The heterozygosity of the trisomic parent may be the reason, as the majority of the tertiaries were isolated from the F_1 population. From the inter-crosses of parental interchange stocks, it is inferred that 10 tertiaries had different chromosomal combinations.

In addition to the primary trisomics[2] and interchange stocks[3], tertiary trisomics will be useful in linkage studies and chromosome mapping in pearl millet.

[3] B. S. GILL and J. L. MINOCHA, Proc. 58th Ind. Sci. Congr. *3*, 756 (1971).

Zusammenfassung. In den Pollenkörnern der F_1-Generation von heterozygotem *Pennisetum typhoides* (Hirse) wird tertiäre Trisomie nachgewiesen.

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Diploidization in *Eleutherodactylus* (Leptodactylidae-Amphibia)[1]

Chromosome mechanisms of evolution, such as rearrangements by translocation, assembly of new favorable linkage groups by centric fusion and polyploidy have been described in Anura. The former two processes do not necessarily involve quantitative alterations of genetic material, but the last implies always duplication of the DNA content. Cytophotometric measurements of DNA content in cells of several species showed that in some instances, increase of DNA content, is not detectable just by the study of the karyotype. This increase is ascribed mainly to tandem chromosome duplications[2–5]. In the present paper we report karyotype diploidization of the polyploid species *Eleutherodactylus binotatus*. *E. binotatus* ($2n = 22$) has fourfold the DNA content of *E. guentheri* ($2n = 22$) and *E. parvus* ($2n = 22$). Concomitantly we found in the former, multivalent meiotic configurations originated by multiple translocations and/or centric fusions.

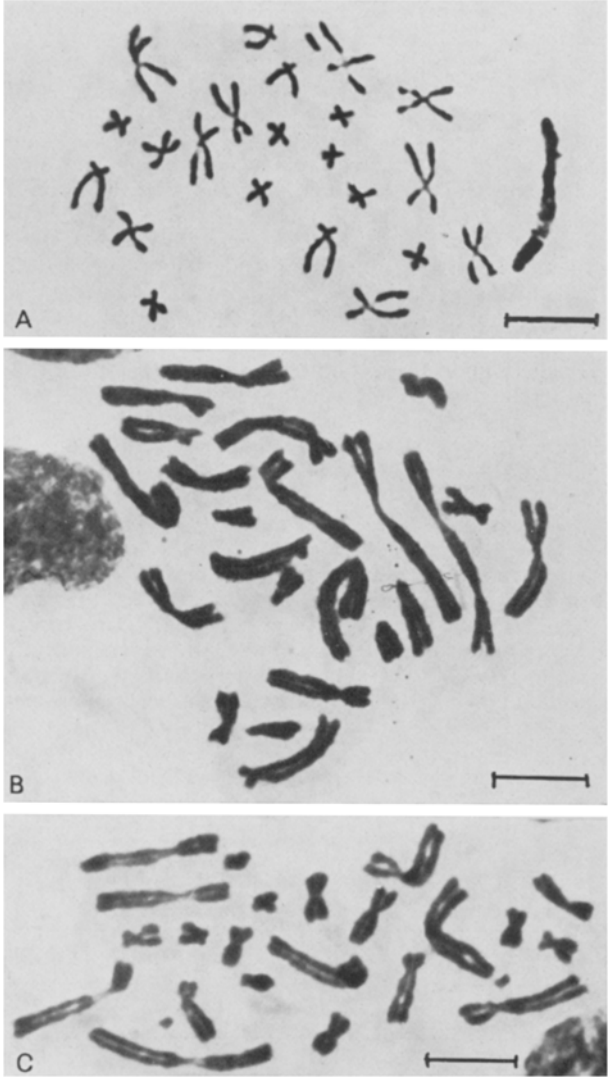

Fig. 1. A) Metaphases of *Eleutherodactylus guentheri*; B) *E. binotatus*; C) *Syphonops paulensis*, at the same magnification. Bar = 10 µm.

The material studied consisted of specimens of *E. binotatus* collected in the State of São Paulo (Ilha Queimada Grande 1 ♂; Boracea 3 ♂) in the State of Guanabara (Rio de Janeiro 1 ♀), and in the State of Bahia (Ilheus 1 ♀). Cytological preparations were obtained by the squash technique of intestine and gonads of animals previously treated with colchicine (1% solution; 0.01 ml/g weight, 2 h prior to the cytological preparation). Small organ fragments were placed into cold distilled water for 15 min, fixed in 50% acetic acid for 15 min, and squashed. The preparations were hydrolized in HCl N at 60 °C for 10 min, and stained by the Feulgen or Giemsa method.

