Journal of Biological Research **5**: 3–21, 2006
J. Biol. Res. is available online at http://www.jbr.gr

— INVITED REVIEW —

Evolutionary cytogenetics in Heteroptera

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Received: 2 September 2005 Accepted after revision: 29 December 2005

In this review, the principal mechanisms of karyotype evolution in bugs (Heteroptera) are discussed. Bugs possess holokinetic chromosomes, i.e. chromosomes without primary constriction, the centromere; a pre-reductional type of meiosis for autosomes and m-chromosomes, i.e. they segregate reductionally at meiosis I; and an equational division of sex chromosomes at anaphase I. Diploid numbers range from 2n = 4 to 2n = 80, but about 70% of the species have 12 to 34 chromosomes. The chromosome mechanism of sex determination is of the XY/XX type (males/females), but derived variants such as an X0/XX system or multiple sex chromosome systems are common. On the other hand, neo-sex chromosomes are rare. Our results in heteropteran species belonging to different families let us exemplify some of the principal chromosome changes that usually take place during the evolution of species: autosomal fusions, fusions between autosomes and sex chromosomes, fragmentation of sex chromosomes, and variation in heterochromatin content. Other chromosome rearrangements, such as translocations or inversions are almost absent. Molecular cytogenetic techniques, recently employed in bugs, represent promising tools to further clarify the mechanisms of karyotype evolution in Heteroptera.

Key words: holokinetic chromosomes, karyotype evolution, molecular cytogenetics.

INTRODUCTION

Cytogenetic studies in Heteroptera date from 1891 with Henking's morphological study on the spermatogenesis of the bug *Pyrrhocoris apterus* Linnaeus (Pyrrhocoridae). He described the presence of a chromatin body (the "X chromosome") that showed an unusual staining during meiotic prophase and a peculiar behaviour during meiosis. The observations of Henking and other cytologists, together with their own observations in males and females of different Heteroptera species, were finally interpreted by Mc Clung (1901) and Wilson (1909b). They suggested a direct relationship between sex determination and presence of either one or two "X chromosomes" in X0/XX systems, or an XY or XX chromosome pair in species with XY/XX systems.

Through a series of publications that appeared between 1897 and 1932, Wilson and Montgomery settled the foundations on Heteroptera cytogenetics. Some years later, between the 1930s and the 1960s, Schrader and Hughes-Schrader contributed significantly to a better knowledge on the organization and function of heteropteran chromosomes and their behaviour during cell divisions (Ueshima, 1979; Manna, 1984).

At present, although approximately 1600 heteropteran species belonging to 46 families have been cytogenetically analyzed, they are not a representative sample of the group (4.2% of approximately 38,000 described species) (Table 1) (Schuh & Slater, 1995). Most studies have been made on male testes, because meiotic cells are more often encountered than mitotic ones, and the earliest reports referred to the male chromosome number and sex chromosome system. No detailed analysis of heteropteran chromosome morphology is possible, since chromosomes are holokinetic (a primary constriction, the centromere, is lacking) and the chromosomes are generally

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relatively small and of similar size. Although DNA measurement is the best means of testing fusion/fragmentation hypothesis in karyotypic evolution of taxa bearing holokinetic chromosomes, this technique has been rarely used in Heteroptera (Schrader & Hughes-Schrader, 1956, 1958; Maudlin, 1974; Papeschi, 1988, 1991; Panzera *et al*., 1992, 1995; Papeschi *et al*., 2001; Grozeva & Nokkala, 2003). Furthermore, cytogenetic studies at the population level in Heteroptera are still scarce. All these facts represent serious constraints in comparative and evolutionary cytogenetics of this insect group.

Recently, molecular approaches such as fluorescent banding and fluorescent *in situ* hybridization with telomeric and ribosomal DNA probes (18S and 28S rDNA), have been carried out in bugs (González-García *et al*., 1996; Sahara *et al*., 1999; Papeschi *et al*., 2003; Rebagliati *et al*., 2003; Cattani & Papeschi, 2004; Cattani *et al*., 2004; Frydrychová *et al*., 2004; Grozeva *et al*., 2004; Hebsgaard *et al*., 2004; Ituarte & Papeschi, 2004; Bressa *et al*., 2005; Vítková *et al*., 2005). These molecular techniques have substantially contributed to a better understanding of the chromatin organization and composition of these holokinetic chromosomes. However, there is still much to be learnt before the mechanisms of karyotype evolution and the role in the speciation of heteropterans is completely clarified.

CYTOGENETICS OF HETEROPTERA: GENERAL FEATURES

The genetic system of Heteroptera has many characteristics that make it unique among most insect groups: the possession of holokinetic chromosomes, a different meiotic behaviour for autosomes and sex chromosomes, a mean chiasma frequency of only one chiasma per bivalent, and a pair of "m chromosomes" in some families.

All heteropteran species possess holokinetic chromosomes (i.e. without a localized centromere). During mitosis, microtubules attach to the entire length of the sister chromatids and at anaphase, they migrate parallel to each other and perpendicular to the polar spindle (Hughes-Schrader & Schrader, 1961). At the ultrastructural level, kinetochore plates, to which spindle microtubules are attached, cover almost all the polar surface of the mitotic chromosomes (Buck, 1968; Comings & Okada, 1972). However, kinetic activity during meiosis is restricted to the telomeric regions and the chromosomes can be regarded as telokinetic (Motzko & Ruthmann, 1984). 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; Cattani *et al*., 2004). The holokinetic nature of heteropteran chromosomes has led to the hypothesis that fusions and fragmentations would be the most frequent mechanisms of karyotype evolution. The non-localized kinetic activity does not have the constraints imposed by a localized centromere in species with monocentric chromosomes. In the latter, acentric fragments or dicentrics are mitotically and meiotically unstable.

