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Heterochromatin characterization in five species of Heteroptera

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Abstract

The amount, composition and location of heterochromatin in *Athaumastus haematicus* (Stål, 1859), *Leptoglossus impictus* (Stål, 1859), *Phthia picta* (Drury, 1770) (Coreidae), *Largus rufipennis* Laporte, 1832 (Largidae) and *Jadera sanguinolenta* (Fabricius, 1775) (Rhopalidae) are analyzed by C-banding and DAPI/CMA fluorescent banding. As the rule for Heteroptera the possession of holokinetic chromosomes and a pre-reductional type of meiosis cytogenetically characterize these five species. Besides, all of them (except *L. rufipennis*) present a pair of m chromosomes. C-banding technique reveals the absence of constitutive heterochromatin in *A. haematicus*, scarce C-positive blocks in *L. impictus* and *J. sanguinolenta*, and C-positive heterochromatin terminally located in *P. picta* and *L. rufipennis*. All C-bands are DAPI bright, except for a DAPI dull/CMA bright band at one telomeric end of the X chromosome in *L. rufipennis*, which probably corresponds to a nucleolar organizing region. The results of the banding techniques are analyzed in relation to the chiasma frequency and distribution in the five species, and it is concluded that there should exist some constraints to the acquisition and/ or accumulation of heterochromatin in their karyotypes.

Introduction

The genetic system of a species is the way of organization and transmission of the genetic material, which determines the balance between coherence and recombination of genes and controls the amount and type of gene combinations. Evolution of genetic systems means the evolution of those mechanisms effecting and affecting genetic variability (Darlington, 1939). Some of the factors that characterize the genetic system of a species are the mode of chromosome organization, the meiotic chromosome behavior and the recombination index.

The possession of holokinetic chromosomes (without a localized centromere) and a pre-reductional type of meiosis cytogenetically characterize heteropteran species. Autosomal bivalents segregate reductionally at anaphase I while the male sex chromosomes divide equationally at first meiotic division. Most heteropterans show as a rule only one chiasma per bivalent, although exceptions are being increasingly reported (Papeschi et al., 2003). Another interesting feature of some families within Heteroptera is the presence of a chromosome pair that behaves differently from the autosomes and the sex chromosomes during meiosis. The so-called m chromosomes are achiasmatic, and associate during first meiotic division forming a pseudobivalent that segregates reductionally at anaphase I (Ueshima, 1979). All these features are distinctive of the genetic systems of Heteroptera.

Despite the general presumption that constitutive heterochromatin is inert material, there is abundant and increasing evidence that constitutive heterochromatin can have important functions in chromosome pairing and segregation, position effect variegation and can even contain genes and other functional DNA sequences (Sumner, 1972). Repeated DNA sequences in insect genomes have been found to be organized according to different patterns. They occur either as families of repeated elements interspersed throughout the genome or as large arrays usually representing satellite DNA sequences (Brutlag, 1980; Blanchelot, 1991). The current knowledge of the organization of single copy and repeated sequences in insects comes mainly from extensive studies on *Drosophila* and early observations in dipteran organisms (Blanchelot, 1991), and more recent studies in homopteran and lepidopteran species (Bizzaro, Manicardi & Bianchi, 1996; Manicardi et al., 1996; Spence et al., 1998; Mandrioli et al., 1999a, b, c; Mandrioli, Manicardi & Marec, 2003; Mandrioli & Volpi, 2003).

The aim of the present contribution is to get further insight into the chromatin organization and constitution of heteropteran holokinetic chromosomes by determining the amount, composition and location of constitutive heterochromatin. Five heteropteran species have been chosen for the present study. From an agricultural point of view, *Largus rufipennis* Laporte, 1832 (Largidae), *Jadera sanguinolenta* (Fabricius, 1775) (Rhopalidae), *Athaumastus haematicus* (Stål, 1859), *Leptoglossus impictus* (Stål, 1859) and *Phthia picta* (Drury, 1770) (Coreidae) are important pests of several families of cultivated and wild plants in our country, including sunflower, tomato, squash, soy, tobacco, potato and various *Solanum* spp. (Schuh & Slater, 1995). From a cytogenetic point of view, *Largus rufipennis* possesses a male diploid number of 13 ($2n = 12 + X0$), chromosomes of relatively large size and a great variability in chiasma frequency and distribution (Mola & Papeschi, 1993; Bressa et al., 1998). *Jadera sanguinolenta* possesses a diploid chromosome number of 13, including 10 autosomes, a pair of m chromosomes and an X0/XX sex determining system. Previous studies have shown that autosomal univalents are a common feature in this species (Bressa et al., 2001). Finally, *A. haematicus*, *L. impictus* and *P. picta* possess a pair of m chromosomes, which is characteristic of most Coreidae (Colombo & Bidau, 1985). In the present work, the heterochromatin amount, type and distribution in these five heteropteran species is analyzed in relation to chiasma frequency and distribution, and the male karyotype and meiosis of *A. haematicus* are described for the first time.

