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Heterochromatin variation in chromosomes of *Anopheles* (*Nyssorhynchus*) *darlingi* Root and *A.*(*N.*) *nuneztovari* Gabaldón (Diptera: Culicidae)

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Abstract

C-banding was used to study the variations in heterochromatic block markings in chromosomes of *Anopheles darlingi* and *A. nuneztovari* from Manaus, State of Amazonas, and Macapá, State of Amapá, Brazil. Both species had two differently shaped X chromosomes and a Y chromosome that was entirely heterochromatic. The X_1 chromosome of *A. darlingi* had markings that extended 1/3 of the total length whereas in the X_2 chromosome the markings were located around the centromeric region. The markings on autosomal chromosomes were concentrated in the centromeric region in both species, with a heterochromatic block in one arm of chromosome II of *A. darlingi*. *A. nuneztovari* had three heterochromatic blocks in chromosome X_1 (longer) and two blocks in X_2 (shorter). X_2X_2 females were not detected in either species. The X_1 and X_2 chromosomes of males were found in *A. darlingi*, whereas in *A. nuneztovari* only the X_1 chromosome was detected. Only intraspecific variation was found in heterochromatic block markings in the sex chromosomes and autosomes in the two populations of both species at each location.

INTRODUCTION

Anopheles (*Nyssorhynchus*) *darlingi* Root, 1926, is the main vector of human malaria in the Amazon region. *Anopheles* (*N.*) *nuneztovari* Gabaldón, 1940, is an important vector in Venezuela and Colombia, but its vectorial capacity in Brazil is controversial (Deane *et al.*, 1948; Deane, 1986; Tadei *et al.*, 1993, 1998).

Chromosomal studies of *A. darlingi* populations from Minas Gerais, Brazil, and of other South American species showed a karyotype of 2n = 6 (Schreiber and Guedes, 1959), as in other *Anopheles* species (Coluzzi, 1988). Rafael and Tadei (1998) reported an identical karyotype (2n = 6) for *A. darlingi* and *A. nuneztovari* populations from the Amazon region.

C-banding analysis of mitotic chromosomes of *Anopheles* species from continental Asia (Baimai *et al*., 1995) revealed species complexes which included *Anopheles dirus* (Baimai, 1984; Hii, 1985) and *Anopheles maculatus* in the Neocellia series (*Cellia*) (Baimai *et al*., 1993). C-banding was reported to be useful for identifying sibling species based on differences in the morphology, quantity and distribution of heterochromatic blocks, principally in X and Y chromosomes (Baimai *et al*., 1993).

In spite of the epidemiological importance of *A. darlingi* and *A. nuneztovari* in the Amazon region, there are no data on C-banding of the metaphase chromosomes of these species. We studied the variation in heterochromatic block markings in metaphase chromosomes to determine the heterochromatic patterns in the Manaus and Macapá populations of these species.

MATERIAL AND METHODS

Two natural populations of *A. darlingi* were sampled, with 20 individuals from Manaus (3°08'S, 60°01'W), Amazonas State, and 14 from Macapá (0°02'S, 51°03'W), Amapá State. For *A. nuneztovari*, 17 individuals from Manaus and 11 from Macapá were analyzed. Slides were prepared from fourth instar larval brain ganglia, treated with a 0.005% colchicine-hypotonic solution, as described by Imai *et al.* (1988). The slides were washed with distilled water, air dried and stored at room temperature for 72 h. C-banding was done using the method of Sumner (1972), with a reduction in the barium exposure time (3 min). The best preparations were photographed using a phase-contrast microscope fitted with a green filter.

RESULTS

The C-banding patterns of 76 out of 103 *A. darlingi* metaphases from Manaus and 57 out of 74 from Macapá, as well as 63 *A. nuneztovari* metaphases out of 86 from Manaus and 46 out of 53 from Macapá were photographed and analyzed. *A. darlingi* and *A. nuneztovari* populations from both localities showed two types of X chromosomes (X_1 and X_2), which differed in the content and distribution of heterochromatic blocks (Figure 1). In *A. darlingi* from Manaus, the sex chromosomes had centromeric markings that extended to 1/3 of X_1 while the Y chromosome was entirely heterochromatic (Figure 2). The X_2 chromosomes of samples from Macapá (Figure 2B) showed fewer markings, which extended only to the centromeric region. These marking patterns were the same as that of *A. darlingi* from