The meiotic behaviour of autosomal bivalents, sex chromosomes and m chromosomes is slightly different. As a rule, autosomal bivalents are chiasmatic, whereas sex chromosomes and m chromosomes are achiasmatic (Ueshima, 1979; Manna, 1984; González-García *et al*., 1996; Suja *et al*., 2000). When autosomal bivalents form only one chiasma terminally located, 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 terminal 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 become functional and no telokinetic activity is observed (Mola & Papeschi, 1993; Papeschi *et al*., 2003). This latter behaviour has been described in *Pachylis argentinus* Berg and *Nezara viridula* (Linnaeus); both species possess a pair of chromosomes carrying a secondary constriction, and when this particular pair presented two chiasmata, the alternative kinetic sites were located next to the secondary constrictions (Papeschi *et al*., 2003). When chiasmata are medially located, the terms pre-reduction and post-reduction lose their meaning. The genetic information located at the chiasma to one pair of telomeric regions divides reductionally, while the information located at the other half of the chromosome does so equationally. We consider meiosis in Heteroptera as pre-reductional taking into account the behaviour of bivalents with one terminal or subterminal chiasma, in which most genetic information segregates reductionally at meiosis I; and the behaviour of fusion/fragmentation trivalents, in which the largest chromosome always segregates from the two smaller ones at meiosis I (Ueshima, 1963; Papeschi,

1994). A post-reductional meiosis has been described in other groups with holokinetic chromosomes (e.g. Juncaceae, Odonata) (Nordenskiöld, 1962; Mola, 1995).

On the other hand, sex chromosomes are achiasmatic and behave as univalents in male meiosis I. They divide equationally at anaphase I and associate at meiosis II through the so-called "touch-and-go pairing". Nevertheless, pre-reduction of the sex chromosomes has been described in three species of *Anisops* (*A. fiebri* Kirkaldy, *A. niveus* Fabricius, *A. sardea* Kirkaldy) (Notonectidae), two species of *Ectrychotes* (*E. abbreviatus* Reuter, *E. dispar* Reuter) (Reduviidae), two species of *Archimerus* (*A. alternatus* Say, *A. calcarator* Fabricius) and four species of *Pachylis* [*P. argentinus* Berg, *P. gigas* Barm., *P. laticornis* (Fabricius), *P. pharaonis* (Herbst)] (Coreidae) and in all the 29 species of Tingidae (Ueshima, 1979; Grozeva & Nokkala, 2001; Papeschi *et al*., 2003). Finally, the m chromosomes are also achiasmatic, but associate at first meiotic division segregating pre-reductionally.

THE m CHROMOSOMES

Wilson (1905) introduced the term "m chromosomes" to describe the smallest chromosome pair in two species of Coreidae which behave differently from both the autosomes and the sex chromosomes during male meiosis. Until present, the m chromosomes have been reported in many heteropteran families (Table 1) (Ueshima, 1979). Although the m chromosomes are generally of small size, they are actually defined by their meiotic behaviour: they are usually unpaired and thus achiasmatic during early meiosis, and at late diakinesis they come close together. At metaphase I, they are always associated end-to-end (touch-and-go pairing) forming a pseudobivalent which segregates reductionally at anaphase I. The second meiotic division is equational for the m chromosomes.

One exception to this meiotic behaviour has been described in *Coreus marginatus* Linnaeus (Coreidae) by Nokkala (1986) and Suja *et al*. (2000), who reported that the m chromosomes were present as a bivalent in some cells.

The m chromosomes generally show allocycly with respect to both the autosomes and the sex chromosomes during male meiosis, but the heterochromatin characterization in this chromosome pair showed that they are by no means completely heterochromatic. The different pycnotic cycle of the m chromosomes reflects differences in chromatin packaging, and this pattern of chromatin condensation is probably related to the regulation of gene expression (Bressa *et al*., 2005). However, there is still no information on the genetic content of the m chromosomes.

Further studies are required in order to clarify the precise behaviour and function of the m chromosomes in different species. Are they always present as univalents or do they form a bivalent? Do they have different behaviour according to the genetic or environmental milieu? Furthermore, which are the mechanisms that guarantee their correct segregation when they are present as univalents? Do they represent some kind of selfish DNA? Nothing can still be said about their function in the genetic system of the heteropteran species possessing them.

SEX CHROMOSOME SYSTEMS

Sex chromosome systems described so far in Heteroptera are simple systems of the types XY/XX (71.4% of approximately 1600 species cytogenetically analyzed) and X0/XX (14.7%), and different multiple systems (X_nY/X_nX_n, X_n0/X_nX_n, XY_n/XX) (13.5%). Neo-sex chromosome systems are very rare and until present they have only been reported in seven species and subspecies (0.5%) (Table 1) (Ueshima, 1979; Bressa *et al*., 1999; Nokkala & Nokkala, 1999; Jacobs, 2004).

Two hypotheses concerning the evolution of the sex-chromosome systems in Heteroptera have been proposed. Ueshima (1979) suggested that the XY system is derived from an ancestral X0 that is commonly encountered in primitive taxa and in phylogenetically related homopteran species (Halkka, 1959). By contrast, based on the presence of a Y chromosome in very primitive heteropteran species, Nokkala & Nokkala (1983, 1984) and Grozeva & Nokkala (1996) suggested that the X0 system is a derived condition from the ancestral XY that is present in the majority of the species cytogenetically analyzed.

