

## C AND AgAs BANDS OF THE OCTAPLOID UNTANHA FROG *Ceratophrys dorsata* (*C. aurita*) (8n = 104, AMPHIBIA, ANURA)

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### ABSTRACT

*Ceratophrys aurita* (*C. dorsata*), a rare octaploid species of Brazilian anuran (8n = 104), has 9 NORs distributed among distinct chromosome groups (6 and 11) with centromeric and telomeric C bands, as analyzed in one male.

This species also presents interpopulational variability of DNA content. The NOR banding pattern shows the occurrence of interspecific polymorphism as compared to *C. ornata*. The number and distribution of NORs, as well as the variability in DNA content are caused by post-ploidy rearrangements.

### INTRODUCTION

Polyploidy is a widespread event among Amphibian which plays an important role in the process of speciation and evolution of some groups. The first examples of natural occurrence of polyploid species were found in anurans of the *Leptodactylidae* family (Beçak *et al.*, 1966, 1967, 1970).

Different levels of polyploidy have been recognized among Ceratophryids, with diploid and tetraploid cryptic species of *Odontophrynus americanus* (2n = 22, 2n = 44) and an octaploid species *Ceratophrys dorsata* (8n = 104) (Beçak *et al.*, 1966, 1967, 1970). Polyploidy has also been reported to occur among the Hylidae, with the description of the tetraploid species *Phyllomedusa burmeisteri* (4n = 52) (Beçak and Beçak 1970a,b) and additional cases of polyploidy reported by Bogart and Tandy (1976). The 4n species of *Odontophrynus* have been extensively used for studies on gene regulation (Beçak and Pueyo, 1970; Beçak and Goissis, 1971; Schmidtke *et al.*, 1976).

Anuran chromosomes have few interstitial bands (Birnstein, 1982). C and NOR bands are easily obtained and have been used for phylogenetic studies (Schmid, 1978; Ruiz *et al.*, 1981).

A wide variability in DNA content has been observed in Anura, with values ranging from 1.06 to 26.92 pg among 70 specimens of Bufonidae, Hylidae, Leptodactylidae, Pseudidae and Microhylidae families (Soma, 1982). In general, among autopolyploid anurans the DNA content is proportional to genome duplication (Beçak and Beçak, 1970b). Variability in rDNA content has been suggested to occur in the genus *Odontophrynus* on the basis of AgAs studies (Ruiz *et al.*, 1981).

In the present study, the genome of *Ceratophrys dorsata* was analyzed for constitutive heterochromatin distribution by C banding, for nucleolar organizer regions by AgAs banding and for DNA content by scanning cytophotometry.

According to Bokermann (1966), the correct taxonomy is *Ceratophrys aurita* (Raddi, 1823), with *Ceratophrys dorsata* (Wied, 1820) being considered as synonymy. *C. aurita* (*C. dorsata*) is geographically distributed in Western and Southern Brazil. The species is extremely rare and the reduced numbers of specimens are mainly observed after periods of rainy weather.

## MATERIAL AND METHODS

Since this material is extremely rare, only 3 specimens were collected over the last 5 years.

Mitotic metaphases were obtained from the intestinal epithelium of one male by the squashing method (Beçak *et al.*, 1967), from a bone marrow cell suspension (Schmid, 1978) and from a testis cell suspension (Evans *et al.*, 1964). C bands were obtained by the method of Sumner (1972), and AgAs bands by the technique of Howell and Black (1981). The metaphase preparations were photographed with a Zeiss photomicroscope.

Nuclear DNA content was determined for two males and one female with a Zeiss Scanning Cytophotometer at  $\lambda = 540$ , using off-line computation. Blood was collected by heart puncture with a heparinized Pasteur pipette. Smears were fixed in methanol for 3 minutes, submitted to HCl hydrolysis for 10 minutes at 60°C, and stained by the Feulgen method for one hour. Automatic scanning steps of 0.5 nm were used. The results were coded on paper tapes using an 8-channel Facit 4070 key punch.

The tapes were then processed with an HP computer to provide a figure with numeral representation of the nuclei, which permitted statistical analysis of nuclear chromatin distribution.

## RESULTS

Cytogenetic analysis of 45 cells from one specimen confirmed the octaploid

karyotype, each cell having 104 chromosomes arranged into 13 groups of 8 homologues each. Groups 1, 4, 6 and 11 are metacentric and the others submetacentric. Some chromosomes in group 6 have a gap adjacent to the centromere, i.e. secondary constrictions. Secondary constrictions were detected in some but not all homologues in group 11 (Beçak, 1967, 1968).

The C-banded karyotypes of *C. aurita* (*C. dorsata*) is presented in Figure 1. The analysis of 20 male cells shows in all chromosomes a large amount of centromeric heterochromatin and a discrete amount of telomeric heterochromatin. The chromosomes in groups 6 and 11 present a large heterochromatin block on the long arm. Groups 12 and 13 have a larger centromeric band.