DNA content was measured in erythrocyte nuclei, of blood smears, stained by the Feulgen method. A Zeiss Scanning Microscope Photometer MPM with a basic step of 0.5 nm at the cytoscan stage, was used. The total magnification used was 100 × (objective) 1.6 × (optovar) 10 × (projective). Measurements were made at extinction at 540 nm. The apparatus was connected to a Facit 4070 and the punched paper tape analyzed by computer.

The blood smears analyzed include *E. binotatus*, *E. guentheri* and *E. parvus*. Smears from *Bufo ictericus* were used as a standard.

The most interesting aspect found in *E. binotatus* was the increased width and length of its chromosomes in relation to most Anura species. Chromosome dimensions in this species resemble that of the Gymnophyona, *Syphonops paulensis* (Figure 1, a, b, c).

[1] This work was supported by the Brazilian CNPq, FAPESP and FEDIB. The authors acknowledge the skilful assistance of M. SOMA.

[2] M. L. BEÇAK, W. BEÇAK and M. N. I. RABELLO, Chromosoma *19*, 188 (1966).

[3] M. L. BEÇAK, L. DENARO and W. BEÇAK, Cytogenetics *9*, 225 (1970).

[4] W. BEÇAK, M. L. BEÇAK, D. LAVALLE and G. SCHREIBER, Chromosoma *23*, 14 (1967).

[5] W. BEÇAK, M. L. BEÇAK, G. SCHREIBER, D. LAVALLE and F. O. AMORIM, Experientia *26*, 204 (1970).

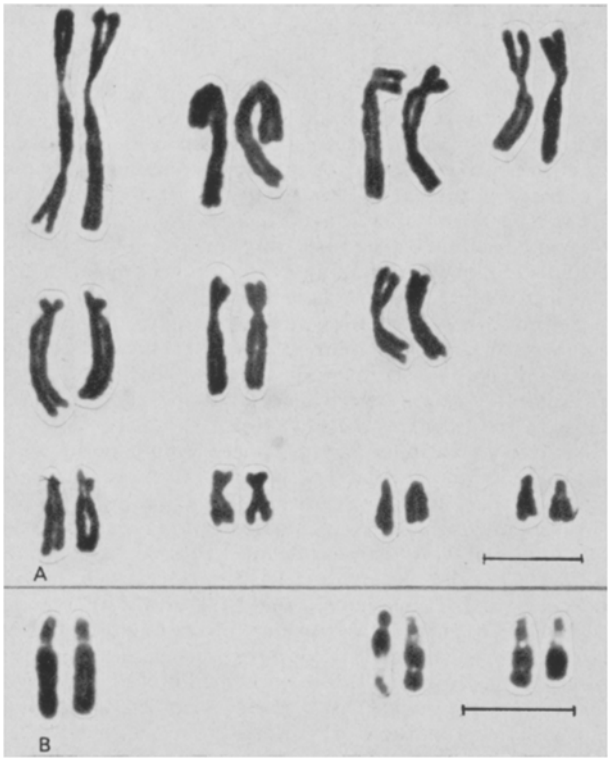

Fig. 2. Karyotype of *E. binotatus* (A); Pairs 8, 10 and 11 showing secondary constrictions (B). Bar = 10 μm.

