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Male meiotic behaviour and nucleolus organizer regions in *Camptischium clavipes* (Fabr.) (Coreidae, Heteroptera) analyzed by fluorescent banding and *in situ* hybridization

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Abstract — All heteropteran species possess holokinetic chromosomes and a pre-reductional type of meiosis. Autosomal bivalents segregate reductionally at meiosis I while the sex chromosomes do so equationally; the m chromosome pair is achiasmatic and also divides pre-reductionally. Previous reports support the hypothesis that during meiosis both ends of a chromosome are able to show kinetic activity, and that those chromosome ends of the autosomes that were kinetically active during the first meiotic division are inactive in the second one.
Camptischium clavipes presents a male karyotype (2n= 21= 18+2m+X0) and a male meiosis that are in agreement with most members of the family Coreidae. Different chromosome banding techniques reveal the presence of small C positive heterochromatin bands at telomeric positions, and a DAPI dull/ CMA bright band at the subtelomeric region of the largest autosomal pair. This band corresponds to the nucleolus organizing region as revealed by *in situ* hybridization.
We used the NOR as a cytological marker and our observations give further support to the hypothesis on the alternate kinetic activity of telomeric regions of autosomes at meiosis I and II in Heteroptera. Besides, the location of the NOR in *C. clavipes* is compared with previous reports in other heteropteran species.

Key words: FISH, fluorescent banding, Heteroptera, holokinetic chromosomes, NOR.

INTRODUCTION

Bugs in the family Coreidae are primarily phytophagous and some of them are of considerable economic importance. The family includes many and diverse forms, particularly in the tropics and subtropics (SCHUH and SLATER 1995; SCHAEFER and PANIZZI 2000). As it is characteristic of Heteroptera the Coreidae possess holokinetic chromosomes, i.e. chromosomes that lack primary constrictions and consequently have non-localized centromeres, and most species of this family present a pair of m chromosomes and an X0/XX sex chromosome determining system. The diploid number of the family ranges from 13 (10A + 2m + X) to 28 (24A + 2m + X_1X_2) with a mode in 21 (18A + 2m + X) (UESHIMA 1979; SANDS 1982; MANNA 1984; COLOMBO and BIDAU 1985; DEY and WANGDI 1988; SATAPATHY and PATNAIK 1989).

Several plant and animal species possess holokinetic chromosomes. During mitosis kinetic activity is distributed along the entire length of the chromosomes and at anaphase sister chromatids segregate parallel to each other and perpendicular to the spindle axis. On the contrary during meiosis, they behave differently depending not only on the group of organisms in which they are present but also on the chromosome type (autosomes, sex chromosomes, m chromosomes) (WHITE 1973; JOHN 1990). Particularly in Heteroptera, kinetic activity during meiosis is restricted to the telomeric regions (telokinetic chromosomes) (MOTZKO and RUTHMANN 1984). According to previous reports both chromosome ends can show kinetic activity during meiosis in such a way that the telomeric regions that were inactive during the first meiotic division become active during the second one (CAMACHO *et al.* 1985; NOKKALA 1985; PÉREZ *et al.* 1997).

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As a rule, autosomal bivalents are chiasmatic while the sex chromosomes and m chromosomes are achiasmatic (UESHIMA 1979; GONZÁLEZ-GARCÍA *et al.* 1996; SUJA *et al.* 2000). When autosomal bivalents present only one chiasma, they orientate at metaphase I with their long axes parallel to the polar axis; they segregate reductionally during meiosis I and equationally during meiosis II. On the other hand, bivalents with two chiasmata orientate equatorially and two different behaviours have been described: a) one chiasma releases first and an axial orientation is finally achieved; or b) alternative sites of kinetic activity next to secondary constrictions become functional and no telokinetic activity is observed (MOLA and PAPESCHI 1993; PAPESCHI *et al.* 2003).

Both in simple and multiple sex chromosome determining systems sex chromosomes behave as univalents in male meiosis; they divide equationally at anaphase I and at the second meiotic division they segregate reductionally, with a few exceptions (UESHIMA 1979; GROZEVA and NOKKALA 2001). Finally, the m chromosomes are a pair of minute elements present in some heteropteran families, which behave differently from both autosomes and sex chromosomes during meiosis; they are achiasmatic and associate at first meiotic division forming a pseudo-bivalent; they segregate reductionally at anaphase I and divide equationally at anaphase II (UESHIMA 1979).