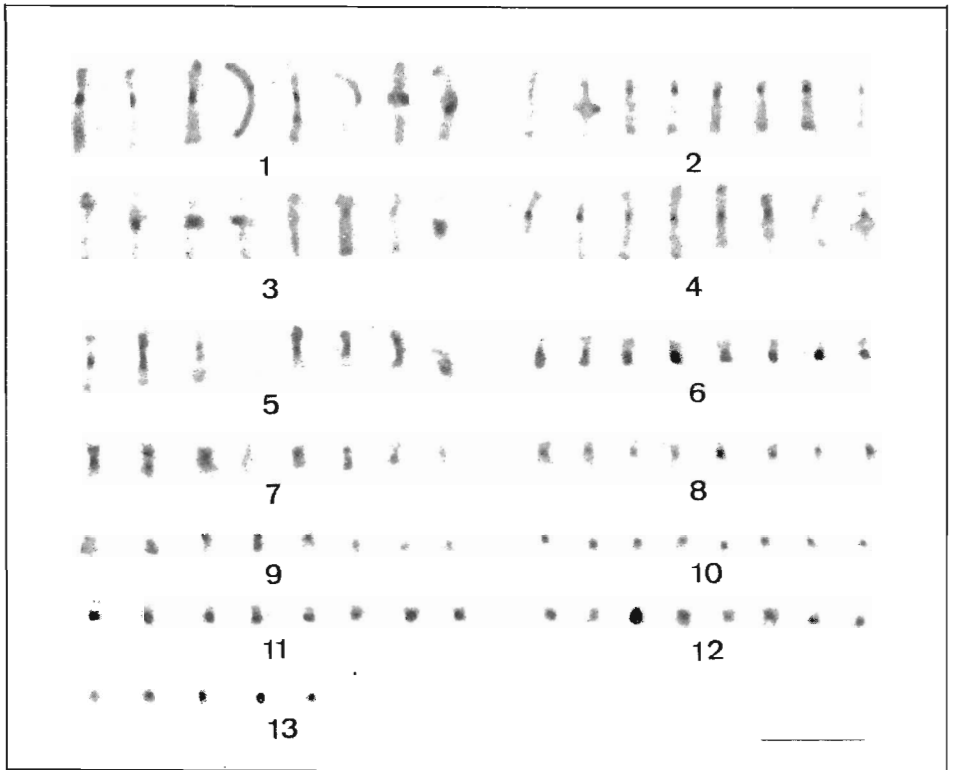


Figure 1 - Centromeric C bands of *C. aurita* (*C. dorsata*  $8n = 104$ ). Bar = 10  $\mu\text{m}$ .

AgAs bands are shown in Figure 2. In all 25 octaploid male cells examined, only 3 chromosomes of group 6 presented a large pericentromeric AgAs band, coinciding with their gaps, and only 6 chromosomes of group 11 exhibited positive NORs in their secondary constrictions. No variability was found.



Figure 2 - Nine NORs in chromosomes of groups 6 and 11 of *C. aurita* (*C. dorsata*  $8n = 104$ ). AgAs staining, Bar = 10  $\mu\text{m}$ .

The cytophotometric data indicated variability in DNA content among the three specimens analyzed (Table I).

The  $8n$  anuran *Ceratophrys aurita* (*C. dorsata*) has a peculiar NOR pattern consisting of 9 NORs distributed between two distinct chromosome groups. The number and distribution of NORs differ considerably from that of *C. ornata* ( $8n$ ) which has 8 NORs (Schmid *et al.*, 1985). The centromeric position of C bands found in *C. aurita* (*C. dorsata*) is similar to that of other anurans such as *C. ornata*.

Cytogenetic analysis of five families of Brazilian anurans has shown that the variation in chromosome number is due to different mechanisms including polyploidy. These studies have shown that the phenomenon favoring the fixation of polyploidy in Anura may possibly be related to characteristics observed during meiosis and

Table I - Variability in DNA content in *C. dorsata* (= *C. aurita*).

Protocol number	Sex	N	DNA/N (pg)	Origin
4407	♂	8n	16.28 ± 0.09	Parelheiros, Santo Amaro, São Paulo
4543	♀	8n	22.71 ± 0.06	Jardim das Palmas, Santo Amaro, São Paulo
5507	♂	8n	21.77 ± 0.22	Bororé, São Paulo

mitosis, i.e. a low crossing-over frequency and the absence of differentiated sex chromosomes (Beçak *et al.*, 1966, 1967, 1968, 1970; Beçak and Beçak, 1970a,b). It was also reported that the variation in NORs may result from postpolyploidy alterations (Beçak and Beçak, 1973, 1974; Ruiz *et al.*, 1981) and that the reduction of gene activity in *O. americanus* 4n was not caused by rDNA elimination (Schmidtke *et al.*, 1976).

The differences in NOR number and position found between *C. aurita* (*C. dorsata*) and *C. ornata* (Schmid *et al.*, 1985) indicate the presence of interspecific polymorphism. The variability in DNA content also suggests interspecific polymorphism. The peculiar position of NORs distributed between different chromosome groups, as well as their excess in the genome of the 8n *C. aurita* (*C. dorsata*) may result from postpolyploid translocations, as suggested for *Odontophrynus* (Beçak and Beçak, 1974).

The study of chromosome banding in *C. aurita* female cells will be of great importance for the establishment of the banding patterns of this species.

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### RESUMO

*Ceratophrys aurita* (*C. dorsata*), uma espécie octaplóide de anuros brasileiros, tem 9 NORs distribuídos entre dois grupos distintos de cromossomos (6 e 11) com bandas C centroméricas e teloméricas. Esta espécie apresenta variabilidade interpopulacional do conteúdo total de DNA. Seu padrão de bandamento NOR indica haver polimorfismo interespecífico, quando comparado com *C. ornata*. O número de distribuição de NORs, bem como, a variabilidade do conteúdo total de DNA foram explicados como causados por rearranjos postpoliploidia.

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