

KARYOTYPES AND PHYLOGENETIC RELATIONSHIPS IN *Drosophila* SPECIES OF THE *annulimana* GROUP (DIPTERA, DROSOPHILIDAE)

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ABSTRACT

Karyotype descriptions of 10 *Drosophila* species of the *annulimana* group are presented and phylogenetic relationships are proposed, based on the probable chromosomal rearrangements and on heterochromatin content variations. The karyotypes of three species (*D. aracataca*, *D. aragua* and *D. schineri*) are described for the first time and, for the other species, the karyotypes are redescribed and compared to those already reported in the literature.

INTRODUCTION

The *annulimana* group is composed of endemic species from the Neotropical Region that have been mainly collected from wet forest domains (Val *et al.*, 1981). It's basic biology, behavior, ecology and geographic distribution is almost unknown.

According to Vilela and Bächli (1990), 15 species of the group are known at present: *D. annulimana*, *D. aracataca*, *D. aragua*, *D. araicas*, *D. arapuan*, *D. ararama*, *D. arassari*, *D. arauna*, *D. breuerae*, *D. gibberosa*, *D. paratarsata*, *D. pseudotalamancana*, *D. schineri*, *D. talamancana* and *D. tarsata*. Many of them are sibling species distinguished only by the male terminalia.

We propose phylogenetic relationships among 10 species of the *annulimana* group, based on karyotypical differences.

MATERIAL AND METHODS

Strains of three species, *D. aracataca*, *D. gibberosa* and *D. pseudotalamancana*, were provided by the National *Drosophila* Species Resource Center, Bowling Green State University, Ohio, USA. The other species strains were each established from a single female collected from nature (Table I). Trappings were carried out in natural habitats, relatively far from human activity areas, following the procedure described by Sene *et al.* (1981).

Table I - Strains analyzed, collection localities and dates.

Species	Strain code	Locality	Collection date
<i>D. annulimana</i>	E54F4	Parque Estadual da Cantareira	23-27/VII/87
	E54F5	São Paulo-SP	
	E54F6	23°27'S, 46°38'W	
	C99E7M	Reserva Ecológica da Cidade	17-23/X/86 c
		Universitária, São Paulo-SP	20-25/XI/86
	E91F1	23°34'S, 46°44'W	28-30/VI/88
	F31F1		22-24/IV/89
F39F1		25-27/IV/90	
<i>D. aracataca</i>	1171.1	Santa Marta, Magdalena, Colômbia	*
	1171.2	San Salvador, Volcan El Boqueron, El Salvador	
<i>D. aragua</i>	E80F1	Santa Maria da Serra-SP 22°40'S, 48°12'W	16-18/IV/88
	E93F23	Estação Experimental e Reserva Ecológica de Mogi-Guaçu-SP	6-8/VII/88
	E93F24	22°17'S, 47°12'W	
<i>D. arapuan</i>	E68F1	Parque Estadual da Cantareira	9-17/XII/87
	E68F2	São Paulo-SP	
	E68F3	23°27'S, 46°38'W	

Continued

Table I - Continued.

Species	Strain code	Locality	Collection date
<i>D. ararama</i>	F19F1	Belém-PA	3-7/XI/88
	F19F2	1°27'S, 48°27'W	
	F19F3		
	F19F4		
	F34F1	Panamá, Panamá	18-20/VIII/89
	F34F2		
F34F3			
<i>D. arassari</i>	E87F3	Parque Estadual de Campos do	10-13/V/88
	E87F4	Jordão-SP	
	E87F5	22°42'S, 45°28'W	
	E87F6		
	E87F7		
<i>D. arauana</i>	E22F38	Reserva Ecológica da Cidade Universitária, São Paulo-SP 23°34'S, 46°44'W	20-22/I/87
	E54F2	Parque Estadual da Cantareira São Paulo-SP 23°27'S, 46°38'W	23-27/VII/87
<i>D. gibberosa</i>	1181	México	*
<i>D. pseudotalamancana</i>	1191	San Salvador, El Salvador	*
<i>D. schineri</i>	E57F10	Peruibe-SP	10-15/IX/87
	E57F11	24°14'S, 46°55'W	

*Provided by the "National Drosophila Species Resource Center, Bowling Green State University", collection date not informed.