The number and location of nucleolus organizing regions have been determined in a few species of Heteroptera by different cytogenetic techniques and different results have been reported. In all of them a single nucleolus organizing region has been observed, but with different locations: at subterminal or medial position, in an autosomal pair or on the sex chromosomes (CAMACHO *et al.* 1985; FOSSEY and LIEBENBERG 1995; GONZÁLEZ-GARCÍA *et al.* 1996; PAPESCHI and BRESSA 2002; PAPESCHI *et al.* 2003; REBAGLIATI *et al.* 2003).

In this work the male chromosome complement and meiotic behaviour of *Camptischium clavipes* (Fabr.) is described and analyzed by C, DAPI and CMA banding; we have also determined the number and location of the nucleolus organizing regions (NORs) by fluorescent *in situ* hybridization. The identification of only one NOR located at the subterminal region of the largest autosomal pair allowed us to use it as a cytological marker and describe the behaviour of this chromosome pair during both meiotic divisions.

MATERIALS AND METHODS

We analyzed 13 males of *Camptischium clavipes* (Fabr.) from Llavallol (Buenos Aires province), Argentina. All the individuals were fixed in the field in 3:1 ethanol: glacial acetic acid and the gonads were dissected under a binocular stereoscopic microscope. The testes were kept in 3:1 at 4°C.

Slides were prepared by the squash-technique in acetic haematoxylin. Some other slides were performed in 45% acetic acid, and the cover slip was removed by the dry ice method. C- and fluorescent banding (DAPI and CMA fluorochromes) were then applied to these slides to reveal different kinds of heterochromatin constitution. Afterwards, some of those slides were hybridized by fluorescent *in situ* hybridization with a rDNA probe according to Papeschi *et al*. (PAPESCHI *et al.* 2003).

Cells at metaphase II were scanned and the chromosomes were measured using the computer application MicroMeasure version 3.3 (REEVES and TEAR 2000).

RESULTS

Karyotype and meiotic behavior - The chromosome complement of *Camptischium clavipes* is 2n= 21= 18+2m+X (males) (Fig. 1a). The complement comprises two large pairs of autosomes, seven smaller pairs of decreasing size, a sex chromosome X, and a pair of m chromosomes, which are the smallest of the complement (Fig. 2).

The X chromosome is positively heteropycnotic and easily identified during early meiotic prophase. After pachytene at the diffuse stage, the autosomal bivalents decondense totally, while the sex univalent remains condensed and positively heteropycnotic. The m pair cannot yet be detected at this stage (Fig. 1b). At diplotene the autosomal bivalents usually display one chiasma per bivalent. The X chromosome remains highly condensed, and the m chromosomes are now distinguishable as two isopycnotic bodies. In some cells a single nucleolus is observed at this stage, generally attached to the largest autosomal bivalent (Fig. 1c). At diakinesis the m chromosomes lie still apart and are slightly negatively heteropycnotic, while the sex univalent is now isopycnotic (Fig. 1d); at this stage the largest bivalent shows one chiasma which can be interstitially (56 cells) or terminally (65 cells) located. At prometaphase I, the m chromosomes are side-by-side, and they remain negatively heteropycnotic (Fig. 1e). During metaphase I all the bivalents and the sex chromosome arrange in a ring, at the center of which lies the m pseudo-bivalent (Fig. 1f). The anaphase I is characterized by the early migration of the m chromosomes, while the rest of the chromosomes do so synchronically (Fig. 1g). At this stage the autosomes divide reductionally, while the X chromosome divides equationally. At telophase I the m chromosomes are found inside the autosomal group, and the sex chromosome is found at its border (Fig. 1h). At met-