Materials and methods

The number and provenience of the male specimens analyzed in the present work are summarized in Table 1. After capturing, all individuals were brought alive to the laboratory and once the gonads were dissected out, they were fixed in methanol:glacial acetic acid (3:1).

Table 1. Number and provenience of adult males included in the present work

Taxa	Males	Provenience (Argentina)	Sample
Athaumastus haematicus (Coreidae)	1	Llavallol (Buenos Aires province)	1
	7	Ruta 36 Km 661 (Córdoba province)	2
	16	Gualeguaychú (Entre Ríos province)	3
	1	Concordia (Entre Ríos province)	4
	9	Merlo (San Luis province)	5
Leptoglossus impictus (Coreidae)	4	Ceibas (Entre Ríos province)	6
	6	Gualeguaychú (Entre Ríos province)	7
Phthia picta (Coreidae)	14	Gualeguaychú (Entre Ríos province)	8
	4	9 de Julio (Buenos Aires province)	9
Largus rufipennis (Largidae)	25	Tornquist Park (Buenos Aires province)	10
Jadera sanguinolenta (Rhopalidae)	10	Martín García Island (Buenos Aires province)	11

Slides were prepared by the squash-technique in acetic haematoxylin. Some other slides were made by the squash technique in a drop of 45% acetic acid, and the cover slip was removed by the dry ice method. Afterwards, C- and fluorescent banding (DAPI and CMA fluorochromes) were then applied to these slides to reveal different kinds of heterochromatin constitution.

Chromosome banding

C-banding was performed according to Papeschi (1988). Fluorescent staining with the GC specific chromomycin A_3 (CMA_3) and AT specific 4′6-diamidino-2- phenylindole (DAPI) was performed according to Rebagliati et al. (2003). Some slides pretreated for C-banding were also stained with CMA_3 and DAPI. Slides were examined with a Leica epifluorescence microscope with appropriate filters combination. Photographs were taken using Kodak color Supra print film 400 ASA.

Results

Male chromosome complement and meiosis

Athaumastus haematicus possesses a male diploid chromosome number $2n = 21 = 18 + 2m + X0$, with two large, two medium-sized and five small autosomal pairs; the X chromosome is similar in size to the smallest autosome and the m pair is noticeably smaller.

From leptotene and until diakinesis the sex chromosome is positively heteropycnotic (Figures 1a–d). At pachytene the X chromosome is localized at the periphery of the nucleus. From diplotene onwards the nine autosomal bivalents as well as the pair of m chromosomes recondense; the m pair continues separated as univalents during almost all the meiotic prophase (Figure 1d and e). At diakinesis the X becomes isopycnotic and the m chromosomes come close to each other (Figure 1e); at metaphase I they are associated in a pseudo-bivalent, which is negatively heteropycnotic and lyes at the center of the ring of autosomal bivalents and the sex univalent (Figures 1f and g). At anaphase I the autosomes and the m chromosomes divide reductionally while the X chromosome segregates equationally (sister chromatids separate to opposite poles) (Figure 1h); all telophase I nuclei show 11 chromosomes ($9A + m + X$). Second meiotic division follows without interkinesis and at metaphase II the autosomes and the X chromosome arrange in a circle with the m chromosome lying at its center (Figure 1i). At anaphase II the X chromosome lags, but is finally included in one of the daughter nuclei; thus, 11 ($9A + m + X$) or 10 ($9A + m$) chromosomes are observed in telophase II nuclei. At late telophase II the X chromosome can still be detected positively heteropycnotic and outside the chromatin mass.

Bivalents present one and sometimes two chiasmata located at terminal or subterminal positions (Figures 1e–g). Chiasma frequency at diakinesis-metaphase I ranges between 9.00 and 9.50 with a mean of 9.12 (Table 2). Figure 2 shows the distribution of relative chiasma frequency of the cells at diakinesis-metaphase I of the 34 analyzed specimens of *A. haematicus* and it can be observed that 88% of the cells present 9 chiasmata.