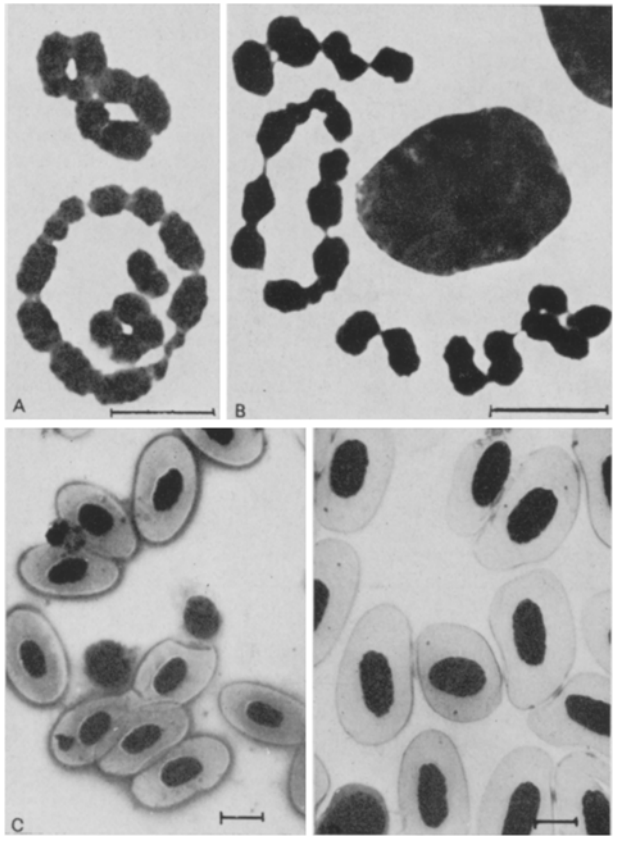

Fig. 3. *E. binotatus* spermatocytes I showing 1 dodecavalent ring, 1 quadrivalent and 3 bivalents (A); 1 dodecavalent ring and 5 bivalents (B); erythrocytes of *E. guentheri* (C) and E. *binotatus* (D) at the same magnification. Bar = 10 μm.

The karyotype presents 22 chromosomes. Pairs 1, 2, 3, 5, 6 and 9 are submetacentric; pairs 4 and 7 are metacentric, and pair 8, 10 and 11 are acrocentric. These 3 acrocentric pairs show a secondary constriction below the centromere (rabbit-ear) and pairs 10 and 11 show another secondary terminal constriction. Pair 2 also shows a secondary constriction at the distal third of the long arm (Figure 2, a, b).

As to the meiosis in the 3 specimens studied, 1 presents normal aspects, and 2 show multivalent rings. The specimen from Queimada Grande shows spermatocytes with 11 annular bivalents and 11 dyads. Both specimens from Boracea show 1 dodecavalent ring and 5 annular bivalents or 1 tetravalent ring and 3 bivalents (Figure 3, a, b). The dodecavalent ring is constituted by chromosomes of different size, symmetrically arranged in a specular image. The presence of this ring indicates a process of multiple translocations similar to the case of *Oenothera lamarckiana*.

Comparing the karyotype of *E. binotatus* with that of *E. guentheri*, already described[6], some differences are noted mainly in relation to the morphology of pairs 8, 10 and 11, which are metacentric in *E. guentheri*. Although the chromosomes of *E. binotatus* are much larger in width and in length in comparison to *E. guentheri*, the relative size remains practically uniform. The small differences noted in some pairs can be ascribed to the multiple translocations.

Apparently the three species *E. binotatus*, E. *parvus* and *E. guentheri* are diploid. Yet *E. binotatus* nuclear DNA content is about 4 times larger than that of the other diploid species. In arbitrary numbers *E. guentheri* presented 134.71 (± 1.45) %, *E. parvus* showed 147.37 (± 3.22) % and *E. binotatus* 513.04 (± 6.15) % of DNA content. These values were calculated by comparison with a standard blood smear of *Bufo ictericus* stained in the same batch of which nuclear DNA content was considered as 100%.

Parallel to DNA and size increase of the chromosomes, an increase in nuclear volume was also found in *E. binotatus* (Figure 3, c, d).

Several hypothesis may be raised to explain this drastic increase of DNA content in *E. binotatus*. Intersticial duplications of DNA[7] in the ancestral diploid species; polyploidy followed by reduction of number through chromosome translocations; and finally polynemy are some of the possibilities. Polyploidy alone or combined with intersticial duplications seems to be the best explanation. In this case the dodecavalent meiotic ring we found in some specimens could indicate multiple post polyploid rearrangements by translocations. According to this hypothesis the specimens from Queimada Grande with normal meiosis, would have already attained the diploid status.

Resumen. El contenido de DNA de *E. binotatus* (*2n* = 22) es cerca de cuatro veces mayor que el de *E. guentheri* (*2n* = 22) y *E. parvus* (*2n* = 22). Ese aumento drástico podría ser resultado de duplicaciones intersticiales y/o poliploidía, con translocaciones múltiples.

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Serviço de Genética, Instituto Butantan, Caixa Postal, 65, São Paulo (Brasil), 19 November 1973.

[6] M. L. Beçak, Caryologia *21*, 191 (1968).

[7] S. Ohno, *Evolution by Gene Duplication* (Springer Verlag, New York 1970).