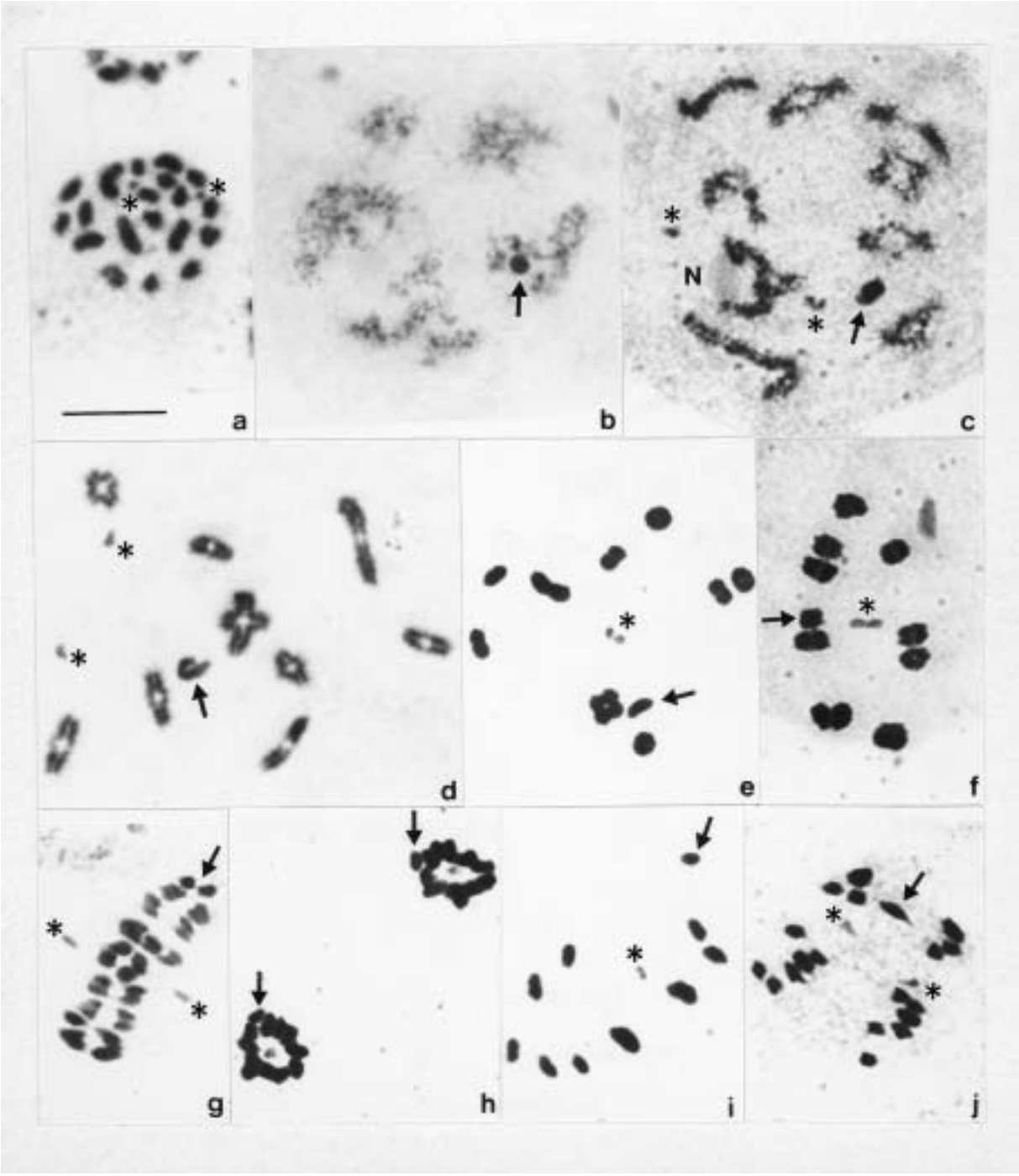

Fig. 1 — Male meiosis of *Camptischium clavipes* (2n= 21= 18+2m+X). a) Spermatogonial metaphase. b) Diffuse stage. c) Diplotene. d-e) Diakinesis. f) Metaphase I. g) Anaphase I. h) Telophase I. i) Metaphase II. j) Anaphase II. N= nucleolus. The arrow points to the X chromosome. *= m chromosomes. Bar= 10 µm.

aphase II the autosomes arrange again in a circle, the m chromosome lying again at its center. The sex chromosome is usually visible a little apart from the autosomal ring (Fig. 1i). At anaphase II the m chromosomes migrate synchronically and the X chromosome can be observed lagging behind the autosomes. (Fig. 1j).

