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The polytene chromosomes of the mosquito *Anopheles bellator* compared with those of *Anopheles cruzii*

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ABSTRACT

A photographic map was made of polytene chromosomes of ovarian nurse cells of *Anopheles bellator* females. The chromosomes of this species have complete or partial homology with those of *A. cruzii*, mainly in the telomeric and centromeric regions. Variability at the single band level was observed as asymmetric bands at seven different positions. One inversion (3Ra) was detected in the 3R arm.

INTRODUCTION

Anopheles bellator is a neotropical species distributed along the Brazilian Atlantic coast, extending to the northeast near Venezuela. It is also found in Trinidad and Tobago (Zavortink, 1973). This species is a primary vector of malaria in Southeastern Brazil and in Trinidad (Forattini, 1962). The immature stages of *A. bellator* have been found associated with the interfoliar space of epiphytic bromeliad flora (Pittendrigh, 1949).

MATERIAL AND METHODS

Samples from two natural populations of *A. bellator* were analyzed: 13 individuals from Guaratuba (23°51'S, 45°50'W) and 12 from Cananéia (25°01'S, 47°55'W). These localities are nearly 280 km apart and have the same type of vegetation; Atlantic forest alternated with cultivated areas. All individuals were collected in the forest.

Adult females were collected and maintained in the laboratory as described by Ramírez and Dessen (1994). Squashes of ovarian nurse cells were prepared from bloodfed females, after fixing the ovaries during 5 min in 50% propionic acid (modified from Lambert, 1983). The cytological preparations were lightly stained with acetic-lactic orcein and analyzed under phase contrast microscopy.

The polytene chromosomes of the ovarian nurse cells were analyzed after they had attained a good level of polyteny, 36 h after bloodfeeding, in females maintained at 25°C.

RESULTS AND DISCUSSION

The polytene chromosomes of the ovaries usually appear as three elements, without chromocentric attachment. One of these chromosomes is short and acrocentric, while the other two are long and submetacentric. The centromeres, which divide the long chromosomes into two arms, right and left, seldom break during the squashing procedures. In *A. cruzii* a related species, it is rare to observe the connection between the two arms of the same chromosomes (Ramírez and Dessen, 1994).

A. bellator chromosomes can be distinguished by different lengths and distinctive banding patterns (Figure 1). They were arbitrarily divided into 44 regions, using easily identifiable bands as landmarks. The

