Indication of the sex chromosome pair bearing Ag-NORs in a brackish water
fish, *Scatophagus argus* showing male heterogamety

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Morphologically distinguishable sex elements were detected in a brackish water teleostean fish, *S. argus*. While the female specimens had 24 homomorphic pairs of rod like chromosomes (2n = 48, NF = 48), in male specimens the karyotype differed having a heteromorphic pair, comprising one metacentric ("Y") and an acrocentric ("X") chromosome (2n = 48; NF = 49). The suspected XX and XY elements showed Ag-NOR locations at and around their centromeric regions, thus indicating some form of association existing between nucleolar organising regions and sex determining genes in this species.

Although over 1600 species of fish have now been karyotypically studied, morphologically distinguishable or heteromorphic sex chromosome elements have only been detected in about 100 species.\(^{1-5}\). During investigation on localization of Ag-NORs in different species of *Indian teleosts*\(^{6-10}\) we have come across an interesting case in which the Y-chromosome could be morphologically distinguished from the other autosomes and X-chromosome and the sex chromosome pair appeared to bear the Ag-NORs which are reported here.

**Materials and Methods**

Altogether 40 specimens of both sexes of *Scatophagus argus* (Family, Scatophagidae) weighing between 20 and 50 g were collected from the Hooghly river near Kakdwip (Estuarine belt of the Sunderbans), West Bengal, India and used after acclimatising them in brackish water cisterns of Central Institute of Brackish water Aquaculture, Kakdwip Research Centre. Since the usual doses of 0.02-0.05% and even 0.1% of colchicine @ 1 ml/100 g body weight, failed to arrest divisional stages at metaphase, a higher dose of 0.2% colchicine @ 1 ml/100 g body weight had to be used to get the satisfactory results. The somatic chromosomes were prepared from kidney and gill tissues of both sexes and germinal chromosomes from testis of male *S. argus*, sacrificing them after 3 hr of injection and by adopting the air drying-Giemsa stain schedule described elsewhere\(^{11}\).

One-step silver nitrate method of Howell and Black\(^{12}\) was followed for localization of Ag-NORs in the metaphase chromosomes.

**Results**

Out of 50 Giemsa-stained well spread metaphase plates studied in both male (Figs 1-3) and female (Fig. 7) *S. argus*, over 70% had 48 chromosomes, which could be ascribed as the diploid number for this species. The male Giemsa-karyotype (Fig. 5) contained 23 homomorphic pairs and a heteromorphic pair comprising one metacentric and an acrocentric chromosome while the female Giemsa-karyotype (Fig. 9) contained all 24 homomorphic pairs. All the chromosomes were acrocentric and very gradually seriated measuring between 1.70 and 0.84 μm in female and 1.68 and 0.92 μm in male except for one metacentric chromosome suspected as the "Y" element which measured 1.80 μm in length. However, in the Giemsa-karyotype of either male or female the X-chromosome could not be distinguished from the other autosomes and was only arbitrarily shown in the karyotypes (Figs 5 and 9).

In silver-stained preparations of metaphase plates in both male (Fig. 2) and female (Fig. 8), one pair of chromosomes showed positive staining for NORs. While in the female sex, a homomorphic pair had their NOR locations at the terminal centromeric regions (Figs 8 and 10), in male, the Ag-NOR locations were heterologous, one chromosome showing its tip NOR positive like that of female while the other was located in the median region.
Figs 1-6—Giemsa stained somatic metaphase complements of male *S. argus* (Figs 1 and 3) and primary spermatocytic late diakinesis (Fig. 4); metacentric "Y" chromosome has been indicated by arrows (Figs 1, 3 and 4); karyotype (Fig. 5) prepared from Fig. 3; silver stained metaphase complement of male (Fig. 2) and its karyotype (Fig. 5); NOR-bearing chromosomes have been indicated by arrows and underlined in karyotypes. Bar = 10 μm.
Figs 7-10—Giemsa stained metaphase complement of female S. argus (Fig. 7) and its karyotype (Fig. 9); silver stained metaphase complement of female (Fig. 8) and its karyotype (Fig. 10); NOR-bearing chromosomes have been indicated by arrows and underlined in karyotype. Bar = 10 μm.
(pericentric) of the metacentric chromosome suspected as the "Y" element.

