

EVIDENCE FOR MULTIPLE SEX CHROMOSOMES IN THE FRESHWATER GOBY, *GOBIONELLUS SHUFELDTI* (PISCES: GOBII-DAE).—Heteromorphic sex chromosomes have been observed in very few fish species. Of the 810 species of osteichthyan fishes listed by Sola et al. (1981), there is evidence of heterogamety for only 29. In fishes with intersexual chromosomal polymorphism, sex chromosomes usually cannot be distinguished from autosomes. Their existence is frequently inferred from sexual differences of general karyotype composition (e.g., male has 19 acrocentric and 29 metacentric chromosomes, while the female has 18 and 30, respectively). Less frequently, heterogamety has been determined by observing atypical meiotic bivalent associations or recognizing

two distinct haploid complements during Meiosis II (Gold, 1979).

Sex determination in the aforementioned 29 species has most often been inferred to be XX:XY, WZ:ZZ or XX:XO. Recently, multiple sex chromosomes have been reported in isolated species of seven families. An $X_1X_1X_2X_2:X_1X_1X_2$ system was proposed by Rishi (1976) for *Callichrous bimaculatus* (Siluridae). Filho et al. (1980) suggested a ZZ:ZW₁W₂ system for *Apereiodon affinis* (Parodontidae). All other species for which multiple sex chromosome systems have been described are believed to have an $X_1X_1X_2X_2:X_1X_2Y$ mechanism (Filho et al., 1980; Levin and Foster, 1972; Murofishi et al., 1980; Thorgaard, 1978; and Uyeno and Miller, 1971, 1972)—males have one less chromosome yet equal arm numbers compared with females. Males in these species consistently have one more banded element than females. This suggests that each species experienced a fusion of an autosome and the Y chromosome that incorporated an autosomal pair into the sex-determining mechanism. Additional evidence for this mechanism in *Megupsilon aporus* (the cyprinodontid species of Uyeno and Miller, 1971), *Allodontichthys hubbsi* (the goodeid species of Uyeno and Miller, 1972) and *Garmanella pulchra* (Levin and Foster, 1972) was found in the formation of a trivalent during late spermatogonial prophase. In *Stephanolepis cirrhifer* (Balistidae), this system was confirmed by the presence of two karyotypes in secondary spermatocytes—one with 17 acrocentric and the other with 15 acrocentric and one metacentric chromosomes (Murofishi et al., 1980). The heteromorphic sex chromosomes are indistinguishable from autosomes in all of these cases of presumed multiple sex chromosomes, except for the Y chromosomes in *Garmanella*, *Megupsilon* and *S. cirrhifer*.

I am reporting the occurrence of multiple sex chromosomes in *Gobionellus shufeldti*. Evidence was obtained from mitotic and meiotic chromosome spreads for sex determination by the $X_1X_1X_2X_2:X_1X_2Y$ model.

Results and discussion.—Twelve specimens of *G. shufeldti* from Irish Bayou in New Orleans, LA were karyotyped by using gill epithelium and testes according to methods developed by Kligerman and Bloom (1977) and modified by J. R. Gold (pers. comm.). Voucher specimens were deposited in the Texas Natural History Collection (TNHC 11549). Both mitotic and meiotic chromosome spreads were obtained. Eighty-nine

