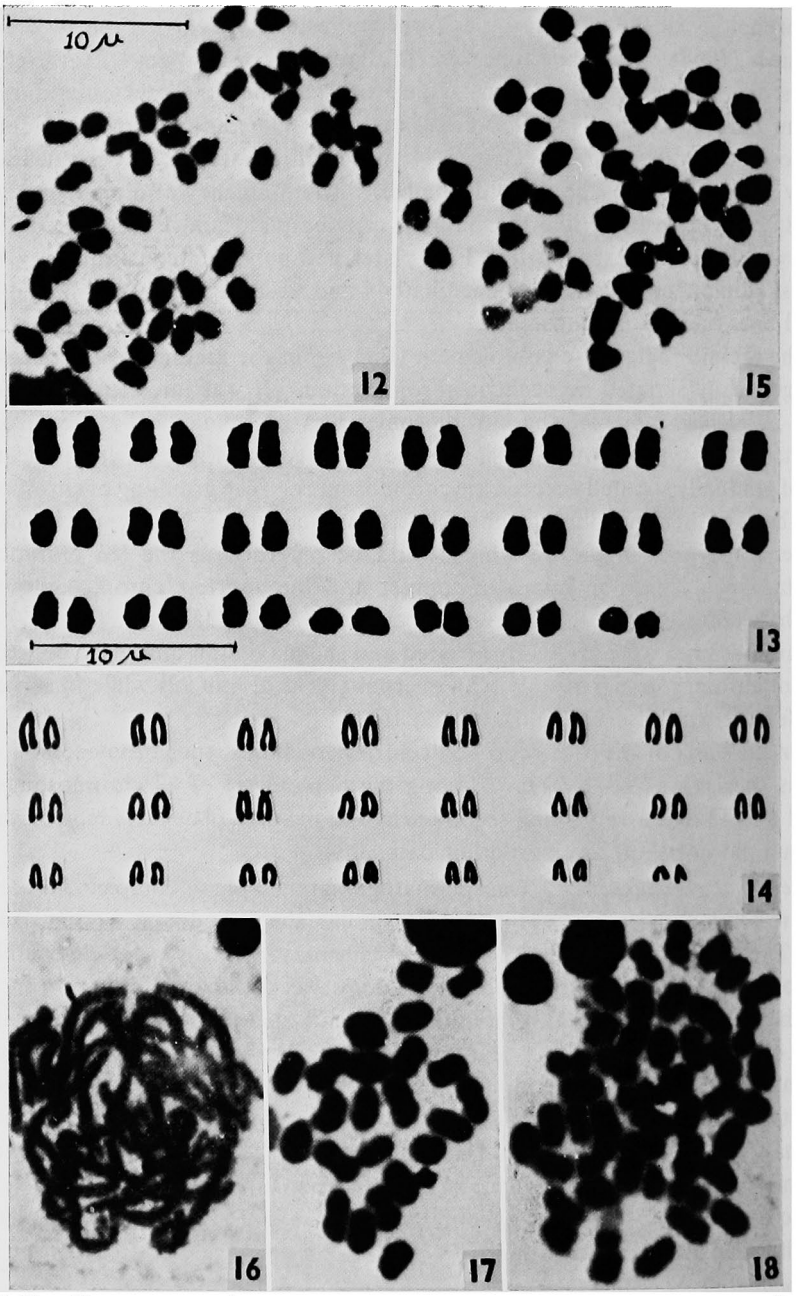
Germinal chromosomes: The spermatogonial metaphase complement consisted of 46 rod-like chromosomes (Fig. 15) of similar shape and sizes as that of kidney cell. The sex chromosomes and 'marker' chromosomes were not detectable and no grouping according to size and morphology was possible.

The behaviour of chromosomes during primary spermatocyte prophase was same as that of *B. glaucus*. There was a growth period. At the pachytene, thin thread-like bivalents were seen in an intermingled condition (Fig. 16). At the late diakinesis or prometaphase I, 23 homomorphic bivalents possessing mostly a single terminal chiasma could easily be seen. No trace of sex bivalent was found. Much condensation and terminalization took place at metaphase I (Fig. 17). Sporadic instance of polyploid metaphase I was also encountered (Fig. 18). In such case no quadrivalent or multivalent structure was found.

3. *Gobioides rubicundus* (Ham.)

