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Metaphase karyotypes of *Anopheles (Nyssorhynchus) darlingi* Root and *A. (N.) nuneztovari* Gabaldón (Diptera; Culicidae)

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ABSTRACT

Metaphase karyotypes of *Anopheles* (*Nyssorhynchus*) *darlingi* and *Anopheles* (*N.*) *nuneztovari* from Manaus and Highway BR-174 (Manaus-Boa Vista), State of Amazonas, and Macapá, State of Amapá, Brazil, showed 2n = 6. They consisted of a pair of metacentric (chromosome II) and a pair of submetacentric (chromosome III) autosomes as well as sex chromosomes X and Y. In the sex chromosomes, the X was acrocentric in *A. darlingi* and submetacentric in *A. nuneztovari*. The Y chromosome was pointed in both species. The chromosomes of *A. nuneztovari* were larger than those of *A. darlingi* except for the Y chromosome. *A. nuneztovari* showed size polymorphism in the X chromosome. Somatic pairing and a strong constriction around the centromeric region in chromosomes II and III were exhibited in both species. The secondary constriction was only detected in chromosome II of *A. darlingi* from the Manaus population and chromosome III from the Macapá population.

INTRODUCTION

The metaphase karyotype in more than 80 species of *Anopheles* studied up to now has a diploid number of six. This chromosome number seems to be a conservative characteristic (Coluzzi, 1982, 1988; Rao and Rai, 1987). Exceptions are *A. maculipennis* and *A. messeae,* which have supernumerary chromosomes (Kitzmiller, 1976). The mitotic karyotype of *Anopheles* includes two pairs of sex chromosomes which exhibit some variation and can be heteromorphic in males (Baimai *et al.,* 1993; Ramírez and Dessen, 1994).

The first study of mitotic chromosomes of the South American subgenus *Nyssorhynchus* was carried out by Schreiber and Guedes (1959). These authors described the karyotypes of *Anopheles darlingi*, *A. strodei, A. albimanus, A. aquasalis, A. noroestensis* and *A. argyritarsis,* and discovered that these species have a chromosome complement of 2n = 6. Morphological variation was detected in the X chromosome, which was metacentric in *A. aquasalis* and *A. noroestensis* and acrocentric in *A. darlingi, A. strodei, A. albimanus* and *A. argyritarsis*. In the latter species it was also subtelocentric (Schreiber and Guedes, *op*. *cit*.).

In the present study karyotypes of *A. darlingi* and *A. nuneztovari* were studied taking into account the absence of research on metaphase chromosomes, which are important for understanding population differentiation of these

species that play an important role as malaria vectors in the Brazilian Amazon (Tadei *et al*., 1993).

MATERIAL AND METHODS

Adult females of *A. darlingi* and *A. nuneztovari* were collected from bovine and/or human bait in Manaus, Amazonas; Highway BR-174 at the 204 km marker between Manaus and Boa Vista, Amazonas State, and Macapá, Amapá State (Figure 1). Table I shows the number of specimens analyzed from each site. Highway BR-174 females were analyzed together with those from Manaus due to the small sample size.

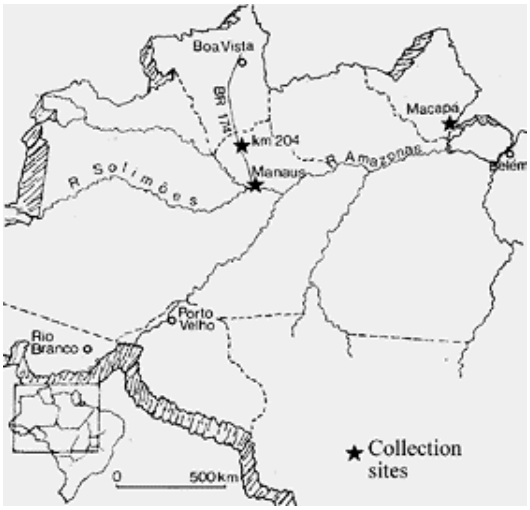

Figure 1 - Collection sites: Manaus and km 204 of Highway BR-174 (Manaus-Boa Vista), Manaus, Amazonas and Macapá, Amapá.

Table I - Number of specimens analyzed of *Anopheles darlingi* and *Anopheles nuneztovari* wild populations from Manaus, BR-174 and Macapá.

Sites	Specimen	
	A. darlingi	*A. nuneztovari*
Manaus	16	9
BR-174	1	2
Macapá	14	10
Total	31	21

Brain ganglia of fourth instar larvae were dissected from each female progeny and used for chromosome slide preparations, following the method described by Imai *et al.* (1988). Mitotic chromosomes from neuroblast cells were photographed after staining with Giemsa, and then analyzed under phase contrast and optovar 1.25X mounted on a Zeiss-Axioplan microscope. All measurements were made using a digital caliper. Chromosomes were numbered according to the nomenclature proposed by Rai (1963). Arm ratios (AR) and relative size (RS%) of all chromosomes were calculated by the method of Beçak (1967) and classified according to Levan *et al.* (1964).