Leptoglossus impictus presents a diploid number $2n = 21 = 18 + 2m + X0$, with one large, three medium-sized and five small autosomal pairs. The m chromosomes are the smallest of the complement and the X chromosome is of small size. During the diffuse stage autosomes decondense completely and the X univalent is detected due to its positive heteropycnosis (Figure 3a). At diakinesis autosomal bivalents generally show interstitial or subterminal chiasmata and the m chromosomes lie separated (Figures 3b and c). At metaphase I the autosomal bivalents arrange in a circle with the m pseudo-bivalent at its center and the X univalent outside it (Figure 3d). After telophase II (Figure 3e) the cell enters the second meiotic division without interkinesis. At metaphase II the arrangement in a circle is not always observed (Figure 3f). In 6 individuals out of the 10 analyzed (2 out of 4 from Ceibas and 4 out of 6 from Gualeguaychú) cells with a pair of univalents were observed (2.5–10.53%). Mean chiasma frequency at diakinesis-metaphase I is 9.03, ranging between 8.92 and 9.40 (Table 2; Figure 2).

Phthia picta has $2n = 21 = 18 + 2m + X0$; the m chromosomes are the smallest of the complement and the X chromosome is medium-sized. At zygotene the positively heteropycnotic X chromosome is readily distinguished. At the diffuse stage the X univalent continues condensed and positively heteropycnotic, while the autosomal

