

Meiotic studies in *Dysdercus* Guérin Méneville 1831 (Heteroptera: Pyrrhocoridae). I. Neo-XY in *Dysdercus albofasciatus* Berg 1878, a new sex chromosome determining system in Heteroptera

María José Bressa^{1,2}, Alba G. Papeschi², Liliana M. Mola² & Marcelo L. Larramendy^{1*}

¹ *Laboratorio de Citogenética y Cátedra de Citología, Facultad de Ciencias Naturales, Universidad Nacional de La Plata, Calle 37 Nro. 668 7mo 'B', 1900 La Plata, Argentina; Fax: (+54) 0221 4 530189;*

E-mail: larramen@museo.fcnym.unlp.edu.ar; ² *Departamento de Ciencias Biológicas, FCEN, Universidad de Buenos Aires, Buenos Aires, Argentina*

* *Correspondence*

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Abstract

The genus *Dysdercus* Guérin Méneville 1831 represents the only taxon within the family Pyrrhocoridae in the New World. Based on morphological features, it has been suggested that American species derived from immigrants from the Old World, most probably from the Ethiopian Region. So far, 10 species from *Dysdercus*, including six species from the Old World and four species from the Neotropical Region have been cytogenetically analyzed. As is characteristic of Heteroptera, they possess holokinetic chromosomes and a prereducational type of meiosis. While the X_1X_20 sex chromosome system has been reported in all cytologically analyzed species of *Dysdercus* from the Old World, the system $X0$ has been found in all but one species from the New World, regardless of the number of autosomes in the complement. In the present study the male meiosis of *D. albofasciatus* Berg 1878 was studied in specimens from four different populations from Argentina. The diploid chromosome number was found to be $2n = 10 + \text{neo-XY}$. The neo-X shows at each subterminal region a positively heteropycnotic and DAPI-bright segment which corresponds to the ancestral X-chromosome. The origin of this neo-XY system involved, most probably, a subterminal insertion of the ancestral X chromosome in an autosome, followed by a large inversion, which included part of the original X chromosome.

Introduction

The genus *Dysdercus* Guérin Méneville 1831 comprises bugs that are often serious pests of cotton both in the Old World and in the New World. They are so-called 'cotton stainers' since, after piercing cotton bolls, they introduce micro-organisms which cause boll rot or discoloration of the lint. Based only on morphological characteristics, it has been suggested that the New World species of *Dysdercus* should derive from immigrants from species of the Old

World, probably having originated from the Ethiopian Region (Van Doesburg 1968). The genus represents the only taxon within the family Pyrrhocoridae in the New World.

Available cytogenetic data on the genus *Dysdercus* is scarce. As is the rule in the order Heteroptera, the genus is cytologically characterized by the possession of holokinetic chromosomes and a prereducational type of meiosis. The autosomal bivalents divide prereducationally while the sex chromosomes are achiasmatic and divide postreductionally. So far, only 10 species

from *Dysdercus*, including six species from the Old World and four species from the Neotropical Region, have been cytogenetically analyzed. All reported species of *Dysdercus* from the Old World, namely *D. cingulatus*, *D. fasciatus*, *D. intermedius*, *D. koenigii*, *D. superstitiosus* and *Dysdercus* sp., are characterized by possession of a similar chromosomal complement of 14 autosomes with an $X_1X_20/X_1X_1X_2X_2$ (male/female) sex chromosome determining system (Ueshima 1979, Kusnetzova 1988). On the other hand, analyses performed on the four New World species have revealed variations not only in the diploid chromosomal number but also in the sex chromosome determining system. *D. chaquensis* (Mola & Papeschi 1997) and *D. ruficollis* (Piza 1947) share a male diploid complement of 13 elements ($12 + X0$) while *D. honestus* (Piza 1947) shows 15 chromosomes ($14 + X0$). Finally, *D. peruvianus* is characterized by the presence of 16 elements in its complement ($14 + X_1X_20$) (Piza 1947, 1951, Mendes 1949).

