Meiotic studies in *Dysdercus* Guérin Méneville, 1831 (Heteroptera: Pyrrhocoridae). II. Evidence on variations of the diffuse stage between wild and laboratory-inbred populations of *Dysdercus chaquensis* Freiberg, 1948

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Dysdercus Guérin Méneville, 1831 comprises insect species that are often serious pests of cotton both in the Old and New World, representing the only taxon from Pyrrhocoridae in the Neotropical Region. The genus is cytologically characterized by possession of holokinetic chromosomes and a prereductional type of meiosis. So far, only seven species from the Old World and five species from the Neotropical Region have been cytogenetically described. In the present report we compare the male meiosis from both natural and inbred populations of *Dysdercus chaquensis* Freiberg, 1948. Our results demonstrated that even though both populations share the same diploid chromosome number, the presence of a diffuse stage was found to be committed to the wild population of the species. Furthermore, the diffuse stage was found in a high frequency in all analysed wild specimens, indicating the long duration of this period among the regular meiosis of *D. chaquensis*. Taking into account that the diffuse stage is connected with an intense and long period of cellular growth, and with an important transcriptional activity, the absence of this stage in all the inbred specimens of *D. chaquensis* could be related with the lack of unfavourable physiological conditions due to the environmental uniformity along seven years of inbreeding.

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The genus *Dysdercus* Guérin Méneville, 1831 comprises medium-sized to large (8–30 mm) insect species that are often serious pests of cotton in the Old World as well as in the New World, representing in the latter the only taxon of the family Pyrrhocoridae. Most species with known biology feed chiefly on seeds and fruits, particularly of the Malvales (SCHUH and SLATER 1995). They are currently referred to as "cotton stainers" since, after piercing the cotton bolls, they introduce microorganisms that cause boll rot or discoloration of the lint. Years ago, VAN DOESBURG (1968), based exclusively on morphological features of the species, suggested a possible origin of the New World forms of *Dysdercus*. Accordingly, the New World species of *Dysdercus* should have been derived from immigrant specimens of the Old World species, probably originating from the Ethiopian Region (VAN DOESBURG 1968).

Despite the wide species diversity of the Pyrrhocoridae, available cytogenetic data on the genus *Dysdercus* are scarce. As is the rule in the order Heteroptera, the genus is cytologically characterized by possession of holokinetic chromosomes and a prereductional type of meiosis. At anaphase I the autosomal bivalents divide reductionally while the sex chromosomes divide equationally and are achiasmatic (UESHIMA 1979). So far, only 11 *Dysdercus* species, comprising six species from the Old World and five species from the Neotropical Region, have been cytogenetically described. *Dysdercus cingulatus*, *D. evanescens*, *D. fasciatus*, *D. intermedius*, *D. koenigii*, *D. superstitiosus*, and *Dysdercus* sp, all six species reported from the Old World, are characterized by sharing similar chromosomal complement of 14 autosomes with an $X_1X_20/X_1X_1X_2X_2$ (male/female) sex chromosome system (UESHIMA 1979; MANNA and DEB-MALLICK 1981; KUZNETSOVA 1988). However, such chromosomal homogeneity is not observed when cytogenetic data from New World species of *Dysdercus* are analysed. Studies performed in the five New World species revealed variations not only in the diploid chromosome number but also in the sex chromosome system. Among these species, *D. chaquensis* (MOLA and PAPESCHI 1997) and *D. ruficollis* (PIZA 1947) share a male diploid complement of 13 elements (12 + X0) while 15 chromosomes (14 + X0) characterize *D. honestus* (PIZA 1947). *Dysdercus peruvianus* is characterized by possessing the highest chromosome number of 16 elements in its complement ($14 + X_1X_20$) (PIZA 1947, 1951; MENDES 1949).

Finally, we have recently described meiotic behaviour of chromosomes in *D. albofasciatus* (12 + neo-XY) (BRESSA et al. 1999).

