A SIMPLE TECHNIQUE TO DETECT SATELLITE AND SATELLITE-STALK IN AMPHIBIA

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THE satellite-bearing and the nucleolar organizer region (NOR) containing chromosomes constitute important landmarks in the construction of karyotype of a particular species. But in conventional stained metaphase preparations, the proper identification of these chromosomes is difficult due to extreme condensation of chromatids. This is particularly true for most of the amphibian species in which, though the chromosomes are much elongated, yet remain in a highly spiralized state—a condition which hampers the clarity of the satellite and the satellite-stalk[1]. Furthermore, proper identification of satellite bearing and/or NOR containing chromosomes is needed, because unlike other vertebrate chromosomes, the chromosomes of amphibia do not respond to conventional G- or Q-banding techniques[2,3]. Recently, we have introduced a simple technique to detect satellite-bearing chromosomes and satellite-stalk to facilitate karyotyping amphibian metaphases. The present communication is a report on the detection of satellite-bearing chromosomes in some Indian Anurans.

Specimens of *Bufo melanostictus* (Schneider; Anura; Amphibia) of either sex were collected from different parts of West Bengal. Metaphase chromosomes from intestinal epithelial cells were prepared after 150 min of *in vivo* colchicine (0.1% solution in sterile distilled water) exposure at a rate 1 ml per 100 g body weight[2,4]. In order to expose satellite region and satellite-stalk the following procedure was applied:

(i) Freshly prepared flame/air dried smears were treated with 0.2N HCl for 10 min at room temperature, rinsed thoroughly in deionized water and dried in air; (ii) HCl-treated smears were kept in 0.025% trypsin (Difco) solution dissolved in amphibian saline (pH 6.8–7.0) using a magnetic stirrer, for 15 min; (iii) Trypsin-treated preparations were repeatedly washed (at least 3 times) in amphibian saline i.e. 0.6% NaCl in distilled water; (iv) Saline soaked preparations were then dipped in absolute ethanol and dried; (v) Preparations were stained in Giemsa