In the order Heteroptera, several sex chromosome determining systems have been described. Among them, simple systems XY/XX (74.7% of the species) and, X0/XX (14.8%), and different multiple systems originated through fragmentation of the X chromosome and less frequently, the Y chromosome (X_n0/X_nX_n , X_nY/X_nX_n , XY_n/XX) (10.3%) are found (Ueshima 1979, Manna 1984). To date, a particular neo-XY system has been reported in only three species (0.2%) (Chickering & Bacorn 1933, Schrader 1940, Jande 1959).

In the present study we analyzed the male meiotic development of *D. albofasciatus* Berg 1878. The results demonstrate that the species is characterized by a diploid chromosome number of 12 elements with a neo-XY sex chromosome determining system which arose, most probably, through a subterminal insertion of the ancestral X in an autosome, followed by a large inversion which included part of the original X.

Materials and methods

Specimens and localities

The material included in the present study comprises 36 adult males obtained from Iguazú National Park (8 specimens), Misiones Province; El Palmar National Park (16 specimens) and Gualeguaychú (2

specimens), Entre Ríos Province; and from the Natural Reserve Martín García Island (10 specimens), Buenos Aires Province, all in Argentina.

Cytogenetic analysis

Immediately after capturing, all specimens were fixed in methanol : glacial acetic acid (3 : 1), and maintained at 4°C until dissection. Afterwards, gonads were dissected free and kept in ethanol 70% (4°C). Slides were made by the squash technique in 2% iron propionic hematoxylin following conventional procedures.

When required, chromosome spreads were stained with 4'6-diamidino-2-phenylindole (DAPI) (Vectashield mounting medium H1200; Vector Laboratories, Burlingame, CA, USA). Fluorescence analysis of the slides was performed in a Zeiss Laborlux fluorescence microscope with Zeiss filter 09.

Results

All specimens from *Dysdercus albofasciatus* possess a diploid chromosome number of 12 ($10 + \text{neo-XY}$, male) with one large, two medium-sized, and two small pairs of autosomes. The largest chromosome corresponds to the neo-X, while the neo-Y is slightly larger than the medium-sized autosomes at mitotic prometaphase (Figure 1a).

Pachytene nuclei are difficult to analyze since it is not possible to individualize the bivalents. However, at this stage, two positively heteropycnotic and DAPI-bright bodies of different size are readily visible. An association between a nucleolus and the biggest heteropycnotic body is often observed. From diplotene onwards, five autosomal bivalents and the neo-XY sex bivalent are distinguished (Figure 1). Autosomal bivalents generally present only one chiasma, except the largest and seldom a medium-sized, which can show two terminal chiasmata. The neo-XY is the largest bivalent and clearly heteromorphic, and shows one and, less frequently, two terminal chiasmata (Figure 1b–d). In some cells, at early diakinesis the sex chromosomes appear associated by a thin thread of chromatin (Figure 1e, f); at late diakinesis, the subterminal positively heteropycnotic and DAPI-bright bodies from the neo-X become isopycnotic.

At metaphase I, both autosomal and neo-XY bivalents show axial orientation in the spindle (Figure 1g).