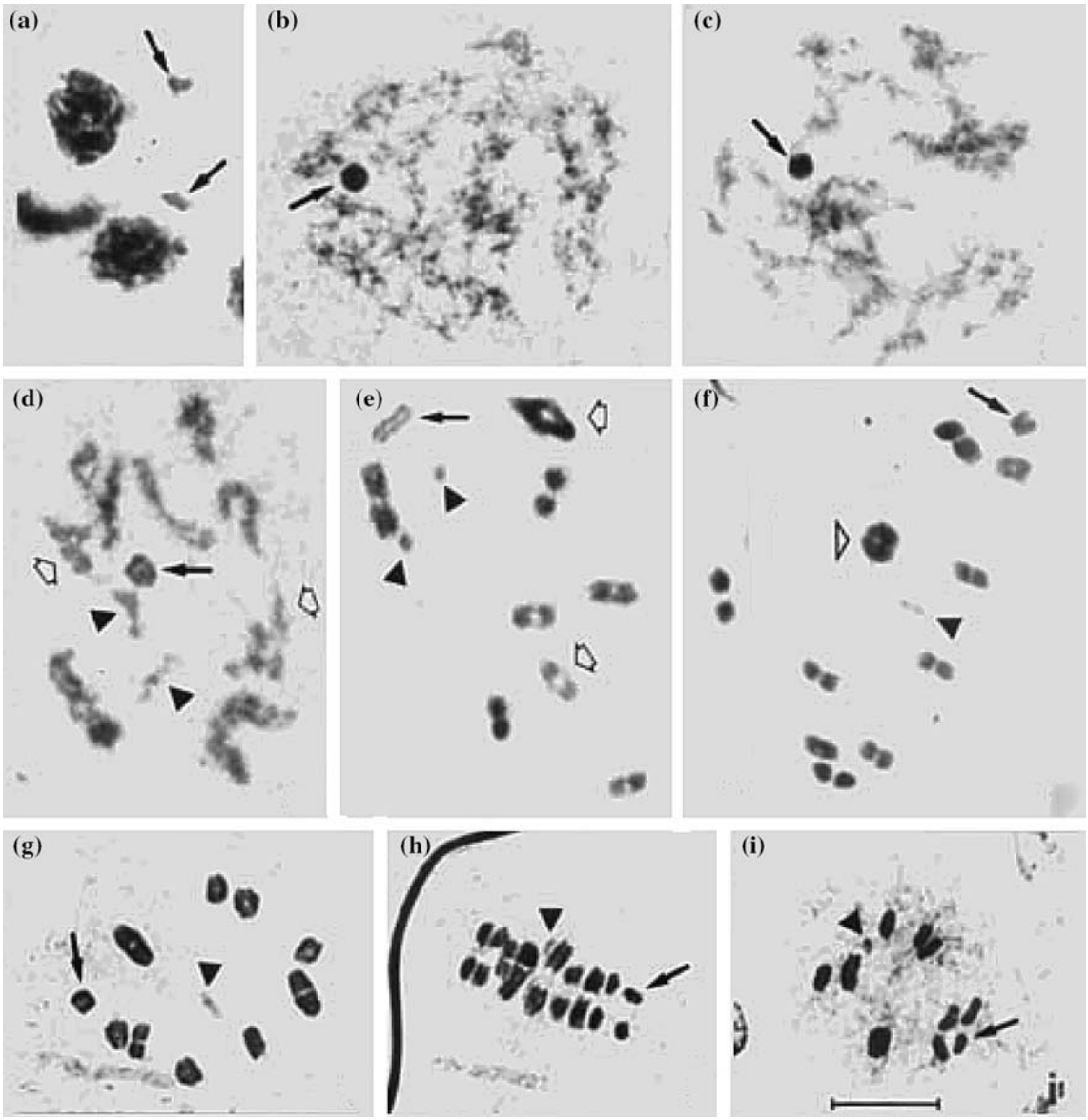

Figure 1. Male meiosis of *Athaumastus haematicus*. (a) Leptotene–Zygotene. (b–c) Diffuse stage. (d) Diplotene. (e) Diakinesis. (f–g) Metaphase I. (h) Anaphase I. (i) Metaphase II. Arrows signal the X chromosome; empty arrows signal bivalents with subterminal chiasmata. Arrowheads point to the m chromosome pair, and empty arrowhead, a ring bivalent. Bar = 10 μm.

bivalents do not decondense completely; positively heteropycnotic regions are observed scattered throughout the nucleus. At diakinesis bivalents present only one chiasma interstitially located (Figure 3g). At metaphase I the autosomal bivalents dispose in a circle and the X univalent lies outside it; the m pseudo-bivalent is negatively heteropycnotic and lies inside the ring (Figures 3h–j). At anaphase I the autosomal bivalents and the m pseudo-bivalent divide reductionally, while the X does so equationally. At metaphase II the autosomes arrange in a circle with the X and the m chromosomes forming part of it (Figure 3k). Mean chiasma frequency at diakinesis-metaphase I is 9.00 (Table 2; Figure 2). A particular feature of *P. picta* is the frequent medial position of the chiasmata, location that can still be observed at prometaphase I and even at metaphase I (Figures 3g–j). In 64% of the analyzed cells at diakinesis-metaphase I belonging to the 18 individuals surveyed, 4

Table 2. Chiasma frequency of *Athaumastus haematicus*, *Leptoglossus impictus* and *Phthia picta*

Species	Provenience	Number of individuals	Chiasma frequency (range)	Mean chiasma frequency
A. haematicus	Llavallol (Buenos Aires province)	1	9	9
	Ruta 36 Km. 661 (Córdoba province)	7	9–9.17	9.1
	Gualeguaychú, Concordia (Entre Ríos province)	17	9–9.5	9.17
	Merlo (San Luis province)	9	9–9.15	9.09
Total		**34**		**9.12**
L. impictus	Ceibas (Entre Ríos province)	4	8.95–9	8.98
	Gualeguaychú (Entre Ríos province)	6	8.92–9.40	9.06
Total		**10**		**9.03**
P. picta	Gualeguaychú (Entre Ríos province)	14	9	9
	9 de Julio (Buenos Aires province)	4	9	9
Total		**18**		**9**

to eight bivalents present medially located chiasmata (Figure 4).

Largus rufipennis and *Jadera sanguinolenta* possess a male diploid chromosome number $2n = 13 = 12 + X0$ and $2n = 13 = 10 + 2m + X0$, respectively. In *L. rufipennis* among autosomal bivalents one larger, three medium sized and two smaller are distinguished; no m pair is present and the X univalent is the smallest of the complement. In *J. sanguinolenta* one autosomal pair is noticeably larger, the m pair is minute and the X chromosome is of small size. Male meiosis in both species has been previously described in detail. Chiasma frequency in *L. rufipennis* ranges from 6.05 to 6.58 with a mean of 6.28, and cells with univalents were observed (0–11.11%). In

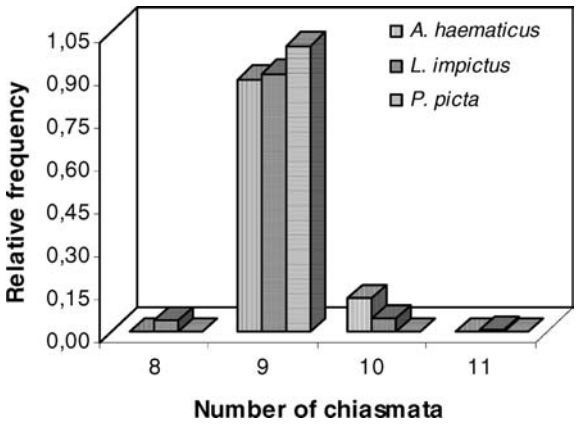

Figure 2. Relative frequency of the number of chiasmata in cells at diakinesis–metaphase I of *Athaumastus haematicus*, *Leptoglossus impictus* and *Phthia picta*.

J. sanguinolenta chiasma frequency ranges from 4.23 to 6.03 with a mean of 5.31, and the number of cells with univalents was striking (0–72.97%) (Bressa et al., 1998, 2001).