Sex chromosome systems with multiple Xs or Ys most probably originated through fragmentation of the ancestral X or Y, respectively. The study of one population of *Belostoma orbiculatum* Estévez & Polhemus (Belostomatidae), polymorphic for the sex chromosome system, provided evidence that supports this hypothesis (Papeschi, 1996). The analysis of two samples from one population of Punta Lara (Buenos

TABLE 1. Number of species cytogenetically analyzed and principal cytogenetic features of heteropteran families

Taxa			Nº of species	2n Range	2n Mode	m chromosomes	Sex Chromosome Systems
Dipsocoromorpha		Dipsocoridae	4	(21-22)		present/absent	X0, XY, XnY
		Schizopteridae	2	30		present	X0
Gerromorpha	Hebroidea	Hebridae	4	(19-27)		absent	X0, XY, neo-system
	Hydrometroidea	Hydrometridae	3	(19-20)		absent	X0, XY
	Gerroidea	Gerridae	21	(19-31)	21/23	absent	X0, XY
		Veliidae	5	(21-25)		absent	X0, XY
	Mesoveloidea	Mesoveliidae	1	35		absent	XnY
Nepomorpha	Nepoidea	Belostomatidae	27	(4-30)	29	absent	XY, XnY, neo-system
		Nepidae	11	(22-46)	43	absent	X0, XY, XnY
	Ochteroidea	Gelastocoridae	1	35		absent	XnY
		Ochteridae	1	50		?	Not determined
	Corixoidea	Corixidae	28	(24-26)	24	present	XY
		Micronectidae	2	(24-30)		absent	XY
	Naucoroidea	Naucoridae	10	(20-51)		present	X0
	Notonectoidea	Notonectidae	14	(24-26)	24	present	XY, Xn0
		Pleidae	3	23		present	X0
Leptopodomorpha	Saldoidea	Saldidae	9	(19-36)	35	present	X0, XY
	Leptopodoidea	Leptopodidae	1	28		absent	XY
Cimicomorpha	Reduvioidea	Reduviidae	125	(12-34)	22	absent	X0, XY, XnY
	Microphysoidea	Mycophysidae	3	14		absent	XY
	Joppeicoidea	Joppeicidae	1	24		absent	XY
	Miroidea	Miridae	168	(14-80)	34	absent	X0, XY, Xn0, XnY
		Tingidae	28	(12-14)	14	absent	X0, XY
	Naboidea	Nabidae	30	(18-40)	18	absent	XY
	Cimicoidea	Anthocoridae	5	(24-32)		absent	XY
		Cimicidae	44	(10-44)	31	absent	XY, XnY
		Polyctenidae	3	(6-12)		absent	XY
Pentatomomorpha	Aradoidea	Aradidae	33	(7-40)	14/27	absent	XY, XnY, neo-system
	Pentatomoidea	Acanthosomatidae	10	(12-16)	12	absent	XY
		Cydnidae	12	(12-31)	12	absent	XY, XnY
		Dinidoridae	7	(14-21)	14	absent	XY, XnY
		Pentatomidae	303	(6-27)	14	absent	XY, XnY, neo-system
		Plataspidae	15	(10-14)	12	absent	XY
		Scutelleridae	21	(12-14)	12	absent	XY
		Tessaratomidae	1	12		absent	XY
		Urostylidae	3	(14-16)		absent	XY
	Lygaeoidea	Berytidae	14	(16-42)	16	absent	XY
		Colobathristidae	1	14		present	XY
		Lygaeidae	402	(10-30)	14/16	present/absent	X0, XY, XnY, XYn
		Piesmatidae	4	(22-24)		absent	XY
	Pyrrhocoroidea	Largidae	11	(11-17)	13/17	present/absent	X0, XnY
		Pyrrhocoridae	21	(12-33)	16	absent	X0, Xn0, neo-system
	Coreoidea	Alydidae	22	(13-17)	13	present	X0, Xn0
		Coreidae	108	(13-28)	21	present	X0, XY, Xn0
		Rhopalidae	25	(13-15)	13	present	X0
		Stenocephalidae	3	14		present	XY, Xn0

Aires province, Argentina) showed that 24 male individuals had the simple system XY, but five specimens from both samples had a multiple system X_1X_2Y. The comparison of area measurements of the sex chromosomes in individuals with $2n = 16$ and $2n = 17$ revealed that the relative size of the X_1 plus the X_2 did not differ significantly from the relative size of the single X. This pointed out that the X chromosome of the simple system has fragmented into two chromosomes of unequal size, one of them a little larger than the Y chromosome (Papeschi, 1996).

To date, neo-sex chromosomes in Heteroptera have been only found in two species of *Lethocerus* (*L. indicus* Lep. et Servielle, *Lethocerus* sp. Mayr) (Belostomatidae) (Chickering & Bacorn, 1933; Jande, 1959), and in *Rhytidolomia senilis* (Say) (Pentatomidae) (Schrader, 1940). In these species, both the ancestral X and Y chromosomes fused each with one chromosome of an autosomal pair, resulting in a neo-X/neo-Y system. The neo-system described in *Hebrus pusillus* Fallén (Hebridae) originated through the fusion of the ancestral X chromosome with one autosome (Nokkala & Nokkala, 1999); but the neo-sex chromosome systems of *Dysdercus albofasciatus* Berg (Pyrrhocoridae) (Bressa *et al*., 1999) and of *Dundocoris nodulicarinus noveni* Jacobs and *D. nodulicarinus septeni* Jacobs (Aradidae) (Jacobs, 2004) have a more complex origin.

In *Dysdercus albofasciatus* the origin of the 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 (Bressa *et al*., 1999). In *Dundocoris nodulicarinus novenus* the neo-XY_1Y_2 system probably originated by the fusion of a large autosome with the X chromosome (neo-X), while the homologue of the fused autosome formed a neo-Y ($= Y_1$) and the ancestral Y chromosome, the Y_2. The neo-XY_1/neo-Y_2 sex chromosome system of *D. nodulicarinus septeni* probably originated by a later fusion of one pair of autosomes with the neo-X and the original Y chromosome (Y_2) (Jacobs, 2004).

HETEROCHROMATIN

Repeated DNA sequences in insect genomes are 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 on the organization of single copy and repeated sequences in insects comes mainly from extensive studies on *Drosophila* and early observations in other dipteran species (Blanchelot, 1991), and more recent studies in homopteran and lepidopteran species (Bizzaro *et al*., 1996; Manicardi *et al*., 1996; Spence *et al*., 1998; Mandrioli *et al*., 1999a, b, c; Mandrioli & Volpi, 2003; Mandrioli *et al*., 2003).

Despite the general presumption that constitutive heterochromatin is an 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, 2003).