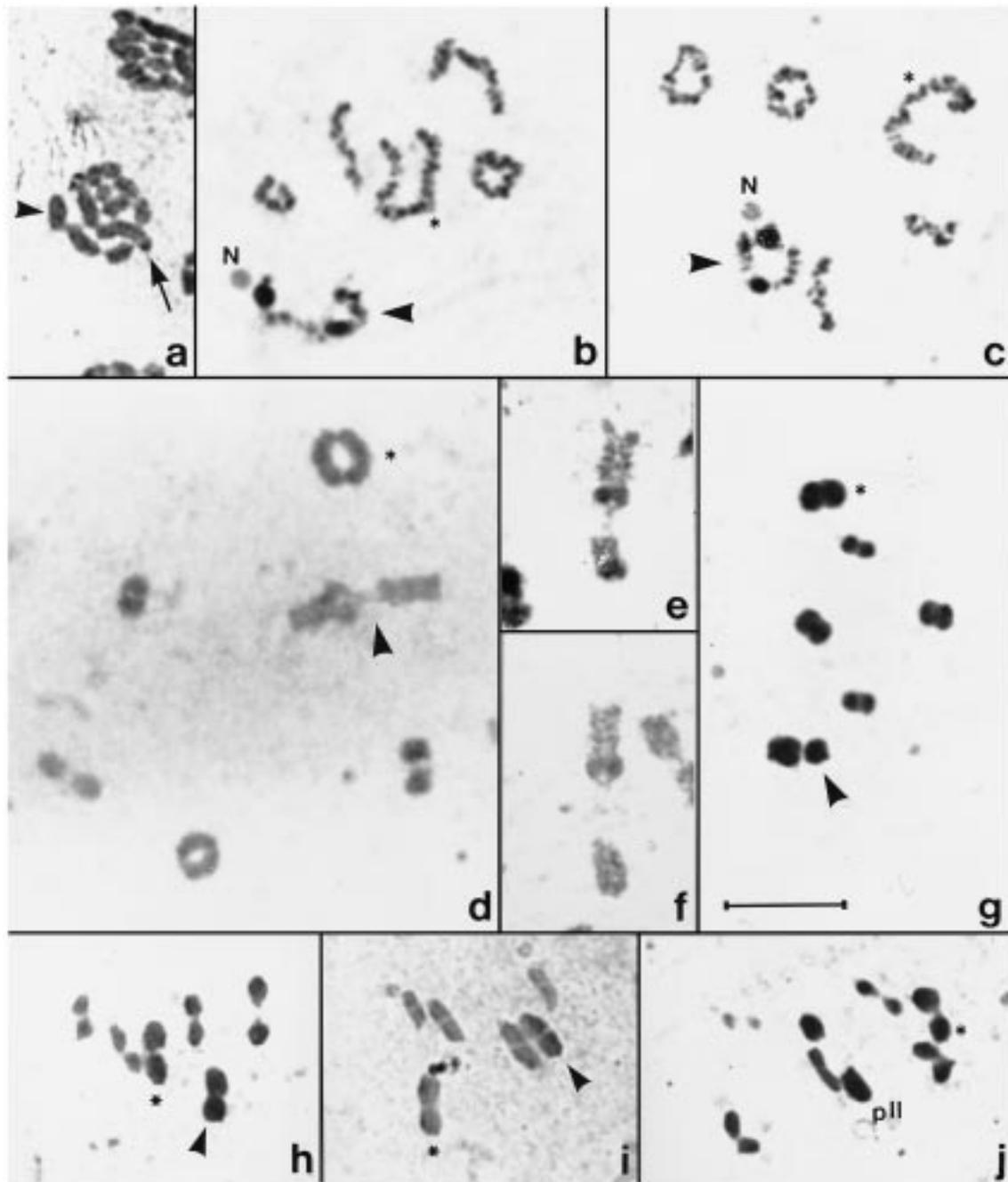


Figure 1. *Dysdercus albofasciatus* Berg 1978. (a) Mitotic prometaphase, arrow points neo-X, arrowhead, neo-Y; (b, c) late diplotene, sex bivalent presents one (b) or two (c) chiasmata; (d) early diakinesis; (e, f) sex bivalents at early diakinesis; (g) metaphase I; (h–j) metaphase II with neo-X (h), with neo-Y (i), and with pseudobivalent (j). (b–d, g, h) Arrowheads: sex chromosomes; asterisks: the largest autosomes; N: nucleolus. Bar represents 10 μ m.

At anaphase I, all chromosomes move synchronously to the poles, resulting in two kinds of metaphase II plates, those with a neo-X (Figure 1h) and those with a neo-Y (Figure 1i), together with five autosomes. Thus, the neo-XY undergoes prereducational meiosis, i.e. a behavior similar to that found in autosomes.

