C-band positive W chromosome in the female Indian frog

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Summary. In the female Indian frog, *Rana tigrina* (2n = 26; NF = 52), the female is heterogametic. The heterochromosomes (ZW) are morphologically indistinguishable, but the W chromosome proves by C-banding analysis to be entirely heterochromatic.

Investigations on the occurrence of sex chromosomes in amphibians are marked by conflicting results. Earlier reports on the presence of heteromorphic sex chromosome pairs in some 15 species of Anura are far from satisfactory[2,3]. The introduction of banding technology, and its application in amphibian cytology, have opened a new avenue to approach the question of sex chromosomes in this group of vertebrates. Schmid et al.[4] first clearly demonstrated the existence of 'heteromorphic' sex chromosomes in males of 2 species of urodeles *(Triturus)* by a banding technique. Further cytological evidence on the occurrence of heterochromosomes in the male of another urodelan species *(Necturus maculosus)* has been furnished by Sessions[5]. These reports point to the presence of male heterogamety in urodeles although they do not accord with genetical data on the axolotl.

The situation is clearly ambiguous in the Anurans. Female heterogamety in *Bufo bufo* and in *Xenopus laevis*[6] was first suggested by analyzing the sex of the progeny of sex-reversed individuals. This has been repeatedly confirmed in *Xenopus*; lastly also by the application of the cell surface H-Y antigen test[7]. However, sex reversal experiments in 4 different species of Asiatic *Rana* have pointed to male heterogamety in these species[8]. Cell surface antigen tests also provided evidence in support of heterogamety in male *Rana pipiens*[7]. Cytological evidence for male heterogamety in a leptodactylid frog has also been published recently[9]. Contrary to these findings, banding analysis of *Pexicephalus adspersus* (= *Rana adspersa*) chromosomes revealed the existence of a heteromorphic (ZW) sex chromosome pair in the female[10]. In the present paper we also report female heterogamety with well differentiated ZW sex chromosomes in the common Indian frog, *Rana tigrina* (Anura; Ranidae).

5 female and 4 male specimens were collected in the Hooghly and Burdwan districts of West Bengal, India. Metaphase chromosomes were prepared from intestine and testis by the methods in use in this laboratory[11–13]. C-banding was performed with certain modifications of the technique originally described by Sumner[14]; the main modification being that $Ba(OH)_2$ denatured slides were dipped into dehydrated alcohol before incubating in 2 × SSC.

Conventional staining of mitotic chromosomes by Giemsa stain at pH 6.8 revealed a 2n number of 26 with a NF = 52. This is in agreement with the earlier observations of Singh and others[3,15]. No morphologically distinguishable sex chromosome pair was identified by the conventional staining method.

An analysis of C-banded metaphase chromosomes permitted the identification of all 13 chromosome pairs. C-band positive constitutive heterochromatin has been noted in the centromeric region of all chromosomes (fig. 1,a). The intensity of stain, however, varied among the different chromosome pairs. In addition to the centromeric heterochromatin,

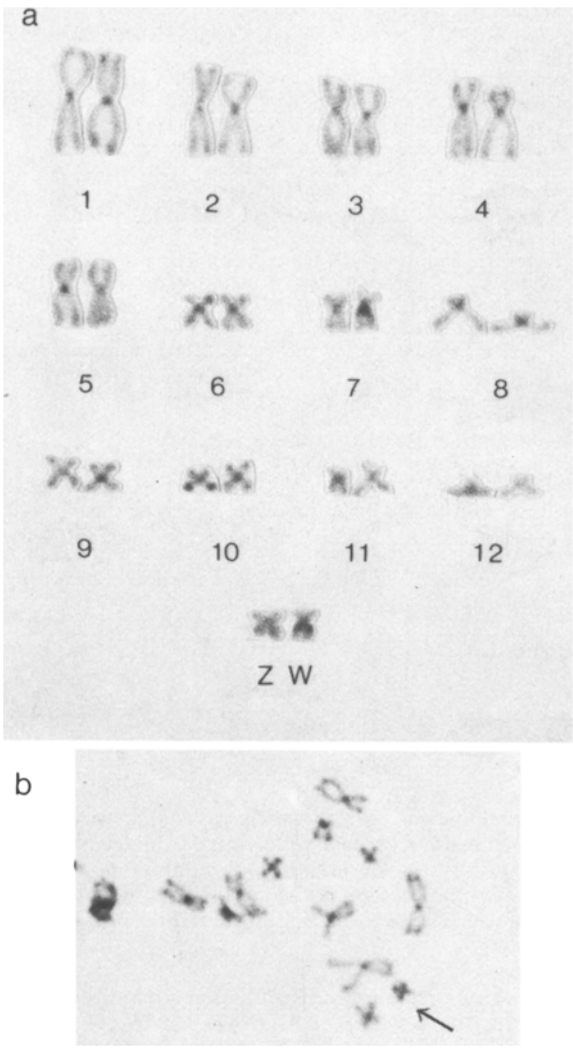

Figure 1. *a* Karyotype prepared from a C-banded metaphase of female *Rana tigrina* showing the distribution of constitutive heterochromatin. *b* Metaphase spread from an aneuploid cell of female bone marrow showing completely heterochromatic W chromosome (arrowed).