Early reports on C positive heterochromatin in heteropterans showed that C bands are terminally located. This led to the suggestion that the principle of equilocal heterochromatin distribution of Heitz (1933, 1935) (i.e. the tendency of heterochromatin of non-homologous chromosomes to be located at similar positions) is also applied to Heteroptera (Solari & Agopian, 1987; Papeschi, 1991; Panzera *et al*., 1995; Cattani *et al*., 2004; Bressa *et al*., 2005). However, recent reports describe a different distribution pattern in some species. In *Tenagobia fuscata* (Stål) (Corixidae), *Triatoma patagonica* Del Ponte (Reduviidae), *Petillia patullicollis* Walk. (Coreidae) and *Nezara viridula* (Linnaeus) (Pentatomidae), while most bands are terminally located, one autosomal pair presents a conspicuous C positive band in an interstitial position (corresponding at least in the latter to a nucleolus organizer region) (Camacho *et al*., 1985; Dey & Wangdi, 1990; Panzera *et al*., 1997; Papeschi *et al*., 2003; Ituarte & Papeschi, 2004). The analysis of 13 species of Tingidae and four species of *Nabis* (Nabidae) using C banding revealed the presence of very scarce C positive heterochromatin located either at telomeric positions or, in some chromosomes, at interstitial ones (Grozeva & Nokkala, 2003; Grozeva *et al*., 2004). Finally, a completely different pattern has been described in *Spartocera batatas* (Fabricius) (Coreidae), in which large heterochromatic blocks are located interstitially in all autosomal pairs (Franco, 2005).

Heterochromatin could be an active component of heteropteran chromosomes. Its accumulation in the karyotype of the species is not a random process and some constraints would regulate its acquisition and/or accumulation in different karyotypes.

There is still little information about heterochro-

matin base composition in Heteroptera, i.e. whether the heterochromatin is enriched in AT or GC bases as revealed by fluorescent banding techniques (DAPI and CMA_3 staining, respectively). Most reports referring to heterochromatin characterization describe C bands as DAPI-bright/CMA_3-dull. The presence of CMA_3 bright bands has been reported in a few species at interstitial or terminal position, either on autosomes or sex chromosomes, and they are generally associated to nucleolus organizer regions (González-García *et al*., 1996; Papeschi *et al*., 2001; Papeschi & Bressa, 2002; Papeschi *et al*., 2003; Rebagliati *et al*., 2003; Cattani *et al*., 2004; Ituarte & Papeschi, 2004; Bressa *et al*., 2005).

Sex chromosomes stain brightly with both fluorochromes, but their fluorescence probably represents differences in chromatin packaging between the autosomes and the sex chromosome rather than differences in base composition (Rebagliati *et al*., 2003).

Very striking differences in the amount and distribution of C positive heterochromatin have been observed in species of *Triatoma* Laporte (Reduviidae). According to Panzera and colleagues (1995), despite the fact that heterochromatin variations represent the main source of karyological differentiation among and within species, they do not appear to affect homologous chromosome pairing and then they should not play a direct role in the speciation process (Panzera *et al*., 1992; Pérez *et al*., 1992; Panzera *et al*., 1995).

CHROMOSOME NUMBER AND KARYOTYPE EVOLUTION

The male diploid chromosome number of Heteroptera ranges from 2n = 4 (*Lethocerus* sp., Belostomatidae) to 2n = 80 (four species of *Lopidea* Uhler, Miridae) (Fig. 1). However, the male diploid number of 14 is the most represented (460 species), followed by the diploid numbers 2n = 16 (186 species), 2n = 34 (92 species), 2n = 12 (89 species), 2n = 13 (80 species), 2n = 21 (70 species), 2n = 22 (76 species), and 2n = 24 (76 species). The Heteroptera comprise eight major groups, and cytogenetic reports are unequally distributed not only among these groups but also within them: Dipsocoromorpha (6 species), Gerromorpha (34 species), Nepomorpha (97 species), Leptopodomorpha (10 species), Cimicomorpha (407 species) and Pentatomomorpha (1016 species) (Table 1). Within the Cimicomorpha, the families Miridae and Reduviidae are the more extensively studied (168 and 125 species, respectively) and within the Pentatomomorpha, the families Coreidae, Lygaeidae and Pentatomidae are the best represented (108, 402 and 303 species, respectively).

Most discussions on karyotype evolution in Heteroptera use the concept of modal chromosome number, that is, the commonest number present in a group. Sometimes this concept can be applied at a family level; more often, it is applicable to lower categories such as tribes or genera. Many times, the modal number is considered the ancestral one for the group under analysis (Ueshima, 1979).

Among the principal mechanisms of karyotype evolution, autosomal fusions and both autosomal and sex chromosome fragmentations can be included (Ueshima, 1979; Manna, 1984; Thomas, 1987; Papeschi, 1994, 1996). Other chromosome rearrangements, such as inversions and reciprocal translocations have been rarely reported (Papeschi & Mola, 1990; Bressa *et al*., 1998; Pérez *et al*., 2004). When analyzing karyotype evolution at the family level, some karyotypes are highly homogeneous, while others show intensive processes of karyotype alterations.

The Pentatomidae are one of the largest families of Heteroptera with approximately 760 genera and 4100 species. The family is worldwide in distribution and well represented in all of the major faunal regions (Schuh & Slater, 1995). Within this family, 303 species and subspecies belonging to 126 genera have been cytogenetically analyzed. The male diploid numbers are between 6 and 27 with a modal number of 14 chromosomes, which is present in 85% of the species. The sex chromosome system is XY/XX, except for only three species: *Macropygium reticulare* (Fabricius) (X_1X_2Y), *Rhytidolomia senilis* (neo-X/neo-Y), and *Thyanta calceata* (Say) (X_1X_2Y) (Rebagliati *et al*., 2005). This family is cytogenetically highly homogeneous and karyotype changes probably played a minor role during speciation.