In most of the individuals from El Palmar National Park and Iguazú National Park (81.2% and 85.7%, respectively) the sex chromosomes can show a particular behavior at the first and/or second division. In some cells at diakinesis-metaphase I, the neo-XY pair is present as univalents that divide equationally at anaphase I; in the resulting metaphase II plates, sex chromosomes associate in a pseudo-bivalent, which undergoes reductional division at anaphase II (Figure 1j). Also, an autosomal pair can be found as univalents in both populations (68.7% and 71.4%, respectively; Table 1).

In the specimens from Gualeguaychú and Natural Reserve of Martín García Island, the chromosome

complement was corroborated. However, the number of cells suitable for meiotic analysis was scarce, not allowing the determination of desynaptic univalents with certainty.

In the sample from El Palmar National Park, four individuals present a B chromosome in a variable percentage of cells (range: 26.2–90.9%; Table 1). The B chromosome always divides equationally at anaphase I and reductionally at the second division, lagging behind the A chromosomes.

Discussion

Our novel finding in *Dysdercus albofasciatus* is the presence of a neo-XY sex chromosome determining system not previously reported in Pyrrhocoridae.

Most heteropteran species present a basic sex chromosome determining system XY/XX, and less frequently X0/XX while derived systems are multi-

Table 1. Meiotic configurations from *Dysdercus albofasciatus* from El Palmar and Iguazú National Parks at diakinesis metaphase I and at metaphase II.

Individual	Diakinesis metaphase I			Total number of cells	Metaphase II		Total number of cells
	6 II	5 II + 2 sex I	5 II + 2 I		Without pseudoll	With pseudoll	
El Palmar National Park (Entre Ríos Province)							
1	13	—	2	15	6	1	7
2	81	5	—	86	1	—	1
3*	37	5	1	43	11	1	12
4	11	2	—	13	—	—	—
5	27	2	1	30	11	1	12
6	15	3	1	19	—	—	—
7	4	1	—	5	4	1	5
8*	11	—	1	12	1	—	1
9	9	1	1	11	—	—	—
10	23	2	2	27	1	—	1
11	8	—	—	8	—	—	—
12	3	—	—	3	—	—	—
13*	11	6	1	18	6	1	7
14*	27	—	1	28	9	5	14
15	2	1	1	4	1	—	1
16	8	4	2	14	—	—	—
Iguazú National Park (Misiones Province)							
1	5	—	—	5	—	—	—
2	81	23	5	109	—	—	—
3	25	12	4	41	16	11	27
4	84	35	1	120	44	5	49
5	25	13	3	41	—	—	—
6	17	11	—	28	3	5	8
7	18	1	1	20	2	—	2

* B chromosome in 90.9%, 85.6%, 60.0% and 26.2% of the cells from specimens 3, 8, 13, and 14, respectively.

ple originated by fragmentation. Two hypotheses concerning the evolution of the sex-determining systems were proposed. Ueshima (1979) suggested that the XY system derived from an ancestral X0 which is commonly encountered in primitive taxa and in homopteran species (Halkka 1959). By contrast, based on the presence of a Y-chromosome in a very primitive heteropteran species, Nokkala & Nokkala (1983) suggested that the X0 system derived from the XY by losing the Y-chromosome during evolution. Our present results on *D. albofasciatus* could support Ueshima's hypothesis, since the neo-XY system has evolved from an ancestral X0 system.

While the presence of a neo-XY system is a common feature in other insects with holokinetic chromosomes such as Odonata (Mola & Papeschi 1994), it is almost absent in Heteroptera. To date, within Heteroptera a neo-XY system has been only

claimed in two species of *Lethocerus* (Belostomatidae) (Chickering & Bacorn 1933, Jande 1959), and in *Rhytidolomia senilis* (Pentatomidae) (Schrader 1940). In these species, the fusion of both the ancestral X and Y chromosomes with a pair of autosomes has occurred, originating a particular neo-XY system which we refer to as the 'neo-X neo-Y system'.