Somatic chromosomes: Only 4 out of 8 specimens survived upto the fixation time after colchicine injection and unfortunately all of them were male. The chro-

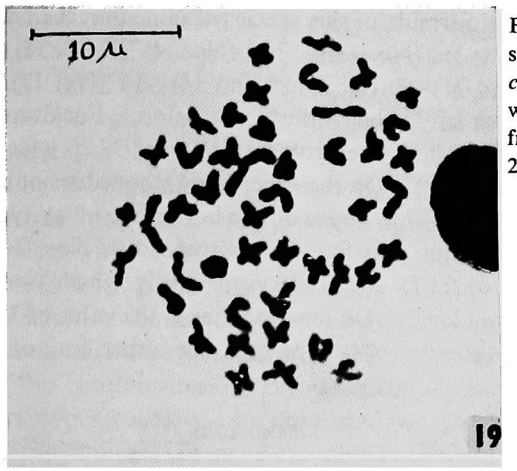
mosome number in different metaphase complements of the kidney of males was found to vary between 44 and 48 with a frequency peak at 46 chromosomes which should be regarded as the diploid number. In 121 metaphases, 113 (93.4%) cells

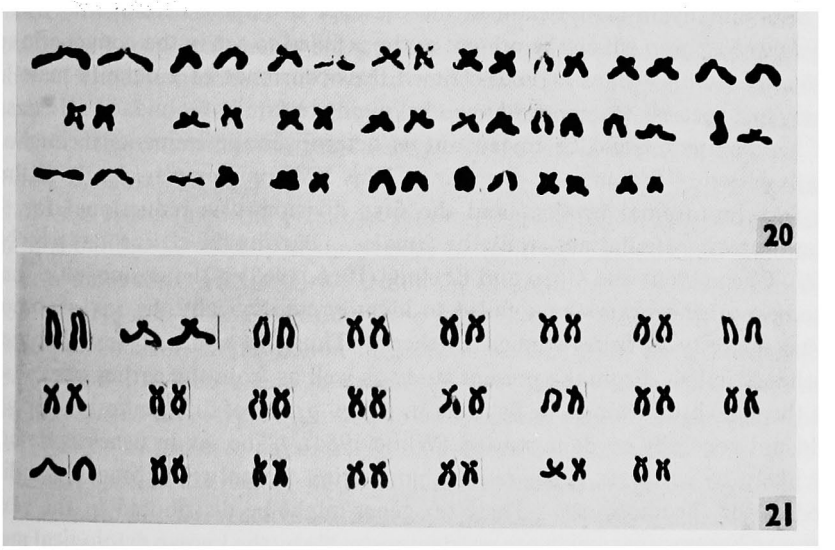


Figs. 12–18. Chromosomes of male *G. giuris*. 12, metaphase with 46 chromosomes from the kidney. 13–14, caryotype. 15, spermatogonial metaphase. 16, pachytene. 17, metaphase I. 18, polyploid metaphase I.

had the diploid constitution while 3 had subdiploid, 1 had hyperdiploid and 4 had polyploid number. Among the 3 subdiploid complements, 1 had 44 and 2 had 45 chromosomes while the hyperdiploid one had 48 chromosomes.

The diploid metaphase complement (Fig. 19) contained 46 chromosomes of various shape and sizes. No 'marker' chromosome or the sex chromosome could be identified in the complement. No size grouping was possible since they were gradually seriated but morphological grouping was made on the basis of metrical





Figs. 19–21. Chromosomes of male *G. rubicundus*. 19, metaphase with 46 chromosomes from the kidney. 20–21, caryotype.

data and by the analysis of caryotype (Figs. 20–21). It consisted of one pair of metacentric, 13 pairs of submetacentric and 9 pairs of rod-like chromosomes. However, sometimes confusion arose about the morphology of some chromosomes when the disposition was not perfect and the chromosome concerned was on the borderline of two morphological types.

The mean length in micra of the longest and the shortest chromosomes in the

metaphase ranged between 2.66 ± 0.32 and 1.59 ± 0.11 micra. The data also indicated that the chromosomes were very gradually seriated. The maximum difference between two adjacent chromosomes was 0.21 micron (Nos. 2 and 3) while in many cases no difference was found at all (Nos. 10–12, 15 and 16, 17 and 18 etc.). The relative percentage length of the different chromosomes also showed a gradual seriation and the maximum difference of 0.44% was found between chromosome Nos. 2 and 3. The value in different chromosomes ranged between 5.54% and 3.31%.