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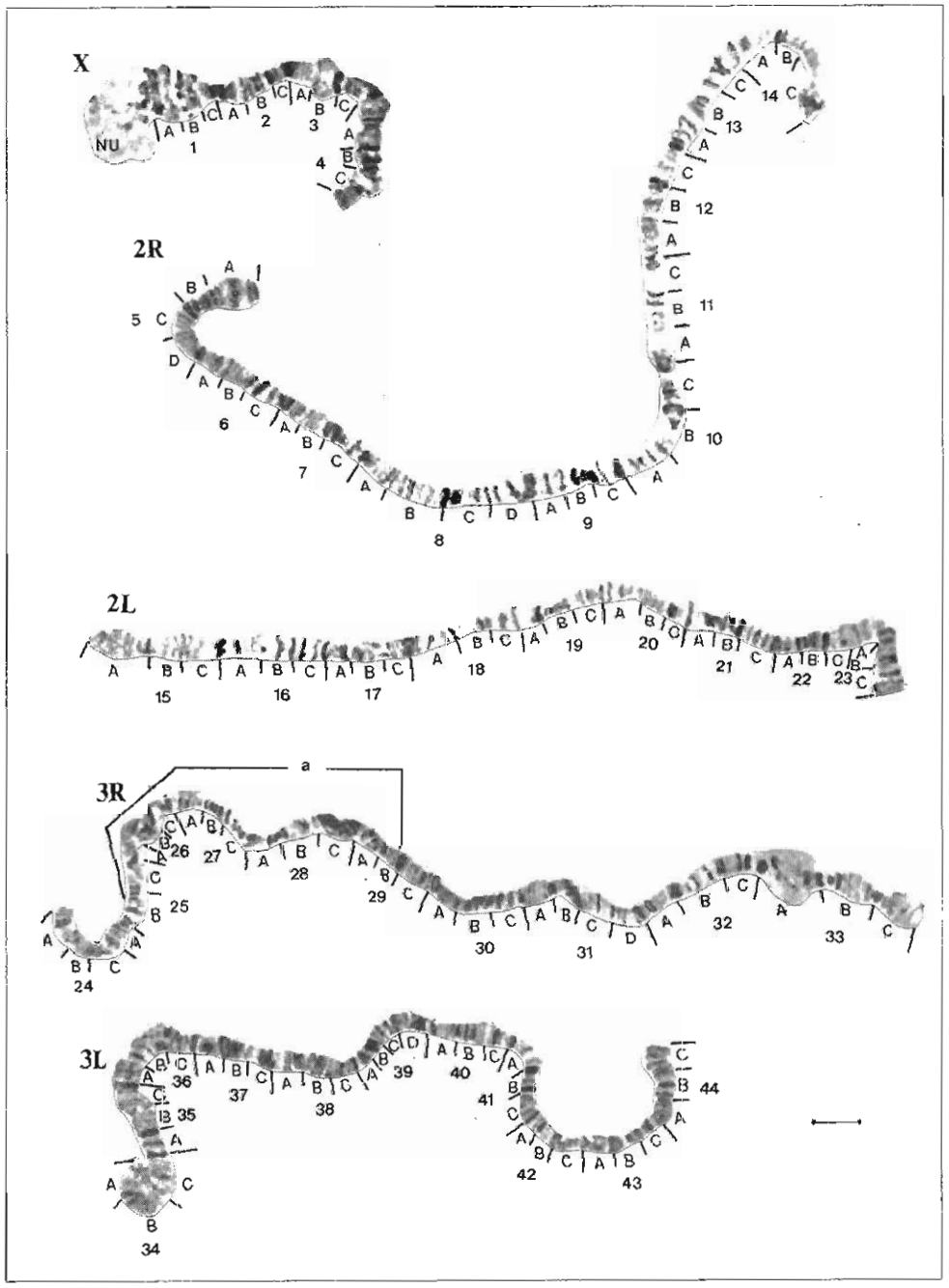

Figure 1 - Photographic map of *Anopheles bellator* ovarian nurse cell polytene chromosomes. The single inversion found, 3Ra, is indicated. The scale represents 10 µm.

chromosomes were designated based on a comparison between their band pattern and that exhibited by *A. cruzzi*. Ramírez and Dessen (1994) named the chromosomes of *A. cruzii* based on their relative lengths in the metaphase configuration. Analysis of both species shows that the shortest chromosome corresponds to the sexual chromosome X. However there is no relation between the size of the long chromosomes and homology of polytene bands. The longest chromosome in *A. bellator* was named 2 because it shows band homology to the intermediate size chromosome 2 of *A. cruzii*. The intermediate size chromosome was designated 3. The nucleolar organizing region (NOR) is located at the tip of chromosome X (Figure 1).

Several segments of *A. bellator* chromosomes show complete or partial homology of band pattern with *A. cruzii*, mainly in the telomeric and centromeric regions. Homology of the distal parts of the arms is common among related species of anophelines, as for example, in the *A. gambiae* complex (Coluzzi and Sabatini, 1967) and among the species of the nearctic group of the Maculipennis complex (Klassen *et al.*, 1965 and Kitzmiller *et al.*, 1967). Table I summarizes the homologies detected between *A. bellator* and *A. cruzii*.

A. cruzii has two alternative sequences of bands in the 3L arm: 3L1 corresponds to that described by Ramírez and Dessen (1994) for the population of Peruíbe, State of São Paulo, and 3L2 is present in several other natural populations (Ramírez, C.C.L., unpublished results). The extremity of arm 3L of *A. bellator* (44C-B) is partially homologous to the telomeric region of the same arm in *A. cruzii* (36A-38C), in those individuals exhibiting type 3L2 of this arm.