RESULTS

Two hundred and twenty-six *A. darlingi* metaphases, 137 from Manaus and 89 from Macapá, as well as 121 *A. nuneztovari* metaphases, 58 from Manaus and 63 from Macapá, were photographed and analyzed. *A. darlingi* and *A. nuneztovari* metaphase chromosomes showed a karyotype of 2n = 6, similar to other anophelines (Figure 2A,B and C,D). The chromosome complement included two pairs of V-shaped (metacentric and submetacentric) autosomes and one pair of sex chromosomes with obvious X-Y dimorphism. The X chromosome was acrocentric in *A. darlingi* and submetacentric in *A. nuneztovari.* The latter species showed size polymorphism. The Y chromosome was pointed in both species.

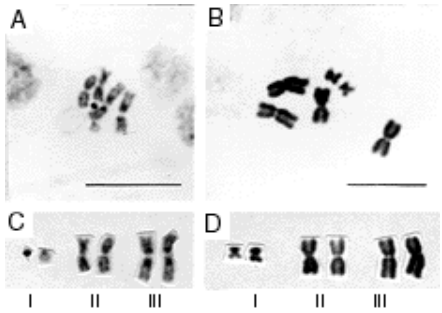

Figure 2 - *Anopheles darlingi*: A = male metaphase from larval neuroblast cells, B = karyotype. *A. nuneztovari*: C = female metaphase; D = karyotype. Scale: 10 µm.

Prometaphase and metaphase chromosomes of the two species showed somatic pairing and a constriction around the centromeric region, as shown in Figure 3A,D of *A. darlingi*. Secondary constriction was only detected in chromosome pair II of *A. darlingi* from the Manaus population (Figure 3B) and in the larger arm of chromosome pair III from the Macapá population (Figure 3C).

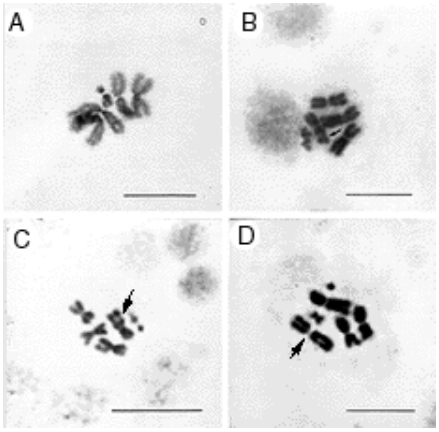

Figure 3 - *Anopheles darlingi*: A = somatic pairing in metaphase chromosomes; B = secondary constriction (arrow) in autosome pair II from the Manaus population; C = secondary constriction (arrow) in autosome pair III from the Macapá population. *Anopheles nuneztovari*: D = metaphase with strong primary constriction (arrow). Scale: 10 µm.

The average lengths (in micrometers) of the size polymorphism in *A. nuneztovari* (Figure 2C,D) were X_1 (large) 1.86 ± 0.25 and X_2 (small) 1.54 ± 0.16. The value of the X chromosome resulted from the average of X large and X small. Statistical analysis showed a significant difference ($t = 3.17$; d.f. = 17; $P < 0.01$).

For the mitotic chromosome measurements of *A. darlingi*, 10 specimens were selected. Five from Manaus and five from Macapá were chosen for similarity of degree of condensation and separated chromatids (Table II). From *A. nuneztovari,* 11 specimens were selected, five from Manaus and six from Macapá. Selection was based on the same criterion used for *A. darlingi* (Table III). Average lengths of the chromosomes of the two species are shown in Figure 4. It can be noticed that *A. nuneztovari* had longer averages for three chromosome pairs, excluding the Y chromosome. Comparison of the average lengths of the three chromosome pairs of both species by *t*-test showed that the result was only significant for the X chromosome ($t = 3.90$; d.f. = 19; $P < 0.01$).

Table II - Mitotic chromosome measurements ($\bar{X}$ ± SD) of *Anopheles darlingi* of 10 specimens from Manaus and Macapá populations. Classification of Levan *et al.* (1964).

Chromosomes	Average length (µm)	Average arm ratio	Relative length (%)	Classification
X	1.28 ± 0.23	-	13.48 ± 2.04	Acrocentric
Y	0.98 ± 0.13	-	10.37 ± 1.57	Pointed
II	3.66 ± 0.72	1.02 ± 0.03	38.71 ± 2.33	Metacentric
III	4.44 ± 0.83	1.33 ± 0.15	46.80 ± 1.82	Submetacentric
Average length of haploid genome = 9.39 ± 1.77 µm.				

