

Cytogenetic and Nucleolar Meiotic Cycle Analyses in *Dysdercus imitator* Blöte, 1931 (Pyrrhocoridae, Heteroptera) from Argentina

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So far, only seven and five species of *Dysdercus* from the Old and New Worlds, respectively, have been cytogenetically analysed. They all have holokinetic chromosomes and a pre-reductional type of meiosis. In the present study the chromosome complement, male meiosis and nucleolar meiotic cycle of *Dysdercus imitator* were analysed. During male meiosis several cytogenetic features are remarkable, namely the presence of a long diffuse stage after pachytene, the finding of one or two ring bivalents per cell in almost all specimens, and the presence of several prenucleolar bodies lasting up to telophase II. The origin and function of these prenucleolar bodies could be related to a particular physiological cycle of the meiocytes.

Key words: Heteroptera, Pyrrhocoridae, *Dysdercus*, holokinetic chromosomes, karyotype, nucleolar meiotic cycle.

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Dysdercus Guérin Méneville, 1831 is the only genus of the about 30 pyrrhocorid genera known from the Old World, which is also present in the New World, mostly in the tropics. Several species of the genus are serious pests of cotton and some other crops in tropical and subtropical areas. It has been suggested that the genus originated in the Old World, and that the American species were derived from immigrants, most probably from the Ethiopian Region (VAN DOESBURG 1968).

So far, from the 12 cytogenetically analysed species, seven belong to the Old World while the remaining five are American species (UESHIMA 1979; MANNA & DEB-MALLICK 1981; MANNA 1984; KUZNETSOVA 1988; BRESSA *et al.* 1999). The Old World species share a diploid chromosome number of 16/18 ($2n = 14 + X_1X_2/0/14 + X_1X_1X_2X_2$, male/female) while the American ones are more heterogeneous, comprising: *Dysdercus albofasciatus* with $2n = 12 = 10 + \text{neo-XY}/10 + \text{neoXneoX}$;

D. chaquensis and *D. ruficollis* sharing $2n = 13/14 = 12 + X_0/12 + XX$; *D. honestus* presenting $2n = 15/16 = 14 + X_0/14 + XX$, and finally *D. peruvianus* with $2n = 16/18 = 14 + X_1X_2/0/14 + X_1X_1X_2X_2$ (MANNA & DEB-MALLICK 1981; KUZNETSOVA 1988; BRESSA *et al.* 1999).

Up till now, only six species of *Dysdercus* have been recorded from Argentina (VAN DOESBURG 1968). These are *D. albofasciatus* Berg, 1878, *D. chaquensis* Freiberg, 1948, *D. immarginatus* Blöte, 1931, *D. peruvianus* Guérin Méneville, 1831, *D. ruficollis* (Linnaeus), 1764 and *D. wilhelminae* Doesburg, 1968. The specimens of *D. imitator* here analysed were caught at Iguazú National Park (Misiones Province) being the first record of this species in the entomofauna of Argentina.

As is the rule in the Heteroptera, all the *Dysdercus* species have holokinetic chromosomes and a pre-reductional type of meiosis, i.e. autosomal bi-

valents divide pre-reductionally while the sex chromosomes segregate post-reductionally (UESHIMA 1979). During meiosis after pachytene, the nucleus enters into a diffuse stage whose duration and characteristics depend on the species. Furthermore, the second meiotic division generally follows the first one without an intervening interkinetic stage (UESHIMA 1979). In most heteropteran species, a single nucleolus can be detected from early meiotic prophase up to the diffuse stage, which disassembles during diplotene or at the latest, at diakinesis. However, in a few species of Coreidae and Plataspidae the presence of nucleoli up to metaphase II and even anaphase II have been reported (YOSIDA 1947; FOSSEY & LIEBENBERG 1995).

In the present report not only the karyotype and the meiotic behaviour of *Dysdercus imitator* but also the nucleolar meiotic cycle are analysed.

Material and Methods

Specimens and localities

The material included in the present work comprises 16 adult males of *Dysdercus imitator* Blöte, obtained from the Iguazú National Park, Misiones Province (Argentina). Five specimens were not included in the meiotic analysis since only spermatids were found in the testes (Table 1).