C- and fluorescent banding

C-banding reveals noticeable differences in the amount and location of heterochromatin among the five species analyzed. No C-band is detected in *A. haematicus* (data not shown) and small C-bands are observed at telomeric position in *L. impictus* (either at one or both telomeric ends) (Figure 5a). All the autosomes and the sex chromosome in *P. picta* (Figure 5b) and *L. rufipennis* (Figure 5c) present heterochromatic C positive bands at telomeric regions; in *J. sanguinolenta* small C positive dots (from 3 to 8) are observed in cells at pachytene and the diffuse stage (Figure 5d) but their presence can no longer be detected from diakinesis onwards.

The X chromosome of all the species analyzed is DAPI and CMA bright during meiotic prophase. All the C-positive bands are also DAPI bright and CMA dull, except for a DAPI dull/CMA bright band located at one telomeric region of the X chromosome of *L. rufipennis* (Figure 6).

Discussion

The genetic system of Heteroptera has many features that make it unique among most insect

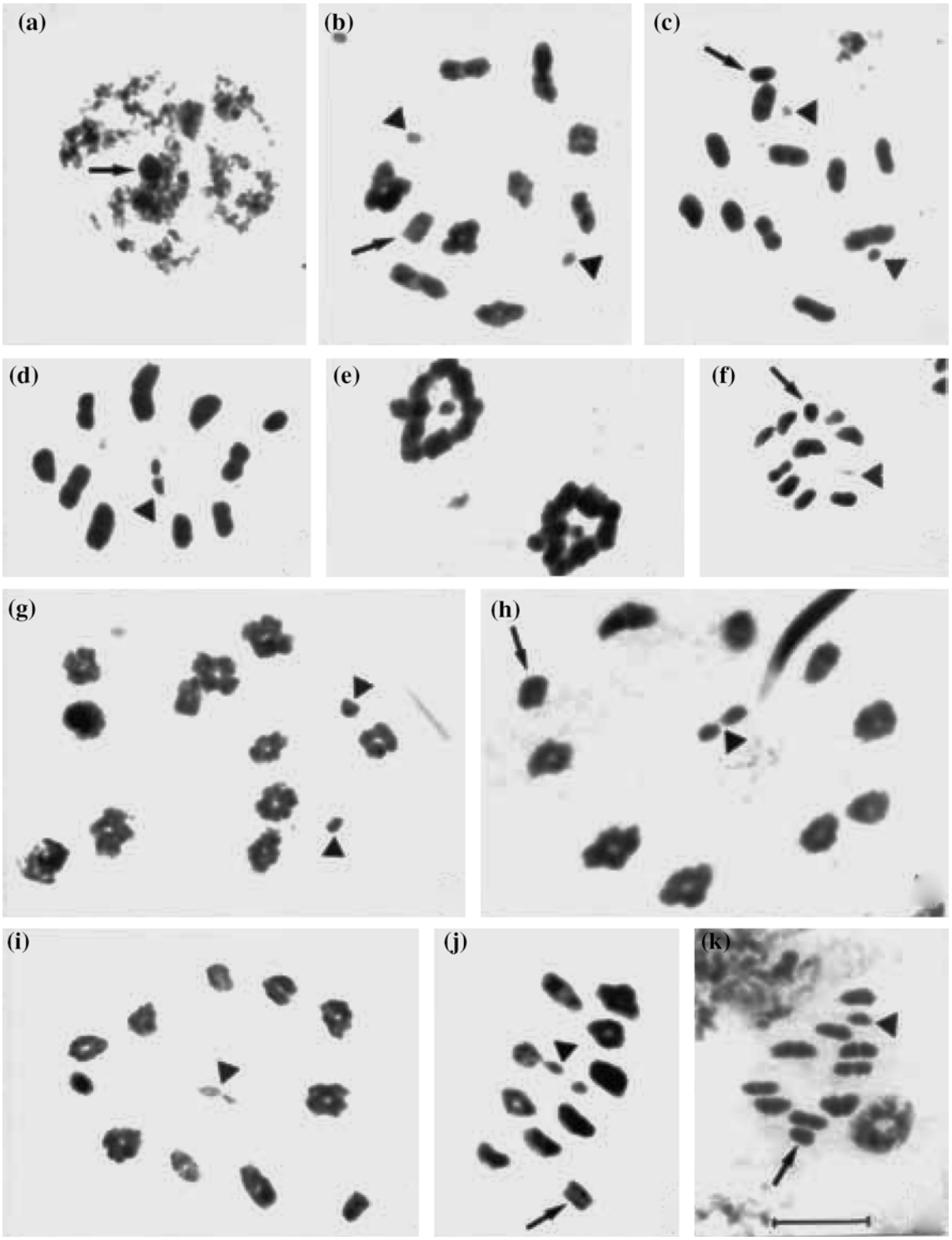

Figure 3. Male meiosis of *Leptoglossus impictus* (a–f) and *Phthia picta* (g–k). *L. impictus:* (a) Diffuse stage. (b–c) Diakinesis. (d) Metaphase I. (e) Telophase I. (f) Metaphase II. *P. picta:* (g) Diakinesis with all bivalents with medial chiasmata. (h–j) Metaphase I with bivalents with medial chiasmata. (k) Metaphase II. Arrows show the X chromosome; arrowheads, the m chromosome pair. Bar = 10 μm.