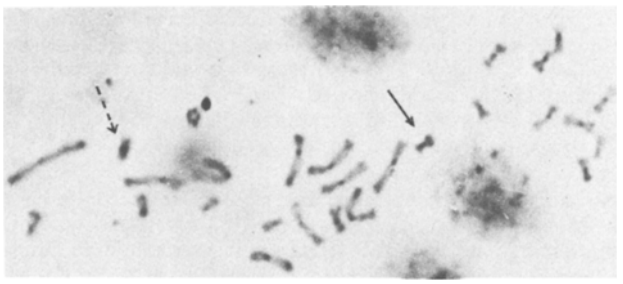

Figure 2. C-banded mitotic metaphase from an intestinal cell of female *R. tigrina*. The completely heterochromatic W chromosome is arrowed. Broken arrow indicates the position of chromosome pair No. 7 with secondary constriction region darkly stained.

telomeric heterochromatin has been recorded in chromosome pairs Nos 1, 3, 4 and 10. An extended and very intense heterochromatic block has been noted in the pericentromeric region of one of the homologues of chromosome pair 7 (figs 1,a and 2) denoting the presence of a secondary constriction in this particular region. By analyzing the C-band karyotype of the female specimens and by comparing the same with that of the male we designated one of the smallest biarmed chromosome pairs as the ZW sex chromosome pair. This pair exhibitied a clear heteromorphism in C-band preparations. The W chromosome is distinguishable by its dense and almost completely C-band positive staining (figs 1,a and b and 2). The Z chromosome, on the other hand, has only inconspicuous centromeric heterochromatin.

Attempts to demonstrate heterochromosomes in Anura were not convincing[16], until Schmid[10] showed by C-band staining the existence of a highly differentiated ZW sex chromosome pair in female *Pexicephalus adspersus* (= *Rana adspersa*). The W chromosome in this species is considerably smaller than the Z, and approximately half of it is composed of constitutive heterochromatin. In *Rana tigrina* we find no size difference, but the W chromosome is composed almost wholly of C-band positive constitutive heterochromatin. So far as we are aware, this is the 1st report on a completely heterochromatic sex (W) chromosome in Amphibia which thus resembles the W chromosome of most snakes and birds[17,18].

Two contradictory schools of opinion try to explain the mechanism of sex chromosome differentiation in vertebrates. According to Ohno[19] and others the morphological differentiation of Z and W (or X and Y) chromosomes evolves through an initial chromosomal rearrangement. Contrary to this, Singh et al.[20] gave priority to an initial heterochromatinization phenomenon. The occurrence of 2 unequal-sized sex chromosomes with an only partially heterochromatinized W in *Rana adspersa*[10] provided evidence in favor of Ohno's hypothesis, whereas the present finding in *Rana tigrina*, of an almost completely heterochromatic W of equal size, may strengthen the view of Singh et al.[20]. Further investigations on Anura should contribute to elucidating the question.

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A tertiary trisomic interchange heterozygote in pea (*Pisum sativum* L.)

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Summary. In M_2 generation of an interchange heterozygote, one off-type plant was isolated which was characterized by the presence of weak and slender stems, with profuse semi-spreading branching, narrow and yellow-green foliage, small and weak pods, and 10 days late-flowering and -maturing as compared to wildtype. The mutant had high pollen and ovule sterility (76-80%) and showed $5^{II}+2^{II}$ (heteromorphic) + 1^{I} (small chromosome) in 85% of the cells. The mutant appeared to be a tertiary trisomic interchange heterozygote. The possible mechanism of the formation of heteromorphic bivalents and a small chromosome are discussed.

Although a few trisomics in pea, primary and tertiary, have been isolated by several workers[3-12], a tertiary trisomic interchange heterozygote (i.e., tertiary trisomic in interchange heterozygote background) seems not to be known yet. The present note deals with the cytomorphological behavior of one such mutant.

One off-type plant was isolated in M_2 generation of the selfed progeny of an interchange heterozygote which was induced through gamma-ray irradiation (10 krad) of the F_1 seeds from the parents, 68 C (from Dr. W. Gottschalk, FRG) and 5064-S (a selection from the original mutant line 5064 - from Dr S. Blixt, Sweden). This parental interchange heterozygote was morphologically similar to normal but had pollen and seed sterility about 83 and 88%, respectively. At metaphase I, the interchange heterozygote had bivalents ($5^{II}+2^{II}$, heteromorphic), quadrivalents ($5^{II}+1$