Table 1

Mean chiasma frequency at diakinesis-metaphase I in specimens of *Dysdercus imitator* from Iguazú National Park (Misiones Province)

Individual	Code	Number of cells	Mean chiasma frequency
1	990493	21	6.52
2	990494	15	7.86
3	990495	0	—*
4	990503	17	7.50
5	990561	61	8.62
6	990562	29	8.72
7	990563	1	—*
8	990564	1	—*
9	990565	10	6.10
10	990566	5	8.20
11	990567	14	7.36
12	990568	31	7.71
13	990570	6	7.66
14	020102	79	6.51
15	020103	0	—*
16	020104	0	—*

*only spermatids were found in the testes

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 70% ethanol at 4°C. Slides were made by the squash technique in 2% iron acetic haematoxylin following conventional procedures.

When required, some slides were made by disrupting and squashing a piece of gonad in 45% acetic acid; the coverslip was then removed by the dry-ice method, and slides were air-dried. Afterwards, Feulgen staining followed by an Ag-staining technique were used as reported elsewhere (PAPESCHI 1988, 1995).

Analysis of the slides was performed with an Olympus BX 50 fluorescence photomicroscope. Cellular images were acquired with a Leica IM50 image manager (Imagic Bildverarbeitung, AG) based on an integrated high-sensitivity monochrome charge-couple device (CCD) camera and the automated image CarioFISH 1.2 software.

Results

Chromosome complement and meiotic behaviour

Dysdercus imitator possesses $2n = 13/14 = 12+X0/12+XX$ (male/female, respectively) and $n = 6+X0$ (male). From early meiotic prophase, the X chromosome is positively heteropycnotic and lies separate from the autosomal chromatin at the periphery of the nucleus (Fig. 1a). Chromosome pairing takes place at synizesis, and at pachytene both the X univalent and a conspicuous nucleolus are clearly distinguished (Fig. 1b). At the diffuse stage, the cell becomes remarkably large and the nucleus acquires an interphase-like appearance. Autosomal bivalents decondense completely, whereas the X remains condensed (Fig. 1c). At diplotene and diakinesis, autosomal bivalents recondense, showing one or two terminal chiasmata (Fig. 1d). Cells with one up to five ring bivalents are observed (Table 1) (Fig. 1d, f, g). At metaphase I, the autosomal bivalents arrange in a circle with the X chromosome lying either at its centre or outside it (Fig. 1e, f). At this stage, three larger, two medium sized and one smaller autosomal bivalents are distinguished, the X being the smallest chromosome of the complement (Fig. 1e). At anaphase I, the autosomes segregate reductionally while the sex chromosome does so equatorially and synchronously with the autosomes (Figs 1h, j; 2a, b). The second meiotic division follows without an interkinetic stage (Figs 1j; 2c). At metaphase II, chromosomes arrange as described for

Fig. 1. *Dysdercus imitator* Blöte, 1931 (Haematoxylin staining). (a) Leptotene; (b) Pachytene; (c) Diffuse stage; (d) Early Diakinesis; (e, f) Metaphase I; (g) Ring Bivalents at metaphase I; (h) Anaphase I; (i) Telophase I; (j) Prometaphase II. N= nucleolus; arrows point at the X chromosome; empty arrows indicate ring bivalents; asterisks show prenucleolar bodies. Photographs have a low magnification in order to show the large increase of cell size after pachytene. Bar = 10 μ m.

Fig. 2. *Dysdercus imitator* Blöte, 1931 (Haematoxylin staining). Higher magnification of Figure 1 h-j. Detail of prenucleolar bodies. (a) Anaphase I; (b) Telophase I; (c) Prometaphase II. Arrows point the X chromosome; asterisks show prenucleolar bodies. Bar = 10 μm .

metaphase I, and at anaphase II the X chromosome segregates synchronously with the autosomes.