In Aradidae, at least 211 genera containing about 1800 species are currently classified in eight subfamilies. Aradids are represented in all major faunal regions with five out of the eight subfamilies being essentially cosmopolitan, but with three being restricted to the Australian-New Zealand-South American arc (Schuh & Slater, 1995). The known chromosome numbers of the Aradidae (33 taxa) range from 2n = 7 to 2n = 48 with two modal numbers of 27 and 14, and sex chromosome systems including XY, X_1X_2Y, $X_1X_2X_3Y$, $X_1X_2X_3X_4Y$ and neo-sex chromo-

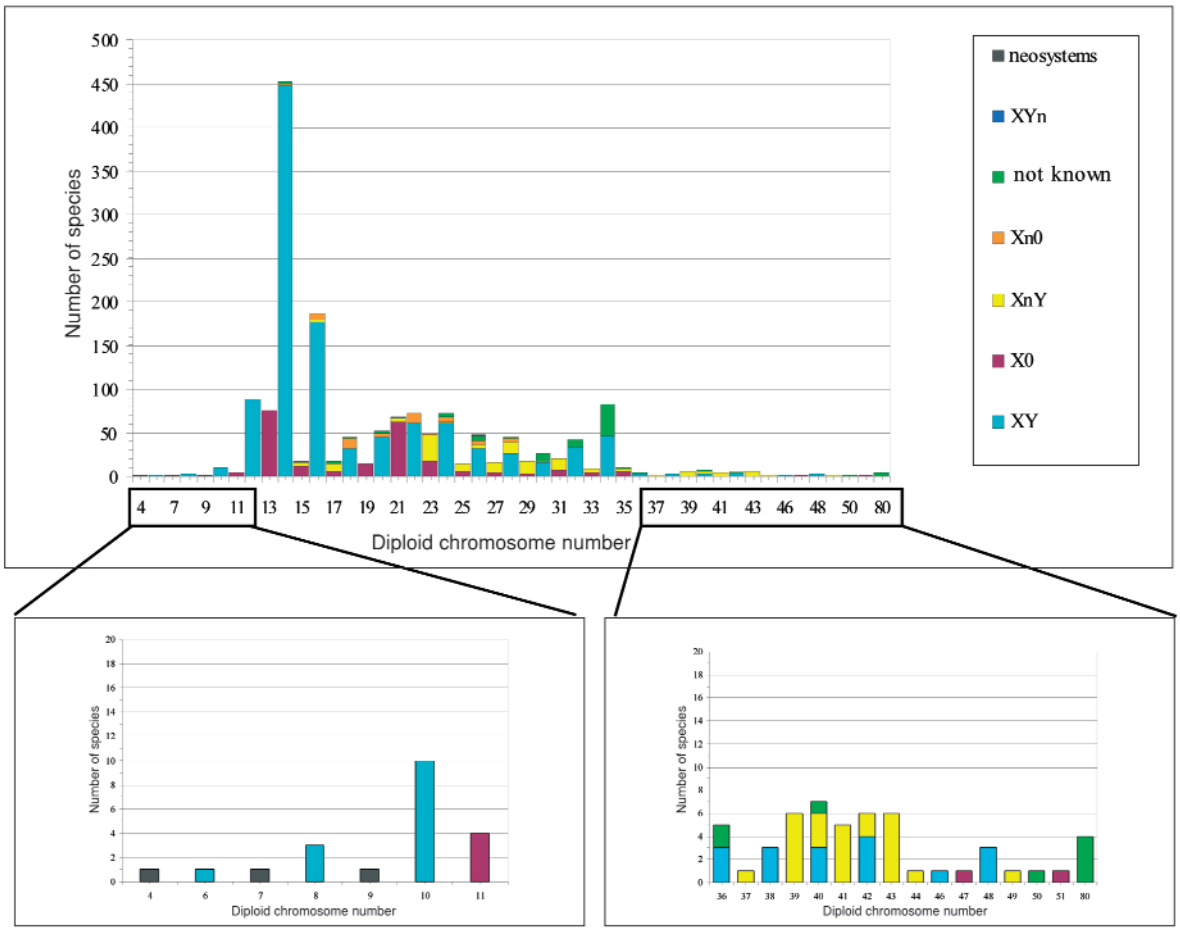

FIG. 1. Male diploid chromosome numbers and sex chromosome systems in Heteroptera.

somes (neo-XY_1Y_2 and neo-XY_1/neo-Y_2) (Jacobs & Groeneveld, 2002; Jacobs, 2004). The diversity in chromosome number and sex chromosome systems found in this family illustrates an intensive process of karyotype evolution. Although it is risky to speculate on the ancestral chromosome number of the Aradidae, Jacobs & Liebenberg (2001) suggested that since 2n = 12 + XY has been encountered in several genera in three of the six subfamilies thus far studied, it would probably represent the ancestral number of the family.

The Coreidae include at least 250 genera and 1800 species that are worldwide in distribution, but are more abundant in the tropics and subtropics (Schuh & Slater, 1995). The diploid number of the family ranges from 13 to 28, with a mode of 21, which is present in 47 out of the 108 species cytogenetically analyzed (43.5%). The Coreidae are also characterized by the possession of a pair of m chromosomes that has been described in 81.9% of the species (Ueshima, 1979; Franco, 2005). The most common sex chromosome system of the family is X0/XX (male/female) (64.3%) followed by the multiple system $X_1X_20/X_1X_1X_2X_2$ (32.1%). A multiple system $X_1X_2X_30$ in males has been ascribed to one population of *Coreus marginatus* Linnaeus (0.9%); and three species of *Acantocephala* (as *Metapodius*), namely *A. femorata* Fabricius, *A. granulosa* (Dallas), and *A. terminalis* (Dallas), have been reported as having an XY/XX system (2.7%). However, the three latter should be considered as a special situation, since the presence of a variable number of supernumeraries (B chromosomes) that associate with the X chromosome complicates the picture (Ueshima, 1979; Papeschi *et al*., 2003; Cattani & Papeschi, 2004; Cattani *et al*., 2004; Bressa *et al*., 2005).