C, DAPI and CMA banding - The C-banding reveals the presence of scarce C positive heterochromatin. Very little C positive bands are seen at the telomeric regions of autosomal bivalents (not shown). All chromosomes stain uniformly with the fluorochromes DAPI and CMA, except for the largest autosomal bivalent and the X chromosome. A DAPI

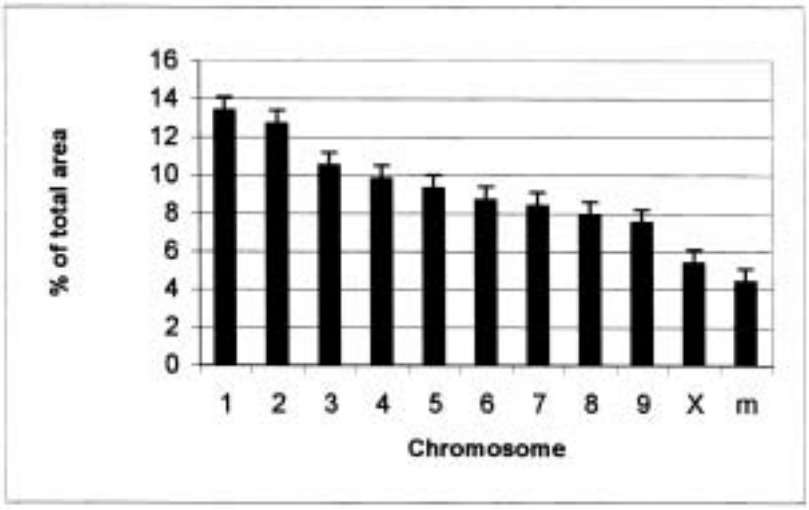

Fig. 2 — Meiotic idiogram of *Camptischium clavipes* performed from cells at metaphase II.

dull /CMA bright band is observed in one of the telomeric regions of the largest autosomal pair (Fig. 3a- d). At metaphase I either this telomeric region (Fig. 3c) (29 cells) or the opposite one (Fig. 3d) (16 cells) is oriented to the poles. During early meiotic prophase up to diakinesis the X univalent is DAPI bright and CMA bright. From this stage onwards the sex chromosome stains as bright as the autosomes. At metaphase II either the CMA bright telomeric region (5 cells) or the opposite one (5 cells) show kinetic activity.

Fluorescent in situ hybridization (FISH) - The rDNA probe hybridizes in one of the telomeric regions of the largest autosomal pair (Fig. 3e- j). Despite the low number of cells at metaphase I (18 cells) and at metaphase II (20 cells) analyzed with FISH we observed that both telomeric regions could be kinetically active at both meiotic divisions. In both metaphase plates the hybridization signals can be seen not only at the telomeric regions that lead migration (Fig. 3f, i, j) (12 cells at metaphase I and 11 cells at metaphase II) but also at the opposite ones (Fig. 3g, h) (6 cells at metaphase I and 9 cells at metaphase II). This bivalent orientates axially at metaphase I and sister chromatids continue associated at the telomeric region leading the anaphasic migration. The second meiotic division follows without interkinesis. After telophase I, the chromosomes arrange at the metaphase plate and sister chromatids begin to separate at the opposite end to the one that was active during anaphase I. The sister chromatids are V-shaped and finally reach an axial orientation. During the second anaphase, the chromosome ends that were inactive at anaphase I acquire kinetic activity.

From the observations performed with the fluorescent banding techniques DAPI and CMA,and the *in situ* hybridization, the NOR telomeric region shows kinetic activity in 65% of the cells at metaphase I (41 out of 63 cells) and in 53.3% of the cells at metaphase II (16 out of 30 cells).

DISCUSSION

The male chromosome complement and meiotic behaviour of *Camptischium clavipes* (Fabr.) is in agreement with the general cytogenetic features of the Coreidae. *C. clavipes* has a diploid number of 21 (male) with a pair of m chromosomes and an X/XX sex chromosome determining system. During meiosis the cell enters in a diffuse stage after pachytene during which the autosomes decondense completely; both metaphase plates present a chromosome arrangement with the autosomes disposed in a circle and the m chromosomes located at its center; the X chromosome forms part of the ring at metaphase I and lies a little apart at metaphase II.