Having the possibility to examine specimens of a natural population from northern Argentina, in the present report we compare the cytogenetic results of both types of populations, and the male meiosis is described in detail. Our results demonstrated that even though both populations share the same chromosomal features and diploid number, the presence of a diffuse stage, not previously reported in the male meiosis, was found to be committed to the wild population of the species.

MATERIAL AND METHODS

Specimens and localities

The material included in the present study comprises 16 adult males obtained from Villa Angela (Chaco Province, Argentina) captured on cotton crop fields. Simultaneously, 11 adult males from the Laboratory of Parasitology, Argentinean Museum of Natural History "Bernardino Rivadavia" (Buenos Aires, Argentina) from a laboratory population with at least 7 years of inbreeding were included. This inbred colony was established from 25 to 30 different pairs of parents kept under a 70 % humidity atmosphere at 30 ± 1°C, with a 12 h light photoperiod, natural diet based on cotton seeds and randomly mating system. Although, two wild specimens (individuals 8 and 12) and four inbred specimens (individuals 21, 23–25) were not included in the chromosome analysis since only spermatids were found in the testes, the former were only suitable for determining presence/absence of diffuse stage (Table 1).

Cytogenetic analysis

Immediately after capturing, all specimens were fixed in methanol–acetic acid (3:1), and maintained at 4°C until dissection. Afterwards, gonads were dissected out and kept in 70 % ethanol (4°C). Slides were

Table 1. *Mean chiasma frequency, percentage of cells with univalents at diakinesis-metaphase I, and percentage of cells at the diffuse stage in the wild and inbred populations of D. chaquensis*

Population	Individual	Code	Number of cells	Mean chiasma frequency	% of cells with univalents	% of cells at the diffuse stage[a]
Wild						
	1	990401	9	6.11	11.11	25.00
	2	990402	21	6.62	0.00	37.13
	3	990403	11	6.00	18.18	30.00
	4	990404	14	6.43	0.00	65.07
	5	990405	27	6.33	0.00	48.17
	6	990504	8	6.25	0.00	10.36
	7	990506	10	6.10	20.00	16.00
	8	990549	nmf	nmf	nd	73.73
	9	990550	60	6.03	5.00	48.93
	10	990551	12	6.16	8.33	39.39
	11	990553	11	6.63	0.00	40.91
	12	990571	nmf	nmf	nd	40.21
	13	990574	14	6.93	0.00	21.63
	14	990603	16	6.50	6.25	39.91
	15	990604	6	6.66	0.00	38.66
	16	990605	6	6.66	nd	29.12
Inbred						
	17	98022	18	6.11	0.00	–
	18	98023	72	6.36	2.78	–
	19	98024	14	6.28	0.00	–
	20	98025	14	6.14	0.00	–
	21	98026	nmf	nmf	nd	–
	22	98027	38	6.34	2.63	–
	23	98028	nmf	nmf	nd	–
	24	98029	nmf	nmf	nd	–
	25	98030	nmf	nmf	nd	–
	26	98031	22	6.54	0.00	–
	27	820696	18	6.16	0.00	–

[a] With regard to all meiotic figures found in testes.
nmf, no meiotic figures suitable for cytogenetic analysis (excepting diffuse stage in those natural specimens).
nd, not determined due to only spermatids were found in testes.

prepared by the squash methodology in 2 % iron acetic 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). Slides were examined with a fluorescence microscope Olympus BX50 with appropriate combination of filters. Cellular images were acquired with the Leica IM50 Image Manager (Imagic Bildverarbeitung AG), based on an integrated high-sensivity monochrome charge-coupled device (CCD) camera and then processed with the CarioFISH 1.2 software.