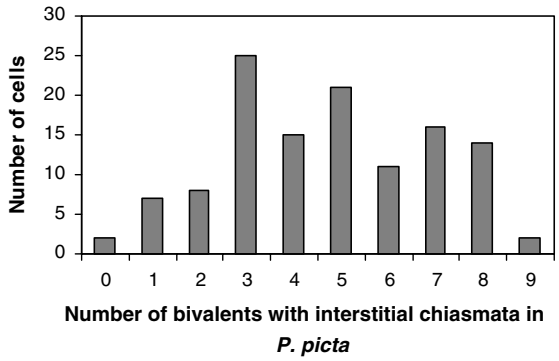

Figure 4. Frequency of bivalents with interstitial chiasmata in cells at diakinesis- metaphase I of *Phthia picta*.

groups: holokinetic chromosomes, a pre-reductional meiosis for autosomes and post-reductional for male sex chromosomes, a mean chiasma frequency of only one chiasma per bivalent, and a pair of m chromosomes in some families. Molecular cytogenetics in Heteroptera is just starting to develop as a new research field worldwide, and very little is known about the chromatin organization and constitution in these holokinetic chromosomes.

The three species of Coreidae here analyzed share the modal diploid number of the family $2n = 18 + 2m + X0$ (male). The cytogenetic characteristics of *Athaumastus haematicus* are very similar to those described for *A. subcarinatus* (Colombo & Bidau, 1985). These characteristics include male karyotype and meiotic behavior, terminal or subterminal position of chiasmata, and presence of bivalents with two chiasmata.

The male karyotype and meiosis of *Leptoglossus impictus* and *Phthia picta* also agree in general

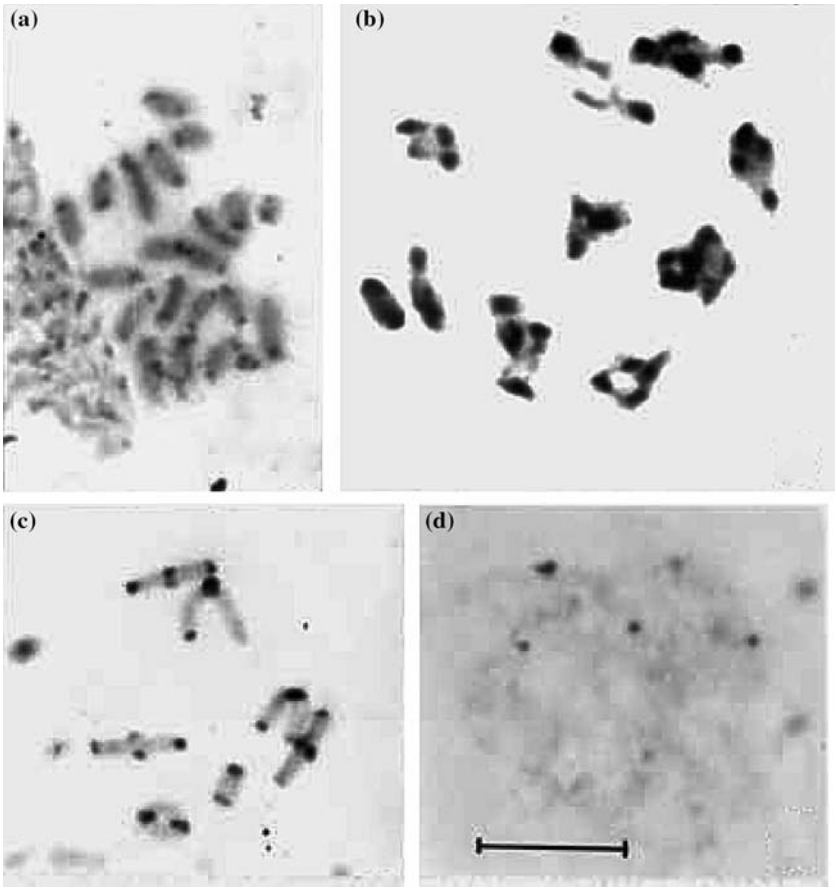

Figure 5. C-banding. (a) Spermatogonial prometaphase of *Leptoglossus impictus*; C-bands at one or both chromosome ends are present. (b–c) Diakinesis of *Phthia picta* (b) and *L. rufipennis* (c); large heterochromatic blocks are observed at telomeric positions. (d) Diffuse stage of *Jadera sanguinolenta*; small C positive dots are detected. Bar = 10 μm.