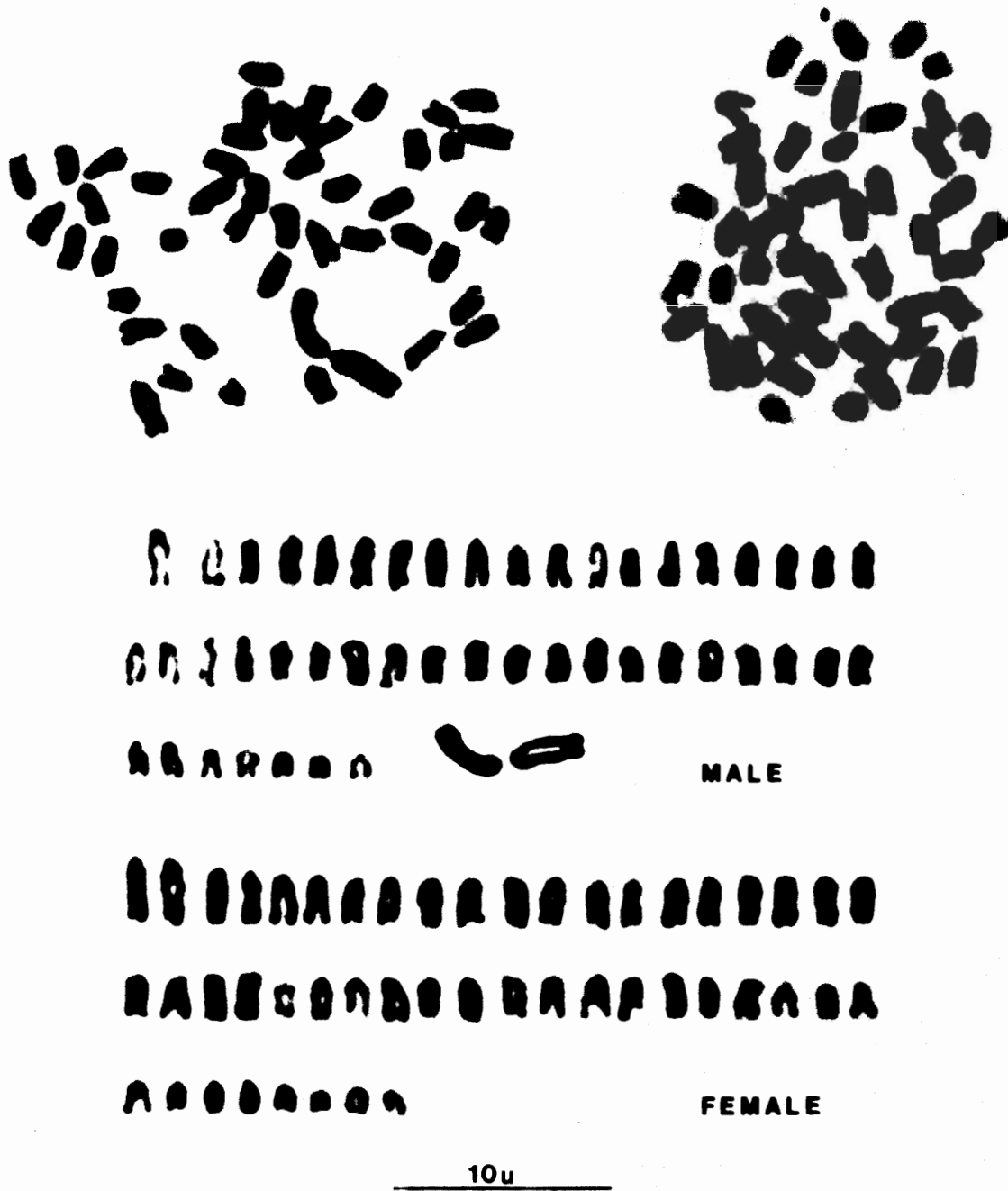


Fig. 1. Above—complete chromosome spreads from which male (l) and female (r) karyotypes were constructed. Below—male and female karyotypes of *Gobionellus shufeldti*.

mitotic cells from four females and 132 cells from eight males were examined for chromosome type and number. Of the female somatic cells observed, 55% had 48 acrocentric or telocentric chromosomes in metaphase (Fig. 1). In males, 60% of the somatic cells showed 46 acrocentric elements and a single, large metacentric chromosome (Fig. 1). Complete spreads

from which the karyotypes were compiled are also shown in Fig. 1. Acrocentric chromosomes were measured and arranged in a descending series and, where possible, were associated with nearest neighbors. Inferences of homology are tentative for most chromosomes because factors such as morphological contortion, differential staining, and differential contraction are sus-



Fig. 2. Late prophase spread from primary spermatocyte illustrating the trivalent and 22 bivalents. Arrows indicate large X (A), small X (B) and Y (C) chromosomes.

pected of confounding the presentation of even a smoothly graded series.

Male somatic metaphase spreads in *G. shufeldti* have one less acrocentric chromosome than female karyotypes, but an equal number of chromosome arms. Examination of meiotic spreads provided two other points of evidence for an $X_1X_1X_2X_2:X_1X_2Y$ system in this species. Late prophase primary spermatocytes had 22 bivalents and one large trivalent in which the two X chromosomes are attached end-to-end to the metacentric Y (Fig. 2). Secondary spermatocyte chromosome spreads (in metaphase II) were characterized by two different haploid numbers—one had 24 acrocentric chromosomes and the other had 23 acrocentric chromosomes and a single large metacentric chromosome (Fig. 3).

Male karyotypes often showed an unpaired acrocentric chromosome that was distinctly larger than the next largest chromosome, while some female karyotypes had instead a pair of acrocentrics that was much larger than all others (Fig. 1). These observations suggest that the largest acrocentrics (single in males, paired in females) constitute one set of the X chromosomes. This identification is inconclusive, however, as long as chromosome homologies within and between spreads are based solely on length. It can be seen in late prophase meiotic spreads (Fig. 2) that the X chromosomes are smaller than the arms of the Y to which they are attached in trivalent formation. Also, one X chromosome is noticeably larger than the other.



Fig. 3. Metaphase II haploid complements showing 23 (A) and 24 (B) chromosomes.

Occasionally the larger of the two X chromosomes was disassociated from the trivalent; this produced meiotic spreads which included an end-to-end bivalent association between the Y and the smaller X, one univalent, and 22 bivalents. Dissociation could have been an artifact of chromosome preparation. It could also have resulted from an absence of chiasmata (White, 1973), which may be attributable to genetic differentiation between the large X and its homologous arm of the Y. Differentiation could be the result of heterochromatinization or inversions in either element, which would presumably be greater for the original X_1 chromosome. Although heterochromatinization is thought to play an important role in evolution of sex chromosomes (Bull, 1978; Kornfield, 1981; Singh et al., 1980) its role in the evolution of sex chromosomes in fishes (Kornfield, 1981; Thorgaard, 1976; de Almeida Toledo et al., 1981) and lower vertebrates in general (Mengen, 1981; Sites et al., 1979) is unclear. The degree of differentiation involved in sex determination in fishes may not need to be very great (Gold, 1979).

There are only two previous reports of heterogamety in gobioid fishes. From histological sections, Nogusa (1955) presented evidence for an XY system in *Odontobutis obscura* (Eleotridae), but with better methods, Nishikawa et al. (1974) and Arai et al. (1974) found 44 acrocentric chromosomes with no evidence of heterogamety. Arai and Sawada (1974) discovered male heterogamety in *Gobiodon citrinus* (Gobiidae) in which males have one less chromosome and two fewer chromosome arms than females. Although not stated, sex determination is presumably XO:XX. This species is not closely related to *G. shufeldti*.

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