Chiasma frequency

Autosomal bivalents often present one terminal chiasma, but up to five ring bivalents are observed (Fig. 1 d, f, g). Mean chiasma frequency in cells at diakinesis-metaphase I varies from 6.10 to 8.72

(specimens 9 and 6, respectively, Table 1). Figure 3 shows the distribution of the relative frequency of chiasmata resulting from cells at diakinesis-metaphase I, which corresponds to 11 specimens included in this study. The relative frequency was calculated as the number of cells with a particular number of chiasmata (range 5-11 chiasmata) divided the total number of cells analysed ($n=290$). This figure evinces that though 6 is the most fre-

Fig. 3. Distribution of the relative frequency of chiasmata among all studied cells in diakinesis-metaphase I. The relative frequency (Y axis) is plotted against the number of chiasmata observed in all cells at diakinesis-metaphase I (X axis).

quent number of chiasmata per cell, 70% of the cells possess a number of chiasmata within the range 7-10. Thus, the median value of the whole cellular population narrowed down to 7 chiasmata per cell (Fig. 3).

Nucleolar cycle

After conventional staining, several extra heteropycnotic positive elements were detected, which varied not only in size but also in number. To determine whether these extra elements were supernumerary chromosomes or prenucleolar bod-

ies, Feulgen staining followed by an Ag-staining technique were performed consecutively onto the same cells. The comparison of the cells after Feulgen staining and Ag-staining allowed us to discard the possibility of such elements being B chromosomes and, on the other hand, to confirm their nucleolar nature. Hence, the methodology rendered the analysis of the nucleolar cycle during meiosis feasible. From leptotene up to the diffuse stage, a conspicuous nucleolus is observed immersed within the autosomal chromatin (Fig. 4a, b). Nevertheless, two nucleoli of different size are seldom observed in some cells. Overall, from diplotene onwards the presence of two nucleoli of different

Fig. 4. *Dysdercus imitator* Blöte, 1931 (silver staining). (a) Leptotene; (b) Diffuse stage; (c) Metaphase I; (d) Metaphase II; (e) Telóphase II; (f) Telophase II pole. N= nucleolus; arrows point to the X chromosome; asterisks show prenucleolar bodies. Bar = 10 μ m.

size is detected in most of the cells. The larger nucleolus is associated to an autosomal bivalent whereas the smaller one has no particular location. As meiosis proceeds, the two nucleoli become no longer detectable being replaced by up to four prenucleolar bodies of different size located randomly in the cytoplasm (Fig. 4c-f). At metaphase I, the prenucleolar bodies are generally found among the chromosomes at the equatorial plate (Fig. 4c). At metaphase II as well as at anaphase II, they lie near the poles (Fig. 4d). At telophase II, the X chromosome is generally observed decondensing lately, and a low number of larger prenucleolar bodies than those present in previous meiotic stages are observed (Fig. 4e, f).

Discussion

Our results in *Dysdercus imitator* reveal that the species possesses a diploid chromosome number of 13/14 (male/female) and an X0/XX sex chromosome determining system. Furthermore, throughout meiosis the presence of several prenucleolar bodies has been revealed as a peculiar meiotic feature of the species.

The seven species of *Dysdercus* cytogenetically described so far from the Old World share a common diploid chromosome number of 16/18 (male/female) and an $X_1X_20/X_1X_1X_2X_2$ sex chromosome determining system (UESHIMA 1979; MANNA & DEB-MALLICK 1981). The six neotropical species are much more distinct not only in the male diploid chromosome number, i.e. from 12 to 16, but also in the sex chromosome determining system (X0, X_1X_20 and neo-XY, males) (UESHIMA 1979; MOLA & PAPESCHI 1997; BRESSA *et al.* 1999).

Considering $2n = 15 = 14 + X0$ (male) as the ancestral chromosome complement from *Dysdercus*, the multiple sex chromosome determining system should have originated through fragmentation of the original X chromosome giving rise to a diploid chromosome number $2n = 16 = 14 + X_1X_20$. This situation is found in *Dysdercus honestus* (MENDES 1947, 1949; PIZA 1947a, 1951) and *D. peruvianus* (PIZA 1947b, 1951; MENDES 1949), respectively. On the other hand, an autosomal fusion between two non-homologous chromosomes could have led to a reduction in the diploid number ($2n = 13 = 12 + X0$), which has been reported in *D. chaquensis* (MOLA & PAPESCHI 1997) and *D. ruficollis* (PIZA 1947b) as well as in *D. imitator* according to the present study. Finally, a later fusion between the original X chromosome and an autosome took place during karyotype evolution, originating a neo-XY sex chromosome determining system. Recently a situation like this has been described in *D.*

albofasciatus, which possesses the lowest diploid chromosome number of the genus reported so far ($2n = 12 = 10 + \text{neo-XY}$) (BRESSA *et al.* 1999).