RESULTS

All specimens of *D. chaquensis*, regardless of the analysed population they belong to, possess a male diploid chromosome number of 13 (12 + X0) with two large, three medium-sized, and one small pairs of autosomes, and the X chromosome similar in size to the smallest pair of autosomes. At leptotene-zygotene the X chromosome is positively heteropycnotic and lies at the periphery of the nucleus (Fig. 1A, 2B). At zygotene and pachytene, chromosomes are too entangled to be clearly individualised. Pachytene is followed by a prominent diffuse stage observed in all wild specimens but not in the inbred individuals. During this stage autosomal bivalents do not decondense completely, with several heterochromatic regions being observed while the X chromosome remains positively heteropycnotic and associated with a conspicuous nucleolus. Furthermore, cell size increases and the nucleus resembles an interphase-like state (Fig. 1B, 2D). The percentage of cells at the diffuse stage among individuals from the wild population reaches values as high as 38.50 % (range 10.36 %–73.73 %, specimens 6 and 8, respectively; Table 1) out of the total number of divisional meiotic figures. From diplotene onwards, six autosomal bivalents and the X chromosome are distinguished. At late diakinesis, the X chromosome becomes isopicnotic (Fig. 1C). At metaphase I, the X chromosome lies in the centre of the ring formed by autosomal bivalents (Fig. 1D, E). At anaphase I autosomal bivalents divide reductionally while the X chromosome divides equationally. Second metaphase follows directly after telophase I (Fig. 1F), without a resting stage. At metaphase II the autosomes dispose at the equatorial plane, adopting a ring configuration with the X chromosome located in its centre (Fig. 1G). At anaphase II, the X chromosome migrates precociously towards one pole (Fig. 1H) and decondenses later at telophase II (Fig. 1I).

Autosomal bivalents generally possess one terminal chiasma (Fig. 1D, E), although the largest bivalent and, less frequently, other bivalents may show two chiasmata (27.29 %) (Fig. 1D, E). In addition, cells with two ring bivalents are observed at very low frequencies (3.53 %) (Fig. 1D). Mean chiasma frequency at diakinesis-metaphase I varies from 6.00 to 6.93, and from 6.11 to 6.54, in wild and inbred populations, respectively (Table 1). The confidence intervals of the arithmetic mean of the wild and inbred populations are (6.39 ± 0.14) and (6.28 ± 0.12), respectively (95 % student's distribution). The student's *t*-test revealed no significant difference between the mean values of the mean chiasma frequency of the wild and inbred populations.

Furthermore, bivalents with interstitial chiasmata were observed in wild and inbred specimens, reaching values of 19.65 % and 35.72 %, respectively (Fig. 1C).

DAPI staining gave concordant results for both populations of *D. chaquensis* (Fig. 2A–D). At early prophase I, the X chromosome is clearly distinguished from the autosomes as a DAPI-bright element as well as at the diffuse stage found in the specimens belonging to the natural population (Fig. 2B–D). At diakinesis all chromosomes are stained homogeneously not revealing DAPI bright bands.

Although with different frequencies, cells with one pair of univalents were observed in both populations of *D. chaquensis*. The percentage of cells with univalents was 3.5-fold higher in the wild specimens (5.24 %, range 5.00 %–20.00 %, specimens 9 and 7, respectively; Table 1) than in those bred under laboratory conditions (1.53 %, range 2.63 %-2.78 %, specimens 21 and 18, respectively; Table 1). In most cases, the origin of the univalents was committed to one medium-sized autosomal pair (28.57 % and 100.00 %, for wild and inbred specimens, respectively). Among the 16 wild males of *D. chaquensis* analysed, regular meiosis, i.e. those divisional figures lacking univalent chromosomes, were observed in 7 specimens (43.75 %). On the other hand, a pair of univalents was found in 6 individuals (37.50 %), while in the remaining three specimens (18.75 %), the presence/absence of univalents was not determined due to the reduced number of cells suitable for cytogenetic analysis. Of the 11 males studied belonging to the inbred population, only five had a regular meiosis (45.46 %) while two individuals presented a variable frequency of cells with a pair of univalents (18.18 %). In the remaining four inbred specimens, the presence of univalents was not determined due either to the reduced number of cells analysed or the absence of meiotic activity in testes (36.36 %).