The stocks were kept at $18 \pm 1^\circ\text{C}$, in 1/4 l flasks containing culture medium prepared with crushed corn (1.2 l of water, 10 g of agar, 18 g of yeast, 40 ml of corn glucose, 80 ml of an aqueous 1.5% malt solution, 10 g of crude soy flour, 80 g of crushed

corn and 15 ml of an alcoholic 10% "Nipagin" solution). When the larvae reached the third instar stage, the flasks were opened and placed inside a larger plastic container with a perforated cover (having holes smaller than the larvae). The larvae left the culture medium and pupated at the bottom of the container. The pupae were transferred to a vessel containing conventional banana culture medium, where they were kept until adult emergence. They were then transferred to flasks containing banana medium, where they were kept for seven days, after which they were transferred to flasks with the crushed corn medium.

The metaphase chromosomes were prepared from the cerebral ganglia (and the surrounding imaginal discs) of third instar larvae, according to the technique described by Baimai (1977), slightly modified: the use of colchicine was suppressed and 3:1 ethanol-acetic acid was used as a fixative.

In the preparations obtained by squashing, the acrocentric and telocentric chromosomes were seen as rods, the submetacentrics, as Js, the metacentrics, as Vs and the microchromosomes look like dots. This nomenclature, "V", "J", "rod" and "dot", employed in earlier works, is still in use. With Baimai's technique (1977), the acrocentrics and telocentrics have the aspect of a V and the submetacentrics and metacentrics look like an X.

Chromosomal classification according to centromere position was made subjectively, as large pericentromeric heterochromatic blocks, made exact localization difficult.

RESULTS

Micrographs of the metaphase spreads are shown in Figures 1 and 2 and a summarized description of each karyotype is presented in Table II. The male karyotypes are schematically represented in Figure 3.

Our metaphase plate description of *D. annulimana* partially coincides with that presented by Dobzhansky and Pavan (1943a and b): "one pair of large Vs, one pair of short rods and three pairs of short Vs". Nevertheless, they misinterpreted the short rods as sexual chromosomes and the males, which would have only one rod, as XO. We did not find, among the many metaphase plates analyzed, any case in which only one of the small acrocentrics appeared. Sometimes a secondary constriction is observed in one of the submetacentric pairs (Figure 1b), but this was never observed in the large metacentric pair. The presence of large pericentromeric heterochromatic blocks may have misled Dobzhansky and Pavan (1943b) who concluded that the large metacentric pair presents strong secondary constrictions in one of the arms.