The chromosome formula of this species obtained on the basis of arm ratio and centromeric index was 1m (No. 22)+13sm (Nos. 4–7, 9–13, 15, 18, 19 and 23)+5st (Nos. 2, 3, 16, 20 and 21)+3t (Nos. 1, 8 and 14)+1T (No. 17). There were some borderline cases which left to the confusing situation. For example, among 13 'sm' type, chromosome No.9 had a centromeric index of 37.26 which was very close to the limit of 'm' type (37.50). On the other hand, the values of chromosome Nos. 7 (27.39), 15 (27.18) and 18 (27.27) were on the borderline of 'st' type (25.00). Among 5 'st' type similar situation was found in chromosome Nos. 2 and 20. They had centromeric indices of 23.75 and 23.16 respectively which were very close to the lower limit of 'st' type (25.00). On the other hand, the value of 12.57 in chromosome No. 21 was on the borderline of 't' type since the upper limit of this type was 12.50.

Discussions

Subrahmanyam (1969) claimed the presence of female heterogamety in *Boleophthalmus boddaerti* which the present authors failed to see in the congeneric species, *B. glaucus*. Only Nogusa (1960) claimed the occurrence of a definite male heterogamety in a species, *Mogrunda obscura* belonging to family Gobiidae. He has found that XY complex could be traced out as heteropycnotic elements throughout the growth period of first meiotic prophase. This XY complex was clearly distinguishable from autosomal bivalent and the first division was reductional for the sex chromosomes. He did not study the females. Barring the claims made by Nogusa (1960), Chen (1969) and Chen and Ebeling (1966, 1968) of heteromorphic sex chromosomes, other workers have failed to identify cytologically the sex chromosomes in vast majority of fishes studied by them. Thus, the state of sex chromosomes in fishes is in fix. From the present study as well as from the earlier ones it seemed that the sex chromosomes in fishes is in a low grade of differentiation for which it could not generally be demostrated (White 1954). The sex in fishes is determined very likely by some sex genes rather than having the entire chromosomes differentiated as sex chromosomes. These sex genes might be distributed in the sex locus on some chromosome which are not demonstrable by the known cytological methods.

According to the list prepared by Nogusa (1960) and the succeeding works (Kaur and Srivastava 1965, Subrahmanyam 1969, Prasad and Manna 1971), the diploid number in different species of this family was determined as 44, 46 and 62 chromosomes of which 46 was found in eight out of 12 species including the present ones. Nogusa (1960) claimed that the chromosomes in different species of this family were rod-like but we found a different picture in our materials. Except *G.*

giuris two other species in our study had a number of metacentric and submetacentric chromosomes.

Most of the earlier authors drew the cytological interrelationships between different species of fishes on the basis of the "Robertsonian principle" or "whole arm transfer" with which the present authors could not agree fully (Manna and Prasad 1971, 1973). The mechanism of the chromosome evolution in the three species under discussion would once again support our earlier view that "pericentric inversion" and/or "centromeric shift" together with some sort of structural rearrangements played the significant role in the evolution of caryotypes in fishes. The diploid number and the morphology of chromosomes based on centromeric index and arm ratio of the three species, *B. glaucus*, *G. giuris* and *G. rubicundus* revealed that they have 2n=46 chromosomes with 78, 46 and 74 so-called "fundamental arms" respectively. Therefore, in these species no "Robertsonian relationship" could be applied in correlating the chromosome number with the morphology of the chromosome. Further, the total of the mean length of the haploid set of chromosomes was 42.21 micra with 16 biarmed chromosomes in *B. glaucus*, 42.68 with no biarmed chromosomes in *G. giuris* and 48.00 micra with 14 biarmed chromosomes in *G. rubicundus*. Therefore, the caryotypes of three species differed considerably but it was not so apparent in the metrical data. Then how to explain the difference in the morphology of the chromosomes in the three species having the same diploid number? The caryotypes could not be correlated assuming only 'mutual translocation' or 'whole arm transfer' in some chromosomes which is the basis of 'Robertsonian principle'. Instead of this, if we assume 16 pericentric inversions in *B. glaucus* and 14 in *G. rubicundus* from *G. giuris* complement, we can account for the presence of variable numbers of biarmed chromosomes in different species having the same diploid number of 46 chromosomes. This would also explain the wide difference in the number of so-called fundamental arms in the three species.