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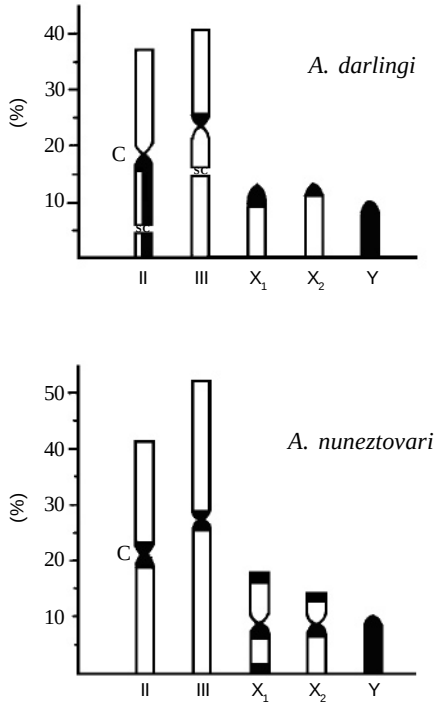

Figure 1 - Diagrammatic comparison of metaphase karyotypes of *Anopheles darlingi* and *Anopheles nuneztovari* from Manaus and Macapá. Only one set of autosomes (II and III) is shown. Variable heterochromatic portions are indicated in black. Chromosomes and heterochromatic portions are shown as a percentage of the total length. c = Centromeric region; sc = secondary constriction.

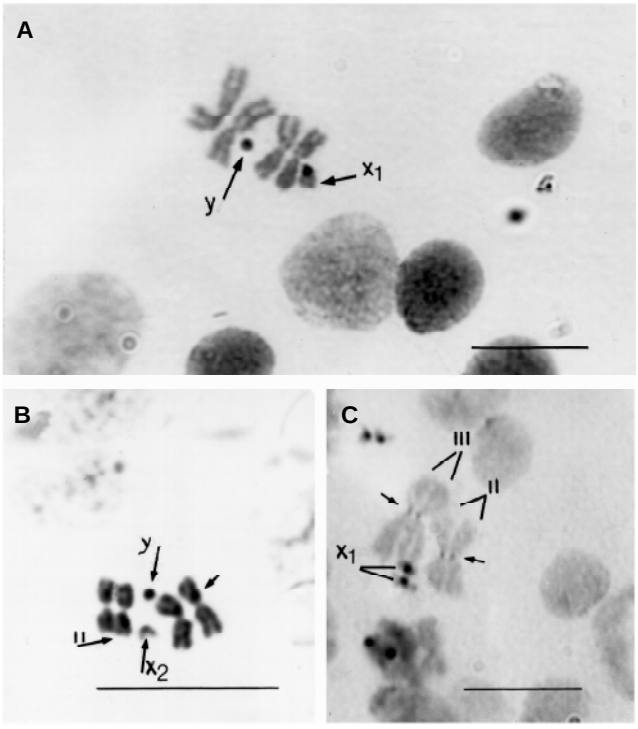

Figure 2 - Metaphase karyotypes from larval neuroblast cells of *Anopheles darlingi.* A = Male from Manaus; B = male from Macapá; C = female from Manaus. Long arrows = X_1X_2 chromosomes. Short arrows = centromeric markings. Bar = 10 μm.

Manaus. Chromosomes with a longer barium exposure (4 min) were more discolored than other preparations, although centromeric markings were seen in autosomes II and III and in the X_1X_1 sex pairs (Figure 2C).

The C-banding pattern in autosomes of the *A. darlingi* population from Macapá was the same as that of *A. darlingi* from Manaus (Figure 1). In these populations, the II and III chromosomes had well-marked centromeric regions (Figure 2B and C). All of the II chromosomes had a band which extended from the centromere along half the length of one arm of the chromatid in each population (Figure 2B).

