CONTRIBUTIONS TO THE CYTOLOGY OF INDIAN ANURA (AMPHIBIA): A STUDY OF CHROMOSOMES IN THE TWO SEXES OF BUFO MELANOSTICTUS SCHNEIDER (BUFONIDÆ)

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INTRODUCTION

The family Bufonidæ of the suborder Procœla has been cytologically well explored and the chromosome numbers of about 14 species of Bufo are so far known to us (Makino, 1951; Wickbom, 1949 a, b). Of these only one Indian species, viz., Bufo stomaticus has been studied (Asana and Mahabale, 1941). In view of our scanty knowledge of the cytology of Indian Bufonidæ, the present investigation was undertaken and a comparative study has been made of both the male and female karyotypes of the common South Indian species Bufo malanostictus Schneider.

The chromosomes have been studied mainly from the spermatogonial and oogonial metaphase plates. The study of the oogonial chromosomes was made from acetocarmine squashes of the ovary. The preparation of good squashes of the ovary presents technical difficulties partly on account of the large quantity of yolk and partly due to the rarity of the suitable stage. Hence, a large number of squashes had to be prepared in order to obtain clear preparations of the required stages.
OBSERVATIONS

1. Spermatogonial metaphase.—Figures 1 and 3 represent a spermatogonial metaphase plate in polar view with 22 metacentric chromosomes. All of them are V-shaped with equal arms and the homologues lie close to one another. The 11 pairs of chromosomes which constitute the complement are shown serially arranged in Fig. 5 from which it may be noted, that of the 11 pairs, 6 are large and 5 are small. All of them have median centromeres.

2. Oogonial metaphase.—Figures 2 and 4 show the polar view of an oogonial metaphase plate and the number of chromosomes can be clearly made out to be 22. The larger chromosomes are seen towards the periphery of the plate surrounding the smaller ones. Fig. 6 represents a serial arrangement of the 22 chromosomes that resolve into 11 homologous pairs. Here also the division of the chromosomes into 2 sets (one large and one small) could very well be seen. All the chromosomes have median centromeres only.

In brief, a study of the spermatogonial as well as the oogonial metaphase chromosomes of *Bufo melanostictus* shows the following features, common in both: (i) the number of chromosomes, i.e., 22, (ii) all the chromosomes are metacentrics only, and (iii) the chromosomes fall into two well-defined groups, i.e., 6 pairs are large and the rest are small.

DISCUSSION

Asana and Mahabale (1941) have also reported that 2n = 22 in *Bufo stomaticus*, the only other Indian species so far studied but they say that 7 pairs of chromosomes are large and 4 are small. In *B. melanostictus*, however, there are definitely 6 pairs of large and 5 pairs of smaller chromosomes in both the male and the female karyotypes. Unfortunately, a more detailed and critical comparison with the findings of the previous investigators could not be made, as a detailed account of the individual chromosomes of *Bufo stomaticus* has not been given by them.

The diploid chromosome number of 22 with 6 large and 5 small pairs of chromosomes has been found to be of uniform occurrence in all the European, North American, South American, African and other Asian species of *Bufo* (Wickbom, 1949 a, b; Makino, 1951). Even the two exceptions to this condition reported by King (1907) and Poska-Teiss (1933) were later proved to be erroneous by Witschi (1933) and Saez et al. (1936), respectively. The striking cytological uniformity and stability of the genus *Bufo*...
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Despite its wide geographical distribution has been pointed out by Wickbom (1949 a). This view is further confirmed by the present investigation.

A comparison of the chromosomes of the male and female karyotypes of *B. melanostictus* shows very clearly that there do not occur any heteromorphic pair or pairs of chromosomes in them. All of them are only regular chromosome pairs without even slight inequality in any pair of any karyotype. It is thus definite that the sex chromosomes which can be identified at the morphological level are absent in the form now studied. After a more thorough and critical study, Wickbom (1945) concluded that “in Anura and Urodela nothing has been brought to light which even suggests the occurrence of morphologically or behaviourly recognisable heterochromosomes in either sex”. Wickbom’s opinion has been endorsed by Matthey (1949, 1951) also, who says that among Amphibia “Sex heterozygosis does not find any morphological expression”. The present investigation goes to strengthen this view further.

**Summary**

1. The chromosomes of both the male and female forms of *Bufo melanostictus* have been studied mainly from the acetocarmine squashes of the testis and the ovary.

2. In both the karyotypes, the chromosome number is 22, all the chromosomes are metacentrics and 6 pairs of chromosomes are large and 5 are small. In other words, the number of chromosomes, their morphology and their division into two well-defined sets are common features shared by the karyotypes of both the male and the female forms.

3. The above findings support the view that sex chromosomes are cytologically not recognisable in *Bufo melanostictus*.

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**EXPLANATION OF PLATE.**

**Fig. 1.** Photomicrograph of spermatogonial metaphase plate—polar view—Acetocarmine squash, × ca. 2,500.

**Fig. 2.** Photomicrograph of oogonial metaphase plate—polar view—Acetocarmine squash, × ca. 2,500.

**Fig. 3.** Spermatogonial metaphase plate—drawing, × ca. 4,000.

**Fig. 4.** Oogonial metaphase plate—drawing, × ca. 3,500.

**Fig. 5.** Homologous pairs of spermatogonial chromosomes serially arranged in order of size, × ca. 5,000.

**Fig. 6.** Homologous pairs of oogonial chromosomes serially arranged in order of size, × ca. 3,500.