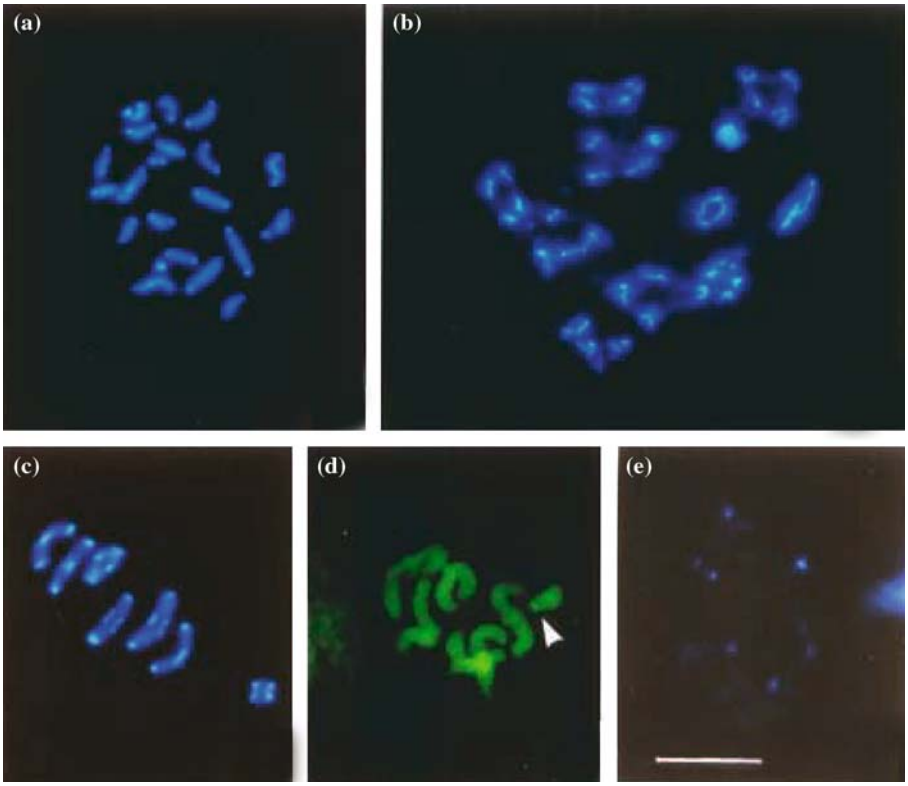

Figure 6. DAPI (a–c, e) and CMA (d) banding. (a) Spermatogonial prometaphase of *Leptoglossus impictus* with DAPI bright bands at one or both telomeric regions. (b) Diakinesis of *Phthia picta* with large DAPI bright blocks in all autosomes; the X chromosome is completely DAPI bright. (c) Metaphase I of *L. rufipennis*; DAPI bright bands are detected at telomeric regions of all the chromosomes. (d) Diakinesis of *L. rufipennis*; the arrowhead points a DAPI dull/CMA bright band at one telomeric end of the X chromosome. (e) Diffuse stage of *Jadera sanguinolenta*; small DAPI bright dots are detected. Bar = 10 μm.

terms with previous reports on these species (Colombo & Bidau, 1985). However, some differences are detected in the degree of chromosome decondensation during the diffuse stage of *L. impictus*; it is incomplete in the samples from Puerto Madryn (Chubut) and El Palmar (Entre Ríos) previously reported but it is almost complete in the samples from Ceibas and Gualeguaychú (Entre Ríos) here analyzed. Furthermore, chiasma frequency ranged between 9.22 and 10.09 in Puerto Madryn sample, was 9.00 in El Palmar sample and ranged between 8.95 and 9.00 in Ceibas, and 8.92 and 9.40 in Gualeguaychú; the presence of univalents was only reported in the last two samples. These differences in the diffuse stage and the recombination index are probably related to differences in the environmental conditions. Since the samples have different proveniences and dates of collection it is not possible to relate the meiotic differences with a particular environmental factor.