Some authors (Nogusa 1960, Post 1965, Roberts 1967) claimed 48 rod-like chromosomes represented the primitive caryotype in fishes. If this be the case, the origin of the three caryotypes of the species under study from primitive number of 48 chromosomes could also be correlated on the assumption of 'pericentric inversion' or ' centromeric shift' mainly in addition to some other type of structural rearrangements responsible for the reduction of 2 chromosomes from the primitive diploid number. That the 'pericentric inversion' and/or 'centromeric shift' was mainly responsible for the caryotypic evolution in three species could be supported from the metrical data. In order to have an idea on amount of structural changes that took place in these species, the mean length in micra and the relative percentage data of different chromosomes irrespective of their morphology could be considered. If *G. giuris* is taken as the standard, a difference of 0.91 micron in No. 1 0.81 in No. 2..... 0.11 micron in No. 23 would be found with *B. glaucus* and 0.31 micron in No. 1, 0.36 in No.2 ..... 0.29 micron in No. 23 with *G. rubicundus*. In no case the difference was even 1 micron. A comparison of data of the relative percentage length of different chromosomes would indicate more clearly that the structural changes took place mainly within and not between the chromosomes as data of any particular chromosome in the three species was not highly variable. Therefore,

we might consider that in the evolution of caryotypes of all these species, the structural alteration in the form of deletion, duplication or translocation played minor role. It was mostly intrachromosomal change in the form of 'centromeric shift' or 'pericentric inversion' which brought the difference in the morphology of the chromosomes in the three species of the present study.

Summary

Caryotype analysis, metrical study and centromeric index of somatic chromosomes from the kidney of three gobiid fishes, *Boleophthalmus glaucus*, *Glossogobius giuris* and *Gobioides rubicundus* and the meiotic chromosomes from the testes of first two species have been investigated. All the three species have 2n=46 chromosomes but the morphology and the metrical data were different. *B. glaucus* had chromosome formula of 6m+10sm+1st+4t+2T and the measurement of the chromosomes ranging between 2.99±0.55 and 1.34±0.15 micra, *G. giuris* had 2t+21T and measurement between 2.35±0.35 and 1.30±0.17 and *G. rubicundus* had 1m+13sm +5st+3t+1T and measurement between 2.66±0.32 and 1.59±0.11 micra. No clear indication of sex chromosomes was found either in the somatic or in the germinal cells. Meiosis was stereotype with the bivalents clearly seen from the diakinesis stage. Chromosomal evolution and interrelationships in the three species have been discussed and the role of pericentric inversion in bringing the morphological differences in the caryotype has been stressed.

References

Chen, T. R. 1969. Karyological heterogamety of deep-sea fishes. Postilla **130**: 1–29.

— and Ebeling, A.W. 1966. Probable male heterogamety in the deep-sea fish, *Bathylagus wesethi* (Teleostei: Bathylagidae). Chromosoma **18**: 88–96.

— 1968. Karyological evidence of female heterogamety in the mosquito fish, *Gambusia affinis* Baird and Girard. Copeia p. 70–75.

Kaur, D. and Srivastava, M. D. L. 1965. The structure and behaviour of chromosomes in five fresh water teleosts. Caryologia **18**: 181–191.

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

Manna, G. K. and Prasad, R. 1971. A new perspective in the mechanism of evolution of chromosome in fishes. Proc. 1st All India Congr. Soc. of Cytol. and Genet. pp. 82.

— 1973. Chromosomes in three species of fish (*Channa*). Nucleus **16**: 150–157.

Nogusa, S. 1960. A comparative study of the chromosomes in fishes with consideration on taxonomy and evolution. Mem. Hyogo Univ. Agric. **3**: 1–62.

Post, A. 1965. Different in vestigations of the chromosome numbers of fresh water teleosts. Z. Zool. Syst. Evolutions-Forsch. **3**: 47–93.

Prasad, R. and Manna, G. K. 1971. A study of somatic and germinal chromosomes of some fifteen species of fresh water fishes. Proc. 58th Ind. Sci. Congr., Part III, p. 633.

Roberts, F. L. 1967. Chromosome cytology of Osteichthyes. Prog. Fish culturist **29**: 75–83.

Subrahmanyam, K. 1969. A caryotypic study of the estuarine fish, *Boleophthalmus boddaerti* (Pallas) with calcium treatment. Current Science **38**: 437–439.

White, M.J.D. 1954. Animal Cytology and Evolution. 2nd Edition, Cambridge University Press.