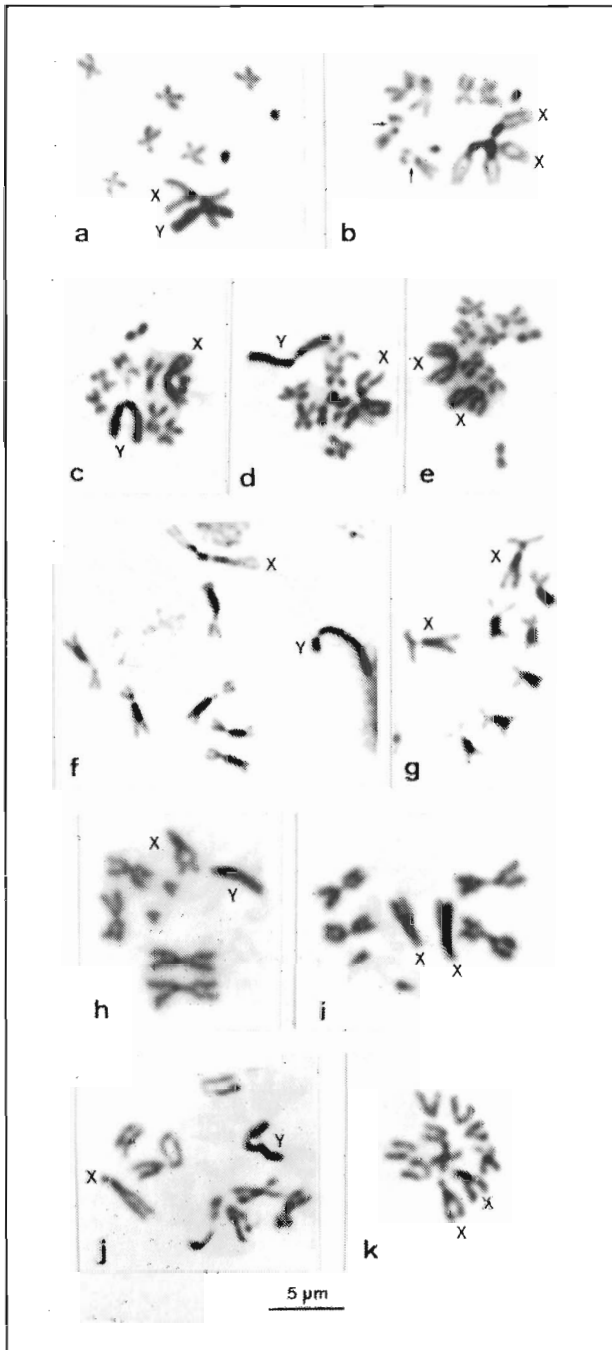


Figure 1 - Metaphase chromosomes of five *Drosophila* species of the *annulimana* group. a and b: *D. annulimana*; c, d and e: *D. aracataca*; f and g: *D. aragua*; h and i: *D. arapan*; j and k: *D. arabama*. The arrows in Figure 1b indicate the secondary constrictions in one of the submetacentric pairs.

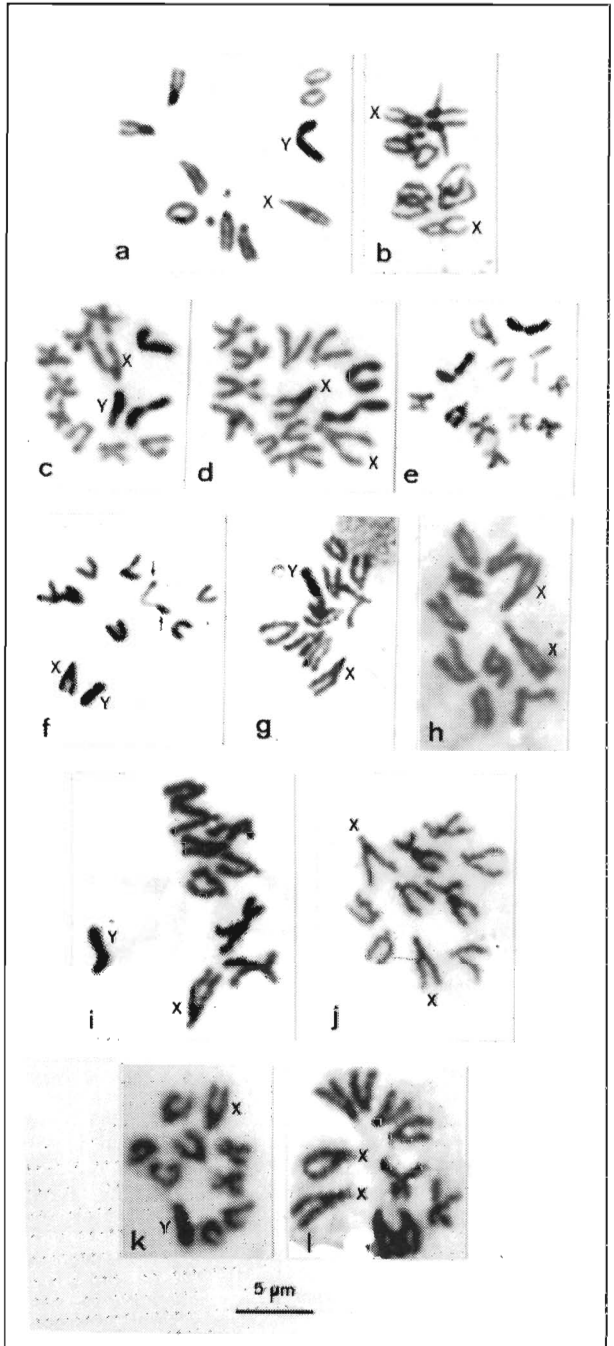


Figure 2 - Metaphase chromosomes of five *Drosophila* species of the *annulimana* group. a and b: *D. arassari*; c, d and e: *D. arana*; f, g and h: *D. gibberosa*; i and j: *D. pseudotalamancana*; k and l: *D. schineri*. The arrows in Figure 2f indicate the heterochromatic regions in the extremities of one acrocentric pair.