Table III - Mitotic chromosome measurements ($\bar{X}$ ± SD) of *Anopheles nuneztovari* X chromosome value resulted from the average of X large and X small. Classification according to Levan *et al.* (1964).

Chromosome	Average length (µm)	Average arm ratio	Relative length (%)	Classification
X	1.72 ± 0.25	1.30 ± 0.17	16.14 ± 1.96	Submetacentric
Y	1.00 ± 0.09	-	8.77 ± 0.79	Pointed
II	4.10 ± 0.89	1.08 ± 0.09	36.66 ± 2.74	Metacentric
III	5.31 ± 1.12	1.36 ± 0.18	47.17 ± 2.78	Submetacentric
Average length of haploid genome = 11.14 ± 2.14 µm.				

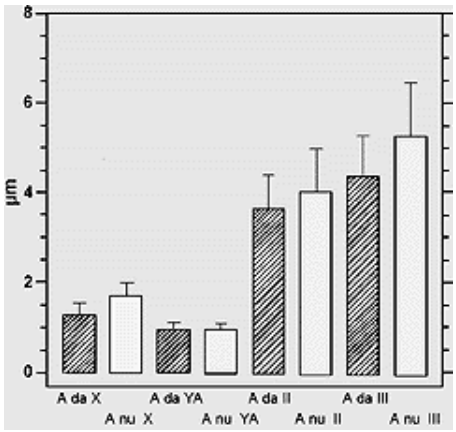

Figure 4 - Average length (X ± SD), in micrometers, of three chromosome pairs of *Anopheles darlingi* (A da*)* and *Anopheles nuneztovari* (A nu).

DISCUSSION

Morphological data obtained from metaphase chromosomes of *A. darlingi* in two Brazilian Amazon populations were similar to those obtained in *A. darlingi* populations from Minas Gerais, Brazil, by Schreiber and Guedes (1959), who also described acrocentric X and Y punctiform chromosomes as well as metacentric (chromosome II) and submetacentric (chromosome III) autosomes. In the *A. nuneztovari* karyotype, described for the first time in this paper, a morphology similar to *A. darlingi* was detected in autosomes and Y chromosome, whereas the X chromosome was submetacentric. This is also the case for mitotic chromosomes from South American species of the *Nyssorhynchus* subgenus, including *A. darlingi*, *A. aquasalis, A. noroestensis* and *A. argyritarsis* (Schreiber and Guedes, 1959, 1961). These authors also detected morphological variation in X chromosomes when they studied the karyotypes of the above mentioned species. This chromosome is metacentric in *A. aquasalis* and *A. noroestensis*, whereas it is acrocentric in *A. darlingi* and *A. argyritarsis*.

Similar chromosome morphology of *A. darlingi* was found in *A.* (*Kerstezia*) *cruzii* by Ramírez (1989), who detected karyotype 2n = 6 in southeastern Brazilian populations (São Paulo), with an acrocentric X chromosome.

Morphometric measurements of *A. darlingi* and *A. nuneztovari* compared to the average X chromosome size of *A. cruzii* (Ramírez, *op. cit.*) had small averages. Taking other *Anopheles* species into account, data from the literature show a sharp variation in mitotic chromosome average length (Rai and Craig, 1961; Kitzmiller, 1963).

Another characteristic detected in chromosomes of the two species studied in this paper was somatic pairing in prometaphase and metaphase. This record is in accordance with Traut *et al.* (1990) who reported somatic pairing to be a common phenomenon in Diptera as well as Culicidae chromosomes, according to Hunter Jr. and Hartberg (1986). Ramírez and Dessen (1994), who studied *A. cruzii* mitotic chromosomes, obtained results that were similar to those obtained in both species studied in this paper. These authors also pointed out somatic pairing in both arms and a strong constriction around the centromeric region.

Secondary constriction is an important aspect of chromosome morphology. In the genus *Anopheles*, Baimai *et al.* (1993) detected a conspicuous secondary constriction in the X chromosome in some cytological preparations from the hyrcanus group. However, in the South American *Anopheles* studied up to now, there have been no records of secondary constriction in mitotic chromosomes of *Anopheles* species (Schreiber and Guedes, 1959). In chromosome preparations of *A. nuneztovari* analyzed in this paper, a secondary constriction was not seen, but one was observed in *A. darlingi* chromosome II from Manaus population (Figure 3B) and chromosome III from Macapá population (Figure 3C). The evolutionary significance of this population difference cannot be explained from the data of this study or from the existing literature.

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