Variability at the single band level was observed as asymmetric bands at the following positions: 15A/B, 17C, 18A, 19A/B, 24B/C, 37A and 37C (see Figure 2). This type of polymorphism is not very frequent among dipteran species, except the Sciarids where asymmetric chromomeres are quite common (Perondini and Dessen, 1988). *A. cruzii* is the only other species in the genus *Anopheles* in which asymmetric bands have been reported (Ramírez and

Table I - Homologies in the band patterns of the polytene chromosomes of *Anopheles bellator* and *Anopheles cruzii*.

Chromosome	Homologies detected		Degree of homology
	Anopheles cruzii	*Anopheles bellator*	
X	3C-3D	1A-1C	Partial
2R	5A	5A	Total
	5B	5B	Partial
2L	18D	23C	Partial
	16A-17A	16B-20C	Total
3R	29B (puff)	33A (puff)	Partial
	19A-C	24A-C	Total
3L (3L2)	36A-38C	44C-B	Partial

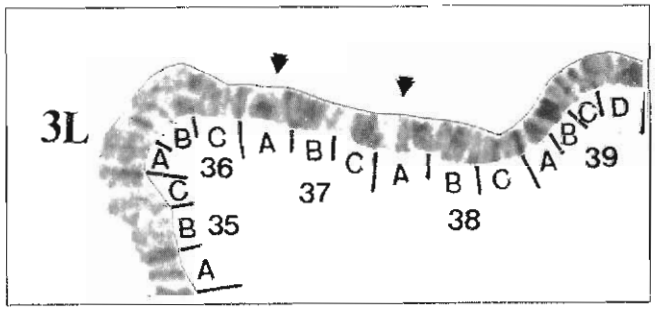

Figure 2 - Magnification of a section of the polytene chromosomes. Photographic map of *Anopheles bellator* to show the asymmetric bands 37A and 37C.

Dessen, 1994). The origin of band asymmetry has been ascribed either to deficiencies or to duplications (Metz, 1937, 1947; Perondini and Dessen, 1988). One of the loci exhibiting structural polymorphism in *Sciara ocellaris* is heterotic, being maintained in the population by an overdominance mechanism (Perondini and Otto, 1991).

Only one inversion was detected in the 3R arm, in individuals from both localities. The breaking points of this inversion are at 25B and 29B. Despite the small size of the sample (n = 25), we can conclude that polymorphism for this inversion is low. While the mean number of inversions per individual in *A. bellator* was estimated to be 0.63, in *A. cruzii* it is much higher, 4.15 (Ramírez and Dessen, 1994). Populations of *A. cruzii* show a much higher degree of chromosomal polymorphism than those of *A. bellator* from the same localities. While *A. bellator* presented only one inversion in the polytene chromosomes, 21 inversions have been found in *A. cruzii* (Ramírez, C.C.L. and Dessen, E.M.B., unpublished results). The amount of genetic polymorphism for a species has been correlated to the degree of ecological diversity of habitats in it's territory (da Cunha *et al.*, 1950; Kumar and Gupta, 1988). The geographic distribution of *A. bellator* and *A. cruzii* in Brazil overlaps. Nevertheless, *A. cruzii* is found in a wide range of ecological niches (Veloso *et al.*, 1956). While *A. cruzii* breeds in bromeliads with varying volumes of water in the interfoliar spaces that are found at different heights in the host trees, *A. bellator* prefers bromeliads from fully illuminated niches, in open areas or at high levels inside the forest (Pittendrigh, 1949; Forattini, 1962). The low genetic polymorphism of *A. bellator* could be a consequence of reduced microhabitat diversity.

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RESUMO

O presente trabalho apresenta o mapa fotográfico dos cromossomos politênicos das células nutritivas do ovário de fêmeas de *Anopheles bellator*. Uma comparação dos cromossomos politênicos de *A. bellator* e *A. cruzii*, uma espécie do mesmo subgênero, mostrou a existência de vários segmentos completa ou parcialmente homólogos, principalmente nas regiões centroméricas e teloméricas. A variabilidade ao nível de bandas foi observada como bandas assimétricas, em sete diferentes posições. Uma inversão (3Ra) foi detectada no braço 3R.

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