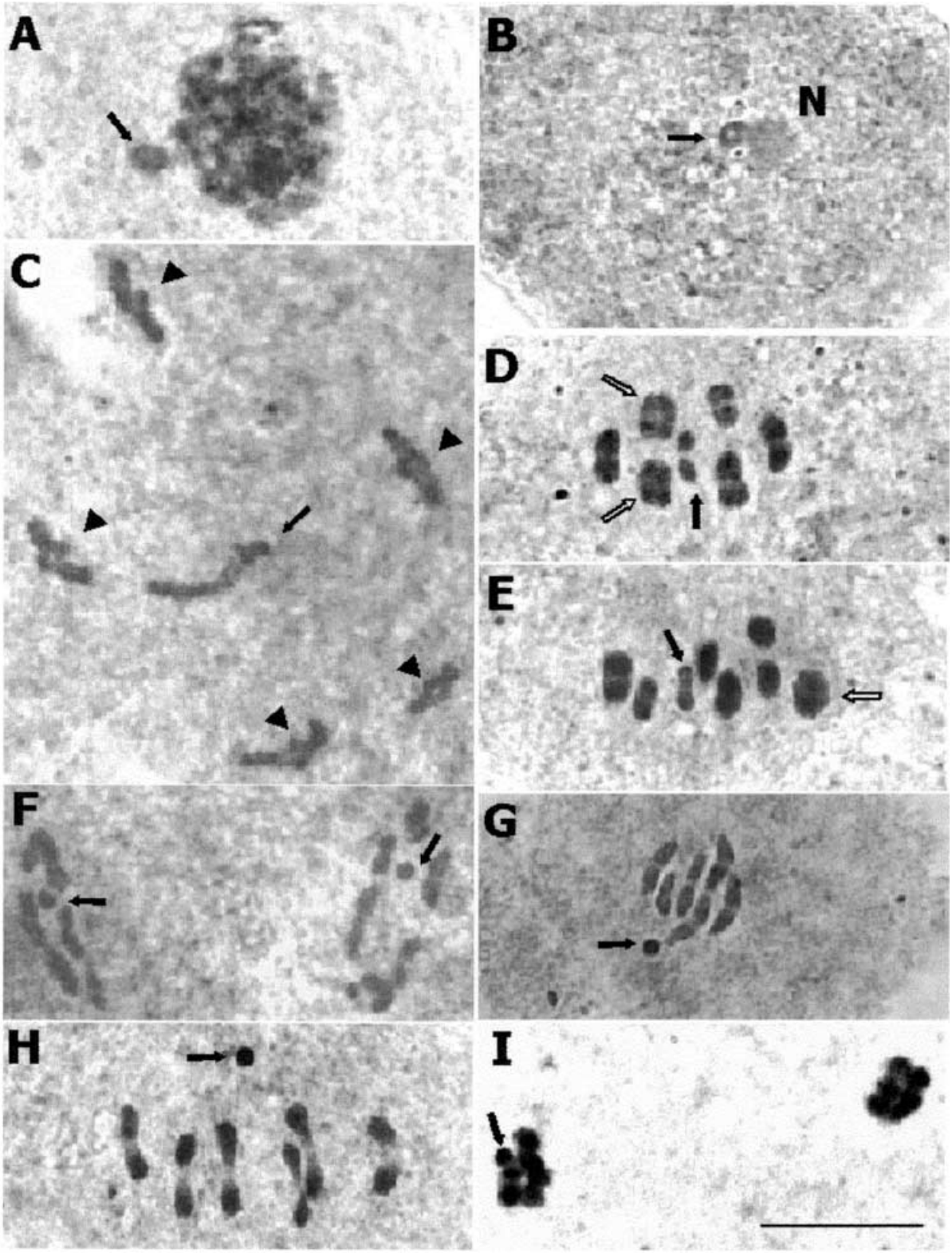

Fig. 1A–I. Wild specimens of *D. chaquensis* Freiberg, 1948. (**A**) Leptotene; (**B**) Diffuse stage; (**C**) Diakinesis with autosomal bivalents showing interstitial chiasmata; (**D**) Metaphase I with the largest autosomal bivalents showing two chiasmata each (polar view); (**E**) Metaphase I with one of the largest bivalents showing two chiasmata (equatorial view); (**F**) Telophase I; (**G**) Metaphase II; (**H**) Anaphase II; (**I**) Telophase II. N = nucleolus. Solid arrows indicate the sex chromosome; empty arrows indicate autosomal bivalents with two chiasmata; solid arrowheads indicate autosomal bivalents with interstitial chiasmata. Bar represents 10 μm.