The m chromosomes were originally described by Wilson (1905) in species of Coreidae, but at present they have been reported in many heteropteran families (Ueshima, 1979). Although the m chromosomes are generally of small size, they are actually defined by their meiotic behavior: they are achiasmatic, behave as univalents during prophase I, and at late diakinesis they come closer and associate forming a pseudo-bivalent that segregates reductionally at anaphase I. The m chromosomes of *A. haematicus, L. impictus* and *J. sanguinolenta* are of small size, but in *P. picta* they are relatively large. Colombo and Bidau (1985) described the presence of a secondary constriction in the m pair of *L. impictus* and *P. picta*, which was not detected in our material. Despite their size differences the m chromosomes behave in a similar way in all the species here analyzed. Suja et al. (2000) reported that the m chromosomes of *Coreus marginatus* (Coreidae)

formed a bivalent in approximately half of the cells at diplotene/diakinesis, and that they were always associated during metaphase I. Further studies are required in order to clarify the precise behavior of the m chromosomes in different species (whether they can be present as a bivalent or only as two univalents, if they are asynaptic or desynaptic, or if they have different behaviors according to the genetic or environmental milieu), and which are the mechanisms that guarantee their correct segregation when present as univalents.

The analysis of constitutive heterochromatin content in these species revealed the absence of C positive bands in *A. haematicus*, the presence of very scarce C positive bands terminally located in *L. impictus* and *Jadera sanguinolenta* and, on the other hand, the presence of conspicuous C positive bands at the telomeric regions of all the autosomes and the X chromosome in *Largus rufipennis* and *P. picta*. This C-banding pattern (C bands terminally located) agrees with most previous reports in Heteroptera (Camacho, Belda & Cabrero, 1985; Grozeva & Nokkala, 2001; Papeschi et al., 2001). The m chromosomes show allocycly with respect to both the autosomes and the sex chromosome during male meiosis, but the heterochromatin characterization in this chromosome pair do not show differences with the rest of the complement: C bands, when present, are terminally located and they are by no means completely heterochromatic. The different pycnotic cycle of the m chromosomes with respect to both the autosomes and the sex chromosome reflects differences in chromatin packaging, and chromatin condensation is also related with the regulation of gene expression. At present, nothing can still be said about the information that the m chromosomes carry or which could be their function in the genetic system of the species possessing them.

The use of fluorescent DNA-binding dyes with different specificities allows a better characterization of heterochromatic regions in terms of their relative enrichment with AT or GC base pairs. The results after DAPI/CMA banding indicate that all the heterochromatin blocks in the species here analyzed are AT rich, with the exception of a DAPI dull/CMA bright band observed at one telomeric region of the X chromosome in *L. rufipennis*. This band probably represents a nucleolus organizing region. Many reports on heteropteran species have described the correspondence of CMA bright bands with NORs (González-García et al., 1996; Papeschi et al., 2003; Rebagliati, Papeschi & Mola, 2003; Grozeva, Kuznetsova & Nokkala, 2004). In all the five species the sex chromosome stains brightly with both fluorochromes. This observation could be due to differences in chromatin condensation between the autosomes and the sex chromosome rather than to differences in base composition, as it has previously been suggested (Rebagliati, Papeschi & Mola, 2003).

When analyzing the amount and location of heterochromatin in the five species under study in relation to chiasma frequency and distribution different situations are encountered. *A. haematicus, L. impictus* and *J. sanguinolenta* present very scarce heterochromatin, and chiasmata are terminally/subterminally located. Both in *L. impictus* and *J. sanguinolenta* cells with univalents are observed; in the latter, the largest autosomal pair is very frequently found as two univalents (Bressa et al., 2001). In *L. rufipennis* there are heterochromatic blocks terminally located, and one or two chiasmata can be observed at subterminal positions. Finally, a very striking feature of *P. picta* is the presence of only one chiasma per bivalent medially located, and large heterochromatic blocks at chromosome ends. This chiasma localization in *P. picta* could be associated with the large heterochromatic blocks observed in this species. It can be suggested that heterochromatin in *P. picta* has accumulated at terminal positions as a consequence of the lack of recombination near the chromosome ends. However, no particular heterochromatin accumulation has taken place either in the m chromosome pair of any of the species here analyzed (which are completely devoid of recombination) or in the largest autosomal pair of *J. sanguinolenta* (which is frequently achiasmatic). This analysis leads as to suggest that although heterochromatin could be an active component of heteropteran chromosomes, some constraints would regulate its acquisition and/or accumulation in the karyotype of the species. Further studies are required to get a better understanding on the organization, structure and composition of heteropteran holokinetic chromosomes, and the possible function of the m chromosomes and the heterochromatin in the genetic system of Heteroptera species.

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