Efforts to study the meiotic behaviour of the suspected XY did not meet with much success as no divisional activity was obtained in some 18 male specimens tried, most of which were sexually immature. However, in one male specimen a few divisional stages of late diakinesis only were obtained (Fig. 4) which showed 24 bivalents with single terminalised chiasma, in all the bivalents confirming the diploid number in the species to be 48 as reported earlier\textsuperscript{,13,14} but not allowing the detailed study of meiotic behaviour of the suspected XY elements. Apparently one X shaped element (Fig. 4, arrow) was found in each of the plates studied which would possibly indicate that the heteromorphic X and the Y successfully paired at least for some of their lengths.

**Discussion**

Khuda-Bukhsh and Manna\textsuperscript{13} reported diploid number of 48 chromosomes in *S. argus* which was later confirmed by Chowdhury et al\textsuperscript{14}. However, while Khuda-Bukhsh and Manna\textsuperscript{13} obtained 48 acrocentric chromosomes in females and 47 rods and one distinctly disposed metacentric chromosome in male *S. argus* collected from Kakdwip, West Bengal, Chowdhury et al\textsuperscript{14} reported 2n=46A+2Sm chromosomes in marine specimens of both sexes of *S. argus* collected from Gopalpur-on-sea, Orissa state. Findings of the present investigation collaborate well with the observations of Khuda-Bukhsh and Manna\textsuperscript{13}. However, in the present study, the Ag-NORs also clearly demonstrate locations on two different sites in the male sex, indicating heteromorphic behaviour of one pair of chromosomes.

Absence of heteromorphic sex pair in overwhelming majority of fish species studied so far has been attributed to their "primitive" state, the sex chromosomes being very little differentiated from autosomes\textsuperscript{15}. Further, out of nearly 100 species showing morphologically detectable sex chromosomes, the most prevalent types of sex determination were XX female : XY male encountered in some 45 species, followed by some 30 species having ZZ male; ZW female while XO female : XX female and multiple sex chromosome mechanisms of *XrXrXrXr* female; *XrXrXrY* male have also been reported in a few other species\textsuperscript{2,5,16-18}. However, the sex chromosome pair suspected in most of these cases were not verified by any banding studies. On the other hand, Park and Grimm\textsuperscript{19} could not find C-band and fluorescent staining technique to be of diagnostic use to differentiate ZW pair in either the European or the American Eels, *Anguilla anguilla* and *A. rostrata*, respectively. Interestingly, in the present study, the location of the NOR in the metacentric "Y" would imply that the other acrocentric NOR bearing chromosome should be "X" chromosome, particularly when two such acrocentric homologues showed the same pattern of NOR location in female sex. Although banding techniques are among the essential tools for the detailed analysis of chromosomes, methodological difficulties have limited the amount of information available on the banded chromosomes of fish\textsuperscript{10,20-24}.

In general 2n = 48 rod like chromosomes are most prevalent among the fish species cytologically studied so far and are believed by many to represent the primitive fish karyotype\textsuperscript{6,16-26}. Occurrence of a single metacentric chromosome in male sex of *S. argus*, therefore, could have been derived by a pericentric inversion of one of the X-chromosomes that would have more male determining genes. Incidentally in the primitive vertebrates the chromosome morphology and behaviour of some sex linked genes indicate that the sex chromosome pair has remained largely homologous and sex determination is believed to be at the genic rather than the chromosomal level\textsuperscript{27}. Differentiation of heteromorphic sex chromosomes probably resulted from inhibition of crossing over between the originally homologous pair, thus isolating the sex determining loci and stabilizing differential accumulation of the two kinds of sex determining genes\textsuperscript{28}.

Morphological differentiation of Z and W or X and Y chromosomes could therefore imply that the initial homologue would either convert into heterologue through structural rearrangement\textsuperscript{28,29} or through initial heterochromatization prior to structural rearrangement\textsuperscript{10}. Our efforts so far to induce C-bands in this species remain unsuccessful and therefore the heterochromatin distribution in the suspected sex chromosome pair remains unknown. Notwithstanding that, however, the present study would raise a possibility of some form of association of NOR-bearing chromosomes and sex determining genes in fish. Usually the Ag-NORs represent the chromosomal sites of 18s and 28s rRNA genes which were presumably transcribed in the proceeding interphase\textsuperscript{10}. The secondary constrictions of
metaphase chromosomes are also associated closely with the nuclear organisar regions. In over 250 species of fish studied so far for their NOR locations, the majority of species have been reported to have two NORs, which have been claimed to be the “primitive” or “fundamental” type in fish. However, the number of NOR varies from 4 to 8 in some species and rarely one in a couple of two. Some species and rarely one in a couple of two and heteromorphism in NOR location has been reported in a few species. On this count S. argus shows NOR locations of fundamental nature but shows sex specific differences in location as also reported in the mosquito fish Aplocheilus panchax in a different manner.

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