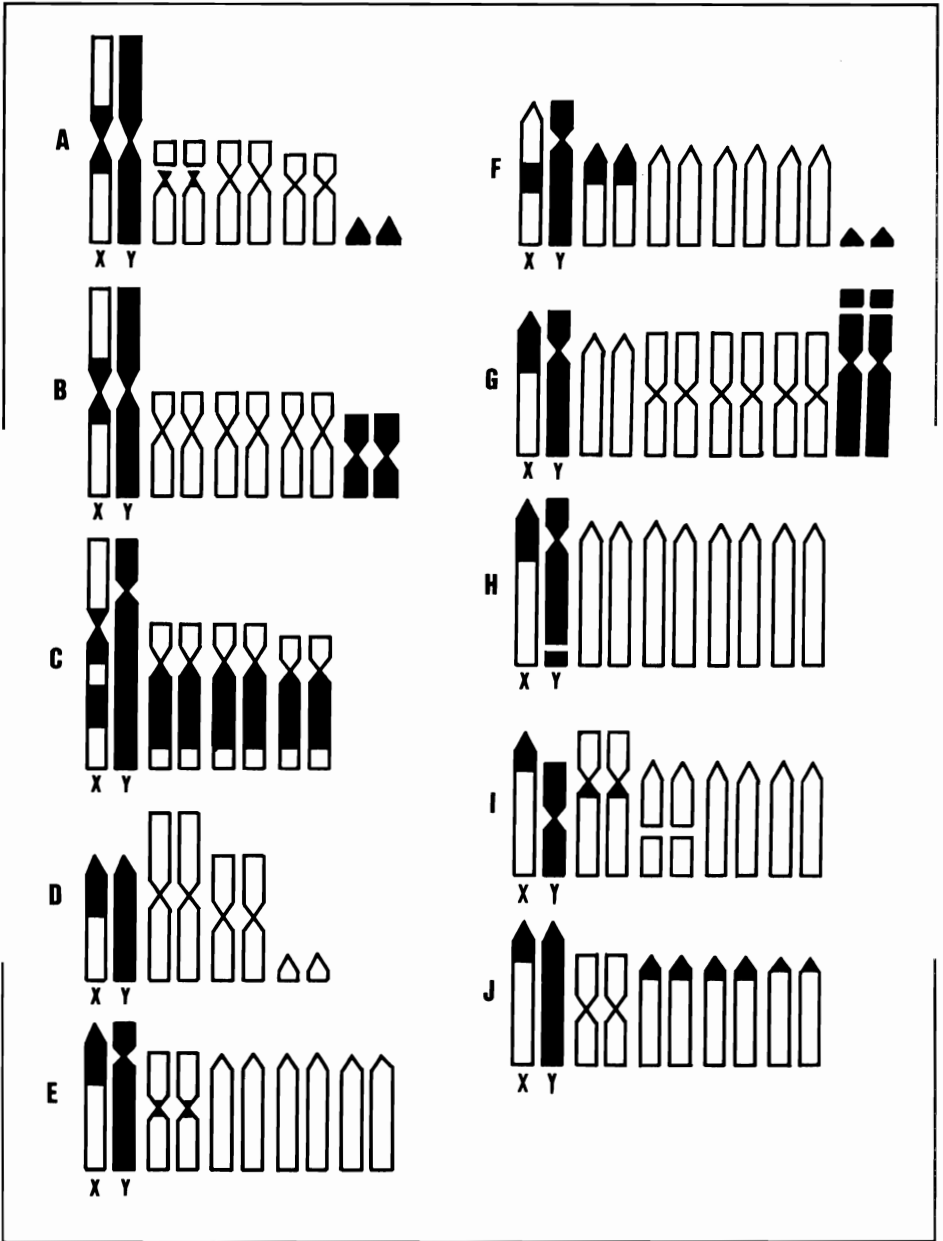


Figure 3 - Schematic representation of the male karyotypes of 10 *Drosophila* species of the *annulimana* group. Heterochromatin is represented in black. A: *D. annulimana*; B: *D. aracataca*; C: *D. aragua*; D: *D. arapuan*; E: *D. ararama*; F: *D. arassari*; G: *D. araua*; H: *D. gibberosa*; I: *D. pseudotalamancana*; J: *D. schineri*.

Table II - Description of the metaphase chromosomes of 10 species of the *annulimana* group.

	X	Y	Autosomal pairs	Other characteristics
<i>D. annulimana</i>	M	M	3SM 1D	One submetacentric slightly larger with a secondary constriction and pericentromeric heterochromatin.
<i>D. aracataca</i>	M	M	4SM	The shortest autosomal pair is totally heterochromatic.
<i>D. aragua</i>	SM	SM	3SM	All chromosomes have large heterochromatic blocks in the long arms. One autosomal pair is slightly shorter.
<i>D. arapuan</i>	A	A	2M 1D	One of the metacentrics is slightly shorter.
<i>D. ararama</i>	A	SM	1M 3A	Centromeric heterochromatin in the metacentric. The X seems to have a satellite.
<i>D. arassari</i>	A	SM	4A 1D	One autosome with a heterochromatic block in the centromeric region. X with a heterochromatic block in the medial region.
<i>D. araua</i>	A	SM	3M 1A 1SM	The submetacentric autosomal pair is larger than the others, totally heterochromatic and has a constriction in one of its arms.
<i>D. gibberosa</i>	A	SM	4A	The Y has a constriction in the long arm.
<i>D. pseudotalamancana</i>	A	M	1SM 3A	One of the acrocentrics has a constriction in the medial region. The submetacentric presents heterochromatin adjacent to the centromere.
<i>D. schineri</i>	A	A	1M 3A	The acrocentrics have heterochromatin in the centromeric region.

M - metacentric; SM - submetacentric; A - acrocentric; D (dot) - very short acrocentric chromosome or microchromosome, always heterochromatic, except in *D. arapuan*.