Previous reports on the number and location of the nucleolus organizing regions in Heteroptera are scarce, and the description has generally been inferred from indirect evidences such as the association of specific chromosomes with nucleoli or the presence of secondary constrictions; however, it has already been established that not all secondary constrictions bear ribosomal DNA (Macgregor 1993). An accurate localization of active and/or total number of NORs through silver impregnation techniques or *in situ* hybridization with rDNA probes, respectively, are very few in this group and the results are quite different. Within Pentatomidae a single NOR has been detected at the telomeric region of one autosomal pair in *Edessa meditabunda* (Re-

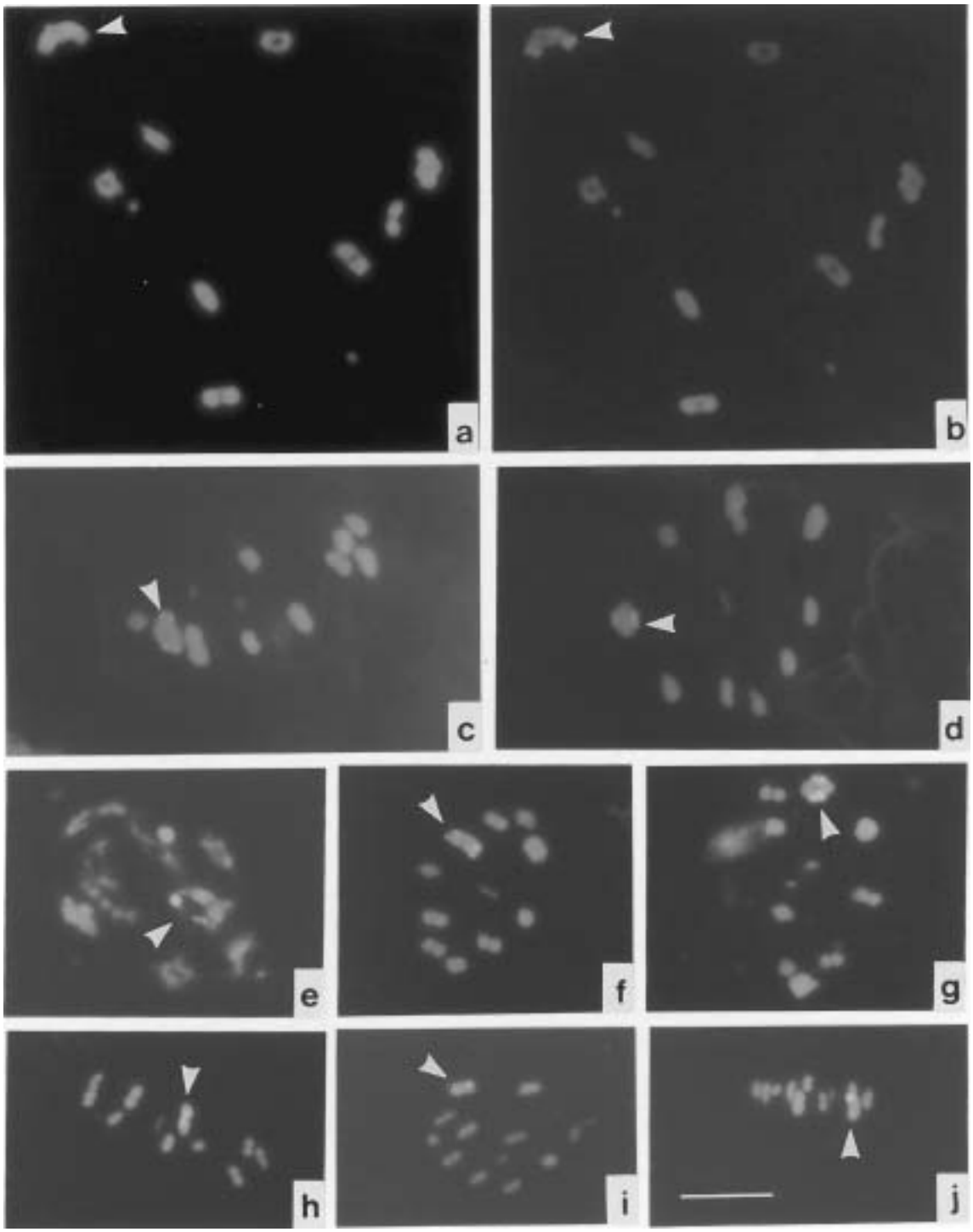

Fig. 3 — DAPI (a), CMA (b-d), and fluorescent *in situ* hybridization with a rDNA probe (e-j). a-b) Diakinesis. c-d) Metaphase I. e) Diplotene. f-g) Metaphase I. h-j) Metaphase II. The rDNA probe hybridizes in the subtelomeric region of the largest autosomal pair (arrowhead). Arrows indicate the DAPI negative/CMA positive signals. Bar= 10 μm.

BAGLIATI *et al.* 2003), at a medial position in one of the largest autosomal pairs in *Nezara viridula* (CAMACHO *et al.* 1985; PAPESCHI *et al.* 2003), and at the telomeric region of the X chromosome in *Graphosoma italicum* (GONZÁLEZ-GARCÍA *et al.* 1996). Within Belostomatidae NORs have been described at the telomeric region of both the X and Y chromosomes in *Belostoma oxyurum* and *B. micantulum*, and at the telomeric region of one autosomal pair in *B. elegans* (PAPESCHI and BRESSA 2002). Finally, within

the Coreidae a single NOR is present in an interstitial position in the largest autosomal pair of *Pachylis argentinus* (PAPESCHI *et al.* 2003), and at a telomeric position in one autosomal pair in *Carlisis wahlbergi* (FOSSEY and LIEBENBERG 1995) and *Spartocera fusca* (CATTANI and PAPESCHI 2004). With the exception of *C. wahlbergi* all these NOR regions are associated with CMA bright bands indicating that the whole rDNA repeating unit is rich in GC. Conversely, the DAPI and CMA bright fluorescence of the X chromosome until diakinesis probably reflects a different degree of chromatin condensation of the sex chromosome rather than differences in base composition, as it has been suggested in *Edessa* species (REBAGLIATI *et al.* 2003).