A striking feature of the X_n0 system in this family is that the X chromosomes are generally intimately associated during male meiosis, and in most species the tightly conjoined Xs were positioned outside the

ring of autosomes on both metaphase plates. Manna (1984) pointed out that in species with the X_n0 sex chromosome system the number of X chromosomes should be confirmed in the spermatogonial complement, because during meiosis the multiple Xs tend to fuse and then appear as a single element. Similar observations have been made by different authors (Manna, 1951; Dutt, 1957; Parshad, 1957; Fossey & Liebenberg, 1995).

Besides the coreids, similar multiple sex chromosome system has been described in several species of Pyrrhocoridae (10 out of 19 species), Notonectidae (3 out of 14 species), Miridae (1 out of 168 species) and Stenocephalidae (1 out of 3 species) (Ueshima, 1979; Manna, 1984; Grozeva & Nokkala, 1996; Grozeva, 1997; Bressa *et al*., 1999; Nokkala & Nokkala, 1999; Bressa *et al*., 2003; Angus *et al*., 2004).

Two heteropteran families are included in the superfamily Pyrrhocoroidea: Largidae and Pyrrhocoridae. The former is represented in all major zoo-

TABLE 2. Diploid chromosome numbers in species of Pyrrhocoroidea

Taxa		2n	2n (male)	References
Largidae				
Larginae	*Largus cinctus* (Herrich-Schaeffer)	11	10+X0	(Wilson, 1909a)
	L. fasciatus Blanchard	13	12+X0	(Vidal & Lacau, 1985)
	L. humilis (Drury)	13	12+X0	(Piza, 1953)
	L. rufipennis Laporte	13	12+X0	(Piza, 1946; Mola & Papeschi, 1993; Bressa *et al.*, 1998)
	L. succintus Herrich-Schaeffer	13	12+X0	(Wilson, 1909a)
	Macrocheraia grandis Gray [= Lohita grandis (Gray)]	15	14+X0	Banerjee, 1958;, Banerjee, 1959; Manna & Deb-Mallick, 1981)
Physopeltinae	*Physopelta cinticollis* Stål	15	12+2m+X0	(Ueshima, 1979; Manna *et al.*, 1985)
	P. gutta Burmeister	17	12+2m+X_1X^2Y	(Manna *et al.*, 1985)
	P. quadrigutta Bergs.	17	12+2m+X_1X_2Y	(Manna *et al.*, 1985)
	P. schlanbuschi (Fabricius)	17	14+X_1X_2Y	(Ray-Chaudhuri & Manna, 1955)
Pyrrhocoridae	*Antilochus conqueberti* (Fabricius)	27	26+X0	(Parshad, 1957; Banerjee, 1958)
	Cenaeus abortivus Gerstäcker	33	32+X0	(Ueshima, 1979)
	Dysdercus albofasciatus Berg	12	10+neo-XY	(Bressa *et al.*, 1999)
	D. chaquensis Freiberg	13	12+X0	(Mola & Papeschi, 1997)
	D. cingulatus (Fabricius)	16	14+X_1X_20	(Sharma *et al.*, 1957; Banerjee, 1958; Manna & Deb-Mallick, 1981)
	D. evanescens Distant	16	14+X_1X_20	(Manna & Deb-Mallick, 1981)
	D. fasciatus Signoret	16	14+X_1X_20	(Banerjee, 1958)
	D. honestus Bloete	15	14+X0	(Piza, 1947b)
	D. imitator Bloete	13	12+X0	(Bressa, 2003)
	D. intermedius Distant	16	14+X_1X_20	(Ruthmann & Dahlberg, 1976)
	D. koenigii (Fabricius)	16	14+X_1X_20	(Ray-Chaudhuri & Banerjee, 1959)
	D. peruvianus Guérin-Menéville	16	14+X_1X_20	(Mendes, 1949; Piza, 1951)
	D. ruficollis Linnaeus	13	12+X0	(Piza, 1947b)
	Dysdercus sp.	16	14+X_1X_20	(Manna, 1951)
	D. superstitiosus (Fabricius)	16	14+X_1X_20	(Banerjee, 1958; Kuznetsova, 1988)
	Iphita limbata Stål	20	14+$X_1X_2X_3X_4X_5X_60$	(Banerjee, 1958)
	Odontopus sexpunctatus Laporte	27	26+X0	(Banerjee, 1958)
	Pyrrhocoris apterus (Linnaeus)	23	22+X0	(Henking, 1891; Wilson, 1909b)
	P. tibialis Stål	23	22+X0	(Takenouchi & Muramoto, 1967; Ueshima, 1979)
	Pyrrhopeplus posthumus Horvath	24	22+X_1X_20	(Parshad, 1957)
	Scantius volucris (Gerstäcker)	19	18+X0	(Parshad, 1957)

geographic regions, but is most abundant and diverse in the tropics and subtropics. Approximately 15 genera and over 100 species are known. The Pyrrhocoridae are chiefly tropical and subtropical, with only a very few species reaching into temperate Holarctic. However, they are found in all major zoogeographic regions. Approximately 30 genera and 300 species are known (Schuh & Slater, 1995).

From the cytogenetic point of view, 10 species of Largidae and 21 species of Pyrrhocoridae are known (Table 2). Six species of the subfamily Larginae lack m chromosomes and have an X0/XX sex chromosome mechanism. The number of autosomes varies between 10 and 14. All the studied species that belong to the other subfamily (Physopeltinae) possess 12 autosomes plus two m chromosomes, but different sex chromosome mechanisms (X0 or X_1X_2Y).