The centromeric region of the X chromosome is heterochromatic, except in *D. arassari*.

The Y chromosome is totally heterochromatic.

The metaphase chromosomes of *D. aracataca* and *D. aragua* are described here for the first time. The chromosomal constitution of *D. aracataca* is very similar to that of *D. annulimana*, and both species differ by a heterochromatic autosomal pair, which is a short metacentric in the first species and a dot in the second one. In *D. aragua* there are considerable heterochromatin in the long arms of the autosomes.

The description of the metaphase plate of *D. arapuan* resembles that presented by Pavan and Cunha (1947): “two pairs of slightly different Vs, one pair of rods of the same size as the largest V, and one pair of tiny dots.”

Pavan and Cunha (1947) described the metaphase plate of *D. ararama* as composed of one pair of large rods with satellites, three pairs of rods and one pair of Js. The description coincides with ours, except for one of the autosomal pairs, considered submetacentric by Pavan and Cunha (1947) and metacentric in the present work. The discrepancy may be due to a misinterpretation or there may be geographic variation in the morphology of this pair, as the population analyzed by Pavan and Cunha was from Bertioiga (SP) and those analyzed in our work were from Belém (PA) and Panamá (Panamá).

The literature description of the metaphase plate of *D. arassari* (Pavan and Cunha, 1947) is “five pairs of rods and one pair of tiny dots”, without any reference to the sex. Either they analyzed only females (in this case the description coincides with that of our work) or the Y chromosome was interpreted as an acrocentric.

The karyotypical description of *D. arauana* coincides with that of Pavan and Nacur (1950): “one pair of large Vs, three pairs of short Vs, one pair of large rods and one pair of short rods”. The submetacentric autosomal pair was totally heterochromatic.