The variations in heterochromatic block markings in X_1, X_2 and autosomal chromosomes of the *A. nuneztovari* from Manaus were the same as that of *A. nuneztovari* from Macapá (Figure 1). The X_1 chromosome (longer) consisted of three heterochromatic blocks (two telomeric and one centromeric) and the X_2 chromosome (shorter) contained two heterochromatic blocks, one of which was telomeric and the other centromeric (Figure 3A, B and C). The X_2 chromosomes of female *A. nuneztovari* had two heterochromatic blocks (Figure 3). The centromeric heterochromatin markings of the autosomes were found in this species (Figure 3D and E).

X_2X_2 *A. darlingi* and *A. nuneztovari* females were not found (Table I). X_1 and X_2 males were found in *A. darlingi* while *A. nuneztovari* males had only the X_1 chromosome.

DISCUSSION

C-banding studies of mitotic and meiotic chromosomes have provided important information on inter- and intraspecific population variation in *Anopheles* species and the technique has proven to be an excellent tool for identifying species complexes (Baimai *et al*., 1993). In this study the analysis of mitotic chromosomes of *A. darlingi* and *A. nuneztovari* described above revealed intraspecific variation in the quantity and distribution of heterochromatic blocks in sex chromosomes and in the centromeric regions of autosomes (Figure 1). Kitzmiller (1977) and Tadei (1985) suggested that in *Anopheles* genus the X chromosome was more sensitive to rearrangements than the autosomes. Intraspecific variation in sex chromosomes through the acquisition of constitutive heterochromatin is a common phenomenon in Southeast Asian anophelines. Baimai *et al.* (1996) reported two types of X chromosomes with floating frequencies in natural populations of *Anopheles willmori*. The X chromosomes in Amazonian populations of *A. darlingi* and *A. nuneztovari* most likely have similar mechanisms of adaptation in order to survive in these populations.

The difference in size between the X_1 and X_2 chromosomes of *A. nuneztovari* may have resulted from the addition to or loss of part of one of these chromosomes. The addition or loss of chromosomal heterochromatin in