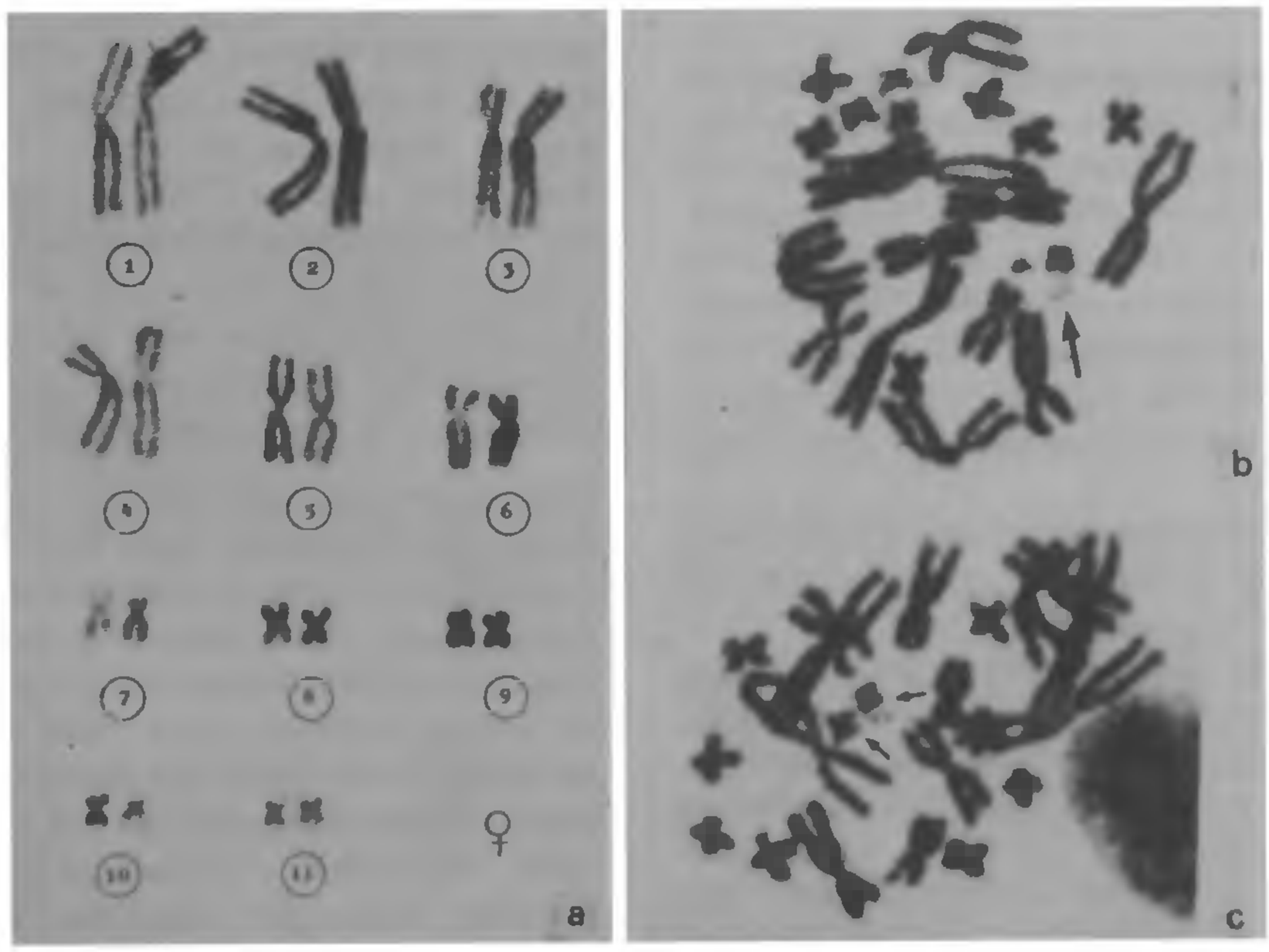

Figure 1a-c. a. Karyotype of *Bufo melanostictus* prepared from conventionally stained female metaphase showing no satellite. **b and c.** Metaphase spreads from male and female specimens after HCl and trypsin treatment showing one or two satellite and satellite-stalks respectively (arrowed).

stain (Gurr R66) diluted in phosphate buffer (pH 6.8) for 10 min (Giemsa stain: buffer = 1:10).

Conventional staining by Giemsa stain without HCl-trypsin pretreatment revealed neither any satellite nor any satellite-stalk in any of the chromosomes of this species (figure 1a). The diploid number of the species is 22 with no cytologically identifiable sex chromosome heteromorphism in any sex. But metaphases subjected to HCl and trypsin treatment revealed the existence of one or two distinct satellite and satellite-stalk bearing chromosomes (figures 1b, 1c). At least 10 well-spread mitotic metaphases were scanned from each of the 40 different specimens of either sex collected from different populations. The existence of satellite-bearing chromosomes has clearly been documented in all metaphases including those in which chromosomes were much condensed. An increase in HCl exposure time up to 30 min and/or trypsin treatment time up to 25 min also exposed satellite stalk but the best result was obtained at 15 min of trypsin exposure and 10 min of HCl treatment at room temperature. Curiously enough, of 6 different populations of *B. melanostictus* studied all showed intra- and inter-population polymorphism in sat-chromosomes. Some of them displayed 2 satellite-bearing chromosomes while others possesed only one such chromosome, and the difference was not sex-specific.

It is known that treatment with 0.2N HCl helps to remove some acid soluble proteins from chromosome arms. Since neither any satellite nor any satellite-stalk were seen after 0.2 N HCl treatment, it seems plausible that some insoluble proteins deposited at the satellite region shield the satellite-stalk, and subsequent trypsinization made the stalk visible by removing the deposited proteins still present. Since an excess digestion with HCl and/or trypsin damages or distorts the morphology of the chromosomes we found that a 10 min HCl and 15 min trypsin treatment is ideal to find the satellite and satellite-stalk in this species.

Our study with other four species of amphibia viz *Bufo himalayanus*, *B. stomaticus* (Bufonidae), *Rana tigrina* (Ranidae) and *Rhacophorus maculatus* (Rhacophoridae) also showed an identical result with this technique.

17 July 1986

1. Chakrabarti, S., *Experientia*, 1979, **35**, 745.
2. Chakrabarti, S., Banerjee, S. N., Neogi, L. N. and Roychoudhuri, S., *Experientia*, 1983, **39**, 321.
3. Schmid, M., *Chromosoma*, 1978, **68**, 131.
4. Banerjee, S. N., *Chromosomal endophenotype of some Indian Anura with reference to c-band distribution and sensitivity to induced aberration*, Ph. D. thesis, Burdwan University, 1986.

DESCRIPTION OF A NEW GENUS, *CRESCENTALEYRODES* FOR *ALEUROLOBUS SEMILUNARIS* (CORBETT) (ALEYRODIDAE: HOMOPTERA) AND TWO NEW COMBINATIONS

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In 1926, Corbett[1] described *Tetraleurodes semilunaris* from *Cymbopogon* sp which was shifted to the genus *Aleurolobus* by Bink[2] in 1983. The present authors collected this species from *Cymbopogon* sp from Burliar (The Nilgiris) in Tamil Nadu in June 1985 although in 1978, Abraham and Joy[3] reported its occurrence on *Cymbopogon flexuosus* for the first time from Kerala in India. A detailed study of the structural features of the pupal case indicated that the species under study did not fit into the generic description of either *Tetraleurodes* or *Aleurolobus* or to any known genera of Aleyrodidae. Hence, a new genus *Crescentaleyrodes* has been proposed to accommodate this species. A study of the available literature also indicated *Aleurolobus monodi* Cohic[4] and *Aleurolobus paulianae* Cohic[4] to be assigned to *Crescentaleyrodes*.

Crescentaleyrodes gen nov
Pupal case: Elongately oval; anterior and posterior marginal setae present, margin irregularly crenate. thoracic and caudal tracheal pore regions may or may not be differentiated; submargin demarcated by an oblong distinct suture from dorsal disc and possesses minute setae and distinct crescent shaped pores arranged at equidistance in submargin; dorsal setae discernible; longitudinal moulting suture reaching submargin transverse moulting suture bends posteriorly and later runs anteriorly meeting submargin; thoracic and abdominal segment sutures marked by paired lateral depressions, abdominal segment 7 shorter than 8; vasiform orifice not