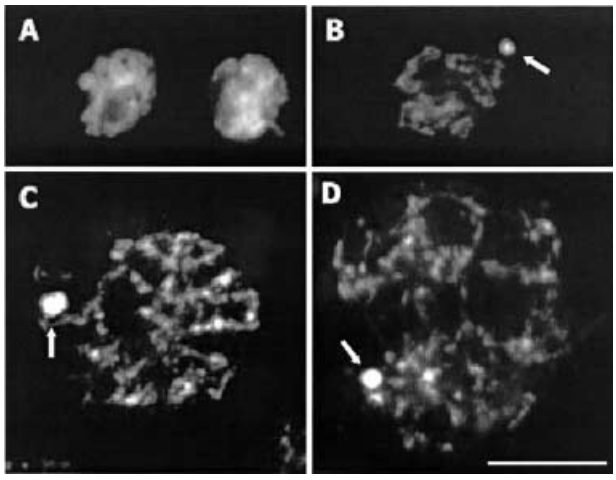

Fig. 2A–D. Wild specimens of *D. chaquensis* Freiberg, 1948. DAPI staining. (**A**) Interphase nuclei; (**B**) Leptotene; (**C**) Pachytene; (**D**) Diffuse stage. Solid arrows indicate the sex chromosome. Bar represents 10 μm.

DISCUSSION

The diploid chromosome number of males of *Dysdercus chaquensis* ($2n = 12 + X0$) was described by MOLA and PAPESCHI (1997) entirely based on specimens with more than three years of inbreeding under laboratory conditions, a colony derived from wild individuals collected in Tolloche (Salta Province, Argentina). The results presented here with specimens belonging to the same colony used in the original description, but with seven years of inbreeding under the same laboratory conditions, are in total agreement with the scarce cytogenetic findings reported by MOLA and PAPESCHI (1997). Moreover, the possibility of analysing wild specimens from another locality from northern Argentina (Villa Angela, Chaco Province) allowed us to reveal the existence of a diffuse stage only present in this population but totally absent in those animal-room specimens included either in the present report or in those employed by MOLA and PAPESCHI (1997) in the original cytogenetic description.

The origin of univalents in a species may result from a variety of causes, both genotypic and environmental, and the precise cause is not always accurately determined. In general, it is difficult to establish whether the univalents observed at metaphase I arise from an asynaptic or desynaptic origin since early prophase I stages, in most organisms, are difficult to be analysed (RILEY and LAW 1965; PAPESCHI and MOLA 1990). In the specimens we studied from both populations of *D. chaquensis*, the asynaptic or desynaptic origin of the univalents cannot be determined due to the characteristics of early meiotic prophase stages of the species. It should be mentioned that the inbred laboratory conditions under which were inbreeding the laboratory specimens were homogeneous along seven years, while the environmental conditions affecting the wild population were most probably heterogeneous but not necessarily detrimental. Thus, an external environmental factor could not be ruled out as a possible inducing cause for enhancing the frequency of univalents in specimens belonging to the wild population.

The pachytene stage is depicted as being followed by diplotene and diakinesis in the general description of different meiotic stages. Moreover, several special structures, e.g. lampbrush chromosomes and dictyotene or diffuse stage, can be mentioned as appearing after pachytene or early diplotene. The latter peculiar meiotic stage is characterized by a diffuse appearance of the chromosomes since the bivalents extend and lose their distinct morphology, a reduced stainability with conventional staining techniques and an interphase-like appearance of the nucleus with a network of chromatin strands (KLÁSTERSKÁ and NATARAJAN 1974; KLÁSTERSKÁ 1976, 1977, 1978). This stage is commonly present in most heteropteran families, including Pyrrhocoridae (UESHIMA 1979).