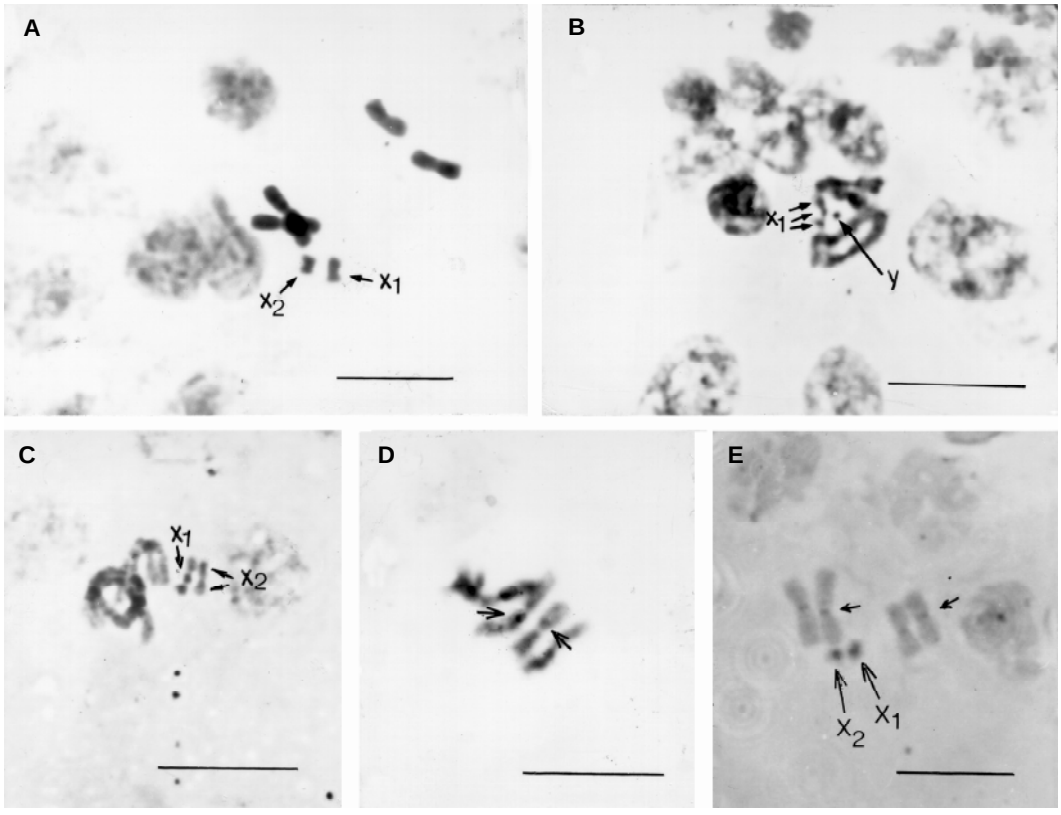

Figure 3 - Metaphase karyotypes from larval neuroblast cells of *Anopheles nuneztovari*. A = Female from Macapá; B = male from Manaus; C, D = females from Manaus; E = female from Macapá. Short arrows = centromeric markings. Bar = 10 μm.

Table I - X_1 and X_2 chromosomes in females and males of *Anopheles darlingi* and *Anopheles nuneztovari* populations from Manaus (MAO) and Macapá (MC).

Nº. of males and females analyzed		Females			Males		
MAO	MC	Chromosome combination	MAO	MC	Chromosome combination	MAO	MC
A. darlingi							
20	14	X_1X_2	7	5	X_1Y	4	7
		X_1X_1	2	1	X_2Y	7	1
		X_2X_2	0	0			
A. nuneztovari							
17	11	X_1X_2	6	3	X_1Y	9	6
		X_1X_1	2	2	X_2Y	0	0
		X_2X_2	0	0			

Anopheles has played an important role in chromosomal evolution in *Anopheles* species (Vasantha *et. al.*, 1982; Baimai *et al.*, 1993, 1996). The X_2 chromosome in Amazonian populations of *A. nuneztovari* could have been derived from the presumed X_1 through the loss of an extra heterochromatic block in the distal end of the chromosome arm.

The heterochromatic blocks of *A. darlingi* and *A. nuneztovari* are similar to those of *Anopheles* (*Kerteszia*) *cruzii*, according to Ramírez (1989) and Ramírez and Dessen (1994, 1996). The inversions in the latter species probably arose from differences in the homolog chromosomes of the same specimen. However, the inversion polymorphism detected in *A. darlingi* (Kreutzer *et al.*, 1972; Tadei *et al.*, 1982; Tadei, 1985) and *A. nuneztovari* (Kitzmiller *et al.*, 1973; Conn *et al.*, 1993) does not necessarily mean that inversions alone positioned the heterochromatic blocks in the chromosomes of these spe-

cies. Rather, these blocks may have originated from differences accumulated during evolution, as proposed by Gatti *et al.* (1982) to account for differences in the heterochromatic patterns of *Anopheles gambiae* and *Anopheles arabiensis*.

The C-banding in the present study in *A. darlingi* and *A. nuneztovari* populations exhibited only intraspecific variation of the heterochromatic blocks in X chromosomes and autosomes. The X chromosomes presented greater variation in the content and distribution of heterochromatic blocks than did the autosomes.

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RESUMO

Pela técnica do bandamento C detectou-se variação de marcação dos blocos heterocromáticos dos cromossomos de *A. darlingi* e *A. nuneztovari* de Manaus, Amazonas, e de Macapá, Amapá, Brasil. Os cromossomos sexuais de ambas as espécies mostraram duas formas de cromossomos X e o Y foi totalmente heterocromático. No cromossomo X_1 de *A. darlingi* a marcação atingiu 1/3 e no cromossomo X_2 foi apenas na região centromérica. Nos autossomos de ambas as espécies as marcações foram constantes nas regiões centroméricas, e o cromossomo II de *A. darlingi* mostrou um bloco heterocromático em um dos braços. *A. nuneztovari* mostrou polimorfismo de tamanho para o cromossomo X, tendo o X maior (X_1) três blocos e o menor (X_2) dois blocos heterocromáticos. Fêmeas homozigotas (X_2X_2) não foram detectadas nas duas localidades. Em machos de *A. darlingi* foram encontrados os cromossomos X_1 e X_2, enquanto que em machos de *A. nuneztovari* somente o cromossomo X_1 foi detectado. Apenas variação intraespecífica de blocos heterocromáticos nos cromossomos X e nos autossomos foi registrada nas duas populações de ambas as espécies estudadas em cada localidade.

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