It has been generally accepted that most Heteroptera possess, as a rule, only one chiasma per bivalent (UESHIMA 1979; MANNA 1984). However, the present observations in *Dysdercus imitator* together with previous findings in other heteropteran species indicate that the frequency of ring-shaped bivalents could be much higher than originally suggested (MOLA & PAPESCHI 1993; BRESSA *et al.* 1998, 1999, 2001; REBAGLIATI *et al.* 2001). The present results in *D. imitator* demonstrate that the mean chiasma frequency in cells at diakinesis-metaphase I is remarkably higher than expected, according to the original suggestions. This analysis clearly reveals that 72% of the specimens of the population are expected to show a mean chiasma frequency between 7.00 and 8.00. In other words, it stresses the presence of at least one or two ring bivalents per cell.

Another notorious feature of meiosis in *D. imitator* is the noticeable increase of cell size during the diffuse stage adopting the well-known interphase-like appearance (KLÁŠTERSKÁ 1977), and that such a large size is not limited to this stage but also persists along the following meiotic stages. Although the presence of the diffuse stage is characteristic for most heteropteran species, in *D. imitator* it seems to acquire a remarkable importance. It has been suggested that the decondensation of the chromosomes and the appearance of nucleoli in the cytoplasm during this stage are events related to intense transcriptional activity (KLÁŠTERSKÁ 1977). In most arthropod species, the nucleoli disassemble at diplotene or diakinesis being the Ag-NORs no longer detectable from metaphase I up to telophase I. Afterwards, the Ag-NORs reappear in early spermatids indicating a recovery of ribosomal RNA transcriptional function, and finally they disappear in late spermatids. However, some exceptions to this general behaviour have been reported. In *Asellus aquaticus* (Isopoda) NORs were visualized by an Ag-staining during the entire process of spermatogenesis and therefore were active in all of its stages (DI CASTRO *et al.* 1983). In *Callicrania seoanei* (Orthoptera), not only the NORs were revealed during both meiotic divisions but also small nucleoli and prenucleolar bodies, spread between the chromosomes, were observed from interkinesis to prometaphase II (SANTOS *et al.* 1987). In *Triatoma brasiliensis* and *T. sordida* (Heteroptera, Reduviidae), the Ag-NORs have been discerned up to metaphase I (GARCÍA TAVARES & VILELA DE AZEREDO-OLIVEIRA 1997). Finally, in *Carlisis wahlbergi* (Heteroptera, Coreidae), the presence of semi-persistent nucleoli was identifiable up to metaphase II (FOSSEY & LIEBENBERG

1995), while in *Acanthocoris sordidus* (Heteroptera, Coreidae) and *Coptosoma punctissimum* (Heteroptera, Plataspidae) the nucleolus was detected in the metaphase plates of the primary and secondary spermatocytes (YOSIDA 1947).

It is known that nucleolus size is related to the biosynthetic activity of the cell and therefore, the size and number of nucleoli or prenucleolar bodies depend on functional characteristics of the cell and could reflect, then, metabolic and functional differences (GARCÍA TAVARES & VILELA DE AZEREDO-OLIVEIRA 1997). The persistence of the nucleoli and prenucleolar bodies observed throughout meiotic stages in *D. imitator* could then be related to intense transcriptional activity in order to produce rRNA required for spermiogenesis. Moreover, the possibility that a high rate of rRNA transcription could be related to an abnormally high rate of synthesis due to a still unknown reason cannot be ruled out. Further studies are required to explain this peculiar nucleolar behaviour in the meiosis of *D. imitator*.

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