Our observations in *D. albofasciatus* lead us to propose a more complex origin of its neo-system. Two meiotic features deserve attention, namely the presence of two positively heteropycnotic and DAPI-bright bodies of different sizes in one of the chromosomes of the sex bivalent and the restriction of crossing-overs to the terminal regions of the sex chromosomes. Assuming that the original sex chromosome determining system in *Dysdercus* was X0/XX, a plausible and most parsimonious hypothesis leading to the neo-system is depicted in Figure 2.

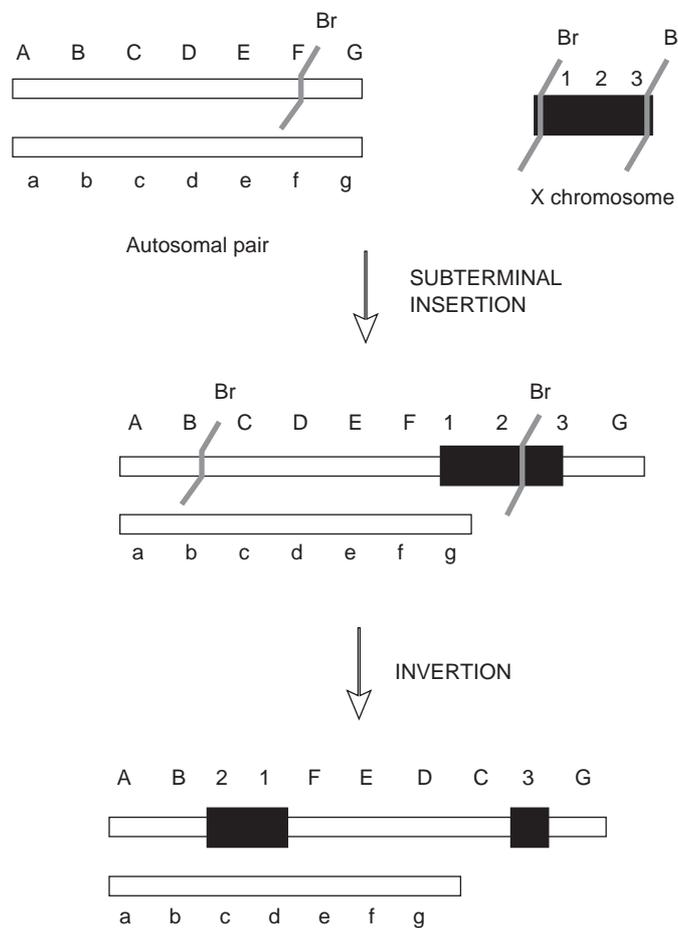


Figure 2. Origin of the neo-XY system in *Dysdercus albofasciatus* Berg 1978. Br = break.

Initially, the ancestral X chromosome was inserted in an autosome in a subterminal position. Afterwards, a large inversion involving most of the neo-X took place, including the ancestral X and originating two X segments of different sizes (Figure 2).

The sex bivalent, and, less frequently, an autosomal bivalent, were observed as univalents. Although pachytene bivalents are too entangled to be analyzed, sex chromosomes appeared connected by a thin thread of chromatin in some cells at early diakinesis, suggesting a desynaptic origin of the univalents. The meiotic behavior of the sex and autosomal univalents, and that of B chromosomes of *D. albofasciatus* is similar to that previously described in *Acanonicus hahni* (Coreidae) and *Largus rufipennis* (Largidae), i.e. equational division at anaphase I, touch-and-go pairing at metaphase II, and reductional second division (Papeschi & Mola 1990, Mola & Papeschi 1993).

Sex chromosomes in Heteroptera, both in simple and multiple systems, divide postreductionally. This fact, together with the constant behavioral pattern observed of desynaptic univalents and B chromosome, suggest the existence of an accurate double regulation of chromosome behavior: one controlling bivalents, and another, the univalents.

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