In *Camptischium clavipes* the NOR is terminally located in the largest autosomal pair and it is also associated with a CMA bright band. The presence of the NOR at one chromosome end allowed us to discern both ends and so to follow the meiotic behaviour of this chromosome pair during meiosis. At diakinesis this chromosome pair presents only one chiasma, which can occur either at a interstitial (46.3 %) or a terminal (53.7 %) position. At metaphase I the NOR telomeric region faced the poles in 65% of the cells while in 35% the opposite end showed kinetic activity. From these observations it can be concluded that both telomeric regions can show kinetic activity at first meiotic division.

In *Nezara viridula* (CAMACHO *et al.* 1985) it has been reported that chiasmata can be formed at the telomeric region near the NOR or at the telomere away from it, suggesting that the presence of a nucleolus organizer region do not interfere with the meiotic recombination. In *Carlisis wahlbergi,* on the contrary, most crossovers occurred in the distal half of the bivalent (FOSSEY and LIEBENBERG 1995) and the authors suggested that the NOR could act as a crossover repellent. In *C. clavipes* we found that both telomeric regions are potentially active at meiosis I but with different frequencies (65% for the NOR telomeric region, 35% for the opposite one). We suggest that when the crossing over occurs in a medial position, kinetic activity at meiosis I could be located at any of the two telomeric regions (either the NOR or the opposite one). On the other hand, terminal chiasmata should only be present at the telomeric region opposite the NOR, since the nucleolus organizing region could represent a hindrance for a recombination event. This hypothesis would explain the different frequencies of both possible orientations of the bivalent at metaphase I.

In this paper we give additional evidence that kinetic activity in monochiasmate autosomal bivalents in Heteroptera is randomly located at one of the two chromosome ends, and that at second meiotic division the chromosome ends that were inactive during meiosis I initiate chromatid segregation at anaphase II. With the present report nucleolus organizing regions have been accurately localized in only ten species of Heteroptera, and the results are highly diverse. It will be necessary to analyze more species before detecting any regular pattern at a particular taxonomic level of the localization of NORs, or attempting any discussion on the chromosome rearrangements that should have taken place during karyotype evolution.

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