Cytologically, Pyrrhocoridae are a heterogeneous family and 21 species belonging to eight genera have been cytogenetically analyzed. Chromosome numbers range from 12 to 33. *Dysdercus* Guérin-Ménéville, the most studied genus within this family, is present both

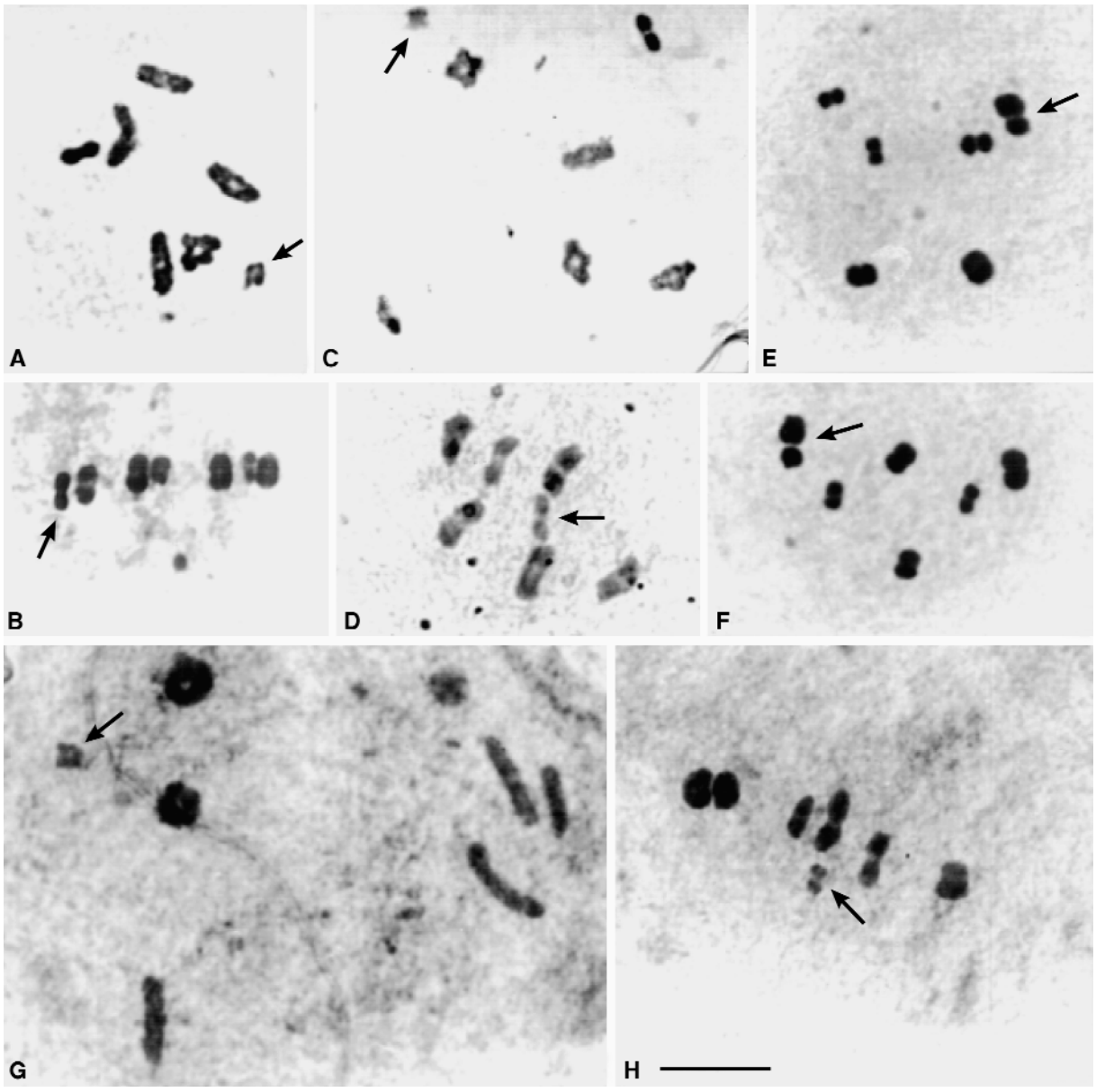

FIG. 2. *Dysdercus chaquensis*: cells at diakinesis (A) and metaphase I (B); *D. ruficollis*: cells at diakinesis (C) and metaphase I (D); *D. albofasciatus*: cells at diakinesis (E) and metaphase I (F); *D. imitator*: cells at diakinesis (G) and metaphase I (H). Arrows point sex chromosome in A-D, G, H and neo-XY bivalent in E, F. Bar = 10 μm. Haematoxylin staining.

in the Old and the New Worlds. So far, from the 13 cytogenetically analyzed species, seven belong to the Old World, while the remaining six are American species (Ueshima, 1979; Manna & Deb-Mallick, 1981; Mola & Papeschi, 1997; Bressa *et al*., 1999, 2002, 2003). The Old World species [(*Dysdercus cingulatus* (Fabricius), *D. evanescens* Distant, *D. fasciatus* Signoret, *D. intermedius* Distant, *D. koenigii* (Fabricius)], *Dysdercus* sp. and *D. superstitiosus* (Fabricius) share a common male diploid chromosome number of 16 and an $X_1X_20/X_1X_1X_2X_2$ sex chromosome system (Table 2) (Manna, 1951; Banerjee, 1958; Ray-Chaudhuri & Banerjee, 1959; Ruthmann & Dahlberg, 1976; Ueshima, 1979; Manna & Deb-Mallick, 1981; Kuznetsova, 1988). 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 system (X0, X_1X_20 and neo-XY, males) (Table 2) (Ueshima, 1979; Mola & Papeschi, 1997; Bressa *et al*., 1999, 2003). Considering 2n = 15 = 14 + X0 (male) as the ancestral chromosome complement from *Dysdercus*, the multiple sex chromosome system should have originated through fragmentation of the original X chromosome giving rise to a diploid chromosome number 2n = 16 = 14 + X_1X_20. Both situations are found in *Dysdercus honestus* (Mendes, 1947, 1949; Piza, 1947b, 1951) and *D. peruvianus* (Piza, 1947a, 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* (Bressa *et al*., 2003). Finally, a later fusion between the original X chromosome and an autosome took place during karyotype evolution, resulting in the neo-XY sex chromosome system in *D. albofasciatus*, which possesses the lowest diploid chromosome number of the genus reported so far (2n = 12 = 10 + neo-XY) (Fig. 2) (Bressa *et al*., 1999).