Wharton (1943) described the metaphase chromosomes of *D. gibberosa* as: “four pairs of rods and the sexual pair: the X is a rod with a proximal constriction and the Y is J-shaped with a distal constriction in the long arm”. Roberts and MacPhail (1985), disagreeing with Wharton’s description (*op. cit.*), described four acrocentric pairs and one submetacentric pair. Our description agrees with that of Wharton (1943). One of the acrocentric pairs of *D. gibberosa* presents a peculiar aspect: in some preparations heterochromatic blocks are seen in the extremities and the block seems larger in one of the chromatids (Figure 2f).

Our description of *D. pseudotalamancana* corresponds to that made by Wheeler (1968) for this species (wrongly identified in that work as *D. talamancana*): “one pair of Vs, three pairs of rods, one rod-shaped X and one V-shaped Y.”

DISCUSSION

Our hypotheses of phylogenetic relationships among the species of the *annulimana* group we analyzed are presented in Figure 4.

We tried to establish the most parsimonious sequence of events that could transform one configuration into another. It was necessary to admit the existence of hypothetical intermediary steps. As the Y chromosome presents great inter and intraspecific size and morphology variation it was not taken into account in the establishment of these relationships. It is frequently observed that in a group of related species some present dots in the metaphase plate and some do not. The absence of the dot may be interpreted as the loss of this chromosome. Wharton (1943) raised the hypothesis, accepted in this work, of dot fusion with other chromosomes.

Analysis of the male terminalia morphology of the *annulimana* group confirms some of the relationships established on the basis of chromosomal analysis. *D. annulimana*, *D. aracataca*, *D. aragua* and *D. arauana* are considered morphologically related because they share, among other similarities, the presence of the aedeagus with two conspicuous lateral projections ("wings") in the dorsal region. The first three species are more closely related because they possess a rough plate in the distal extremity of the aedeagus, which is absent in *D. arauana* (Breuer and Pavan, 1950; Vilela and Pereira, 1982; Vilela and Val, 1983). From the analysis of the metaphase chromosomes, these four species could be included in a subgroup, in which *D. aragua*, *D. aracataca* and *D. annulimana* would be more closely related because they present an X-autosomal fusion, absent in *D. arauana*.

D. arapuan and *D. arassari* are also more closely related phylogenetically to one another than they are with the other species of the group, which is confirmed by morphological studies (Breuer and Pavan, 1950).

The other three species (*D. pseudotalamancana*, *D. ararama* and *D. schineri*) that can be chromosomally placed in the same subgroup, do not show close morphological relationships. *D. schineri* is considered by Pereira and Vilela (1987) to be an atypical member of the *annulimana* group.

The position of *D. gibberosa* in the phylogeny based on metaphase chromosomes remains uncertain. *D. gibberosa* could be related to *D. schineri*, *D. ararama* and *D. pseudotalamancana*, or it could derive by fusion of the dot, from an ancestor with a karyotype similar to that of *D. arassari*.

ACKNOWLEDGMENTS

The authors thank Dr. J.S. Yoon, from the National *Drosophila* Species Resource Center, Bowling Green State University, Ohio, USA, who kindly provided *Drosophila* strains. J.C.R. Magalhães, allowed collecting at his farm (Barreiro Rico) in Santa Maria da Serra, SP. FAPESP (proc. no. 87/0161-8) and CNPq (proc. no. 840470/89 and 840167/91) granted the fellowships to the first author. The Departamento de Biologia do Instituto de Biociências provided facilities to perform this work. The Instituto de Botânica and the Instituto Florestal, of the Secretaria do Meio Ambiente de São Paulo, gave authorization to collect in parks and natural

reserves. We thank Dr. A.B. da Cunha for reading an early draft, Dr. C.R. Vilcla, for suggestions during the development of this work, for his help in the fieldwork, for the species identification and for the review of the manuscript, and an anonymous referee for improving the manuscript.

Publication supported by FAPESP.

RESUMO

São apresentadas descrições dos cariótipos de 10 espécies de *Drosophila* do grupo *annulimana* e propostas relações filogenéticas entre elas, tomando por base os prováveis rearranjos cromossômicos e alterações na quantidade de heterocromatina que ocorreram durante a evolução do grupo.

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(Received May 25, 1992)