Another consequence of the diffuse stage is related with the fact that whenever this stage is present, the chromosomes must pass through very profound changes in their organisation between pachytene and diakinesis due to the cyclic decondensation-condensation phenomenon. At late diplotene, the bivalents can already be discerned as conspicuous entities but some remnants of fine chromatin connections between different bivalents can persist, and give them an appearance of sticky chromosomes. Such type of interconnections of bivalents by chromatin strands have been commonly observed both in plant and animal meiosis (MOENS 1964; WHELAN and HORNBY 1969; KLÁSTERSKÁ and NATARAJAN 1974; KLÁSTERSKÁ 1977). Eventhough this type of interbivalent connections is frequently observed among insects (UESHIMA 1979), signs of this morphological feature have not been observed in the *D. chaquensis* specimens we analysed here. A similar situation has been reported to occur in some other insects, including the grasshopper *Stethophyma* (Orthopthera) (KLÁSTERSKÁ 1976) as well as many species of Heteroptera (WILSON 1925). Whether the absence of interchromosomal associations is a common phenomenon for those insects possessing a diffuse stage as a normal step in the first meiotic division or is restricted only to a particular group of species remains unknown.

From the available data, all reports agree in demonstrating that eventhough wide variation exists among organisms in regard to the diffuse stage, at

this stage the nuclei recede to a greater or less degree towards a condition of resting nucleus. This process is related with an intense and long period of cellular growth, being more evident in oocytes than in spermatocytes or pollen mother cells. Furthermore, this stage has been associated with an intense transcriptional activity characterized by the extension or unwinding of the chromosomal thread and the appearance of nucleoli-like bodies in the cytoplasm (MONESI 1967; KLÁSTERSKÁ and NATARAJAN 1974; KLÁSTERSKÁ 1977). In agreement with this observations, our findings demonstrate the presence of a big nucleolus in the cellular cytoplasm of the spermatocytes at this stage, being the X chromosome generally associated with it.

It has been suggested that the timing of reproduction, nymphal development, migration and diapause of *Dysdercus* species depend on the diet, temperature, relative humidity, precipitation, and photoperiod (STADLER et al. 1987). Accordingly, the absence of the diffuse stage in all the inbred specimens of *D. chaquensis* included in the present report and most probably those studied by MOLA and PAPESCHI (1997) should be related with the lack of unfavourable physiological conditions due to the environmental uniformity along seven years of inbreeding. In good agreement with this suggestion, is the high frequency of cells that were found at this stage in all wild specimens we analysed, indicating the long duration of the period among the regular meiosis of *D. chaquensis*. Furthermore, the high preponderance of the diffuse stage in male meiosis supports the idea that this period is mostly committed to an important biosynthetic activity as suggested elsewhere (KLÁSTERSKÁ and NATARAJAN 1974; KLÁSTERSKÁ 1976, 1977, 1978). A comparative analysis performed simultaneously on both natural and laboratory inbred specimens on other heteropteran species known to possess a diffuse stage is required in order to confirm whether the lack of unfavourable physiological conditions induces the absence of such period. The results we present here not only support this assumption but also represent to the best of our knowledge the first cytogenetic evidence of the absence of a particular meiotic stage in a population bred under laboratory conditions. Since such stage was never observed in any of the inbred individuals, a larger series of these latter specimens should be analysed in order to confirm our observation. However, at present there is no possibility of obtaining more males from this population since the inbred population is not any longer available. Finally, the hypothesis of the existence of a polymorphism in the presence/absence of the diffuse stage among the specimens of *D. chaquensis* initially employed for the establishment of the inbred population cannot be ruled out as a plausible explanation for our observations.

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