The Belostomatidae, often referred to as giant water bugs, are distributed worldwide, although their greatest diversity is in the tropics. According to Lauck & Menke (1961), the 146 species of the family can be grouped into three subfamilies: Lethocerinae (including the cosmopolitan genus *Lethocerus* Mayr), Horvathininae (represented only by the genus *Horvathinia* Montandon), and Belostomatinae (with the genera *Belostoma* Latreille, *Diplonychus* Laporte, *Hydrocyrius* Spinola, and *Limnogeton* Mayr). Cytogenetic reports on Belostomatidae refer to the male chromosome complement and meiosis of seventeen *Belostoma* species, three *Dyplonychus* species and seven *Lethocerus* species (Table 3). The modal diploid chromosome number is 2n = 26 + X_1X_2Y (present in 11 species of *Belostoma*), although in both subfamilies Belostomatinae and Lethocerinae, species with a noticeable reduction in diploid number are encountered: 2n = 14 + XY (three species of *Belostoma*), 2n = 6 + XY [*B. oxyurum* (Dufour) and *L. americanus* Leidy], and 2n = 2 + neo-X/neo-Y (*Lethocerus* sp.) (Chickering, 1927b; Chickering & Bacorn, 1933; Papeschi, 1992; Papeschi & Bressa, 2002). Our results, together with previous cytogenetic information on the family, led us to propose an ancestral karyotype 2n = 26 + XY, from which the present karyotypes 2n = 26 + X_1X_2Y should have derived through the fragmentation of the ancestral X chromosome. The analysis of a population of *Belostoma orbiculatum*, polymorphic for the sex chromosome system, lend support to this hypothesis (Papeschi, 1996). The DNA content has been determined in nine Argentine species of *Belostoma* and there are significant differences in the DNA content among them (Table 3). Heterochromatic C positive bands are terminally located, and since they are DAPI-bright and CMA_3-dull, they probably represent AT-rich DNA sequences (Figs 3, 4). The comparison of the DNA and heterochromatin content among the species suggests that both the amount of C positive heterochromatin and C negative chromatin changed during evolution. The species with reduced chromosome complements are also characterized by a low DNA content and very scarce C positive heterochromatin. We have suggested that these karyotypes probably originated from the ancestral chromosome complement through chromosome fusions. Besides, the scarce C positive heterochromatin implies either that the ancestral karyotype was devoid of this kind of heterochromatin, or else, that it became lost during the fusion events.

Finally, fluorescence *in situ* hybridization with an rDNA probe (Fig. 5) and DAPI and CMA_3 banding (Fig. 4) in *B. oxyurum* (2n = 6 + XY), *B. micantulum* (Stål) (2n = 14 + XY), and *B. elegans* (Mayr) (2n = 26 + X_1X_2Y) revealed the presence of a NOR at the telomeric region of the X and Y chromosomes in the first two species, but at the telomeric region of an autosomal pair in the latter. In the three species, the

TABLE 3. Diploid chromosome numbers, DNA and heterochromatin content in Belostomatidae

Taxa		2n	2n (male)	DNA (1C) (pg)	% Het.	References
Belostomatinae	*Belostoma bifoveolatum* Spinola	29	26+X_1X_2Y	1.21	60	(Papeschi, 1991)
	B. bergi (Montandon)	29	26+X_1X_2Y	–	–	(Papeschi & Bressa, 2004)
	B. cummingsi De Carlo	29	26+X_1X_2Y	–	–	(Papeschi & Bidau, 1985)
	B. dentatum (Mayr)	29	26+X_1X_2Y	1.93	58	(Papeschi & Bidau, 1985; Papeschi, 1991)
	B. dilatatum (Dufour)	29	26+X_1X_2Y	–	–	(Papeschi, 1992)
	B. discretum Montandon	29	26+X_1X_2Y	1.02	38	(Papeschi, 1992)
	B. elegans (Mayr)	29	26+X_1X_2Y	1.55	60	(Papeschi & Bidau, 1985; Papeschi, 1988, 1991)
	B. elongatum Montandon	29	26+X_1X_2Y	1.74	59	(Papeschi, 1992)
	B. gestroi Montandon	29	26+X_1X_2Y	1.13	37	(Papeschi, 1992)
	B. martini (Montandon)	29	26+X_1X_2Y	1.11	25	(Papeschi, 1991)
	B. micantulum (Stål)	16	14+XY	0.88	15	(Papeschi, 1988)
	B. orbiculatum Estévez & Polhemus	16	14+XY	–	–	(Papeschi, 1996)
	B. plebejum (Stål)	16	14+XY	–	–	(Papeschi, 1994)
	B. oxyurum (Dufour)	8	6+XY	0.53	30	(Papeschi & Bidau, 1985; Papeschi, 1988)
	B. indentatum (Haldeman)	29	26+X_1X_2Y	–	–	(Ueshima, 1979)
	B. flumineum (Say)	24	22+XY	–	–	(Chickering, 1916, 1927a)
	Belostoma sp.	24	22+XY	–	–	(Mongomery, 1901, 1906)
	Diplonychus annulatus (Fabricius)	28	26+XY	–	–	(Jande, 1959)
	D. rusticus (Fabricius)	28	26+XY	–	–	(Bawa, 1953; Jande 1959)
	D. subrhombeus (Mayr)	28	26+XY	–	–	(Jande, 1959)
Lethocerinae	*Lethocerus annulipes* (Herrich-Schaeffer)	28	26+XY	–	–	(Papeschi, 1992)
	Lethocerus americanus Leidy	8	6+XY	–	–	(Chickering, 1927b)
	L. griseus (Say)	28	26+XY	–	–	(Chickering, 1927b)
	L. indicum Lep. et Ser.	26	24+neoX-neoY	–	–	(Banerjee, 1958; Bagga, 1959; Jande, 1959)
	L. melloleitaoi De Carlo	28	26+XY	–	–	(Papeschi & Bressa, 2004)
	Lethocerus sp.	4	2+neoX-neoY	–	–	(Chickering, 1927a, 1932; Chickering & Bacorn, 1933)
	L. uhleri (Montandon)	ca. 30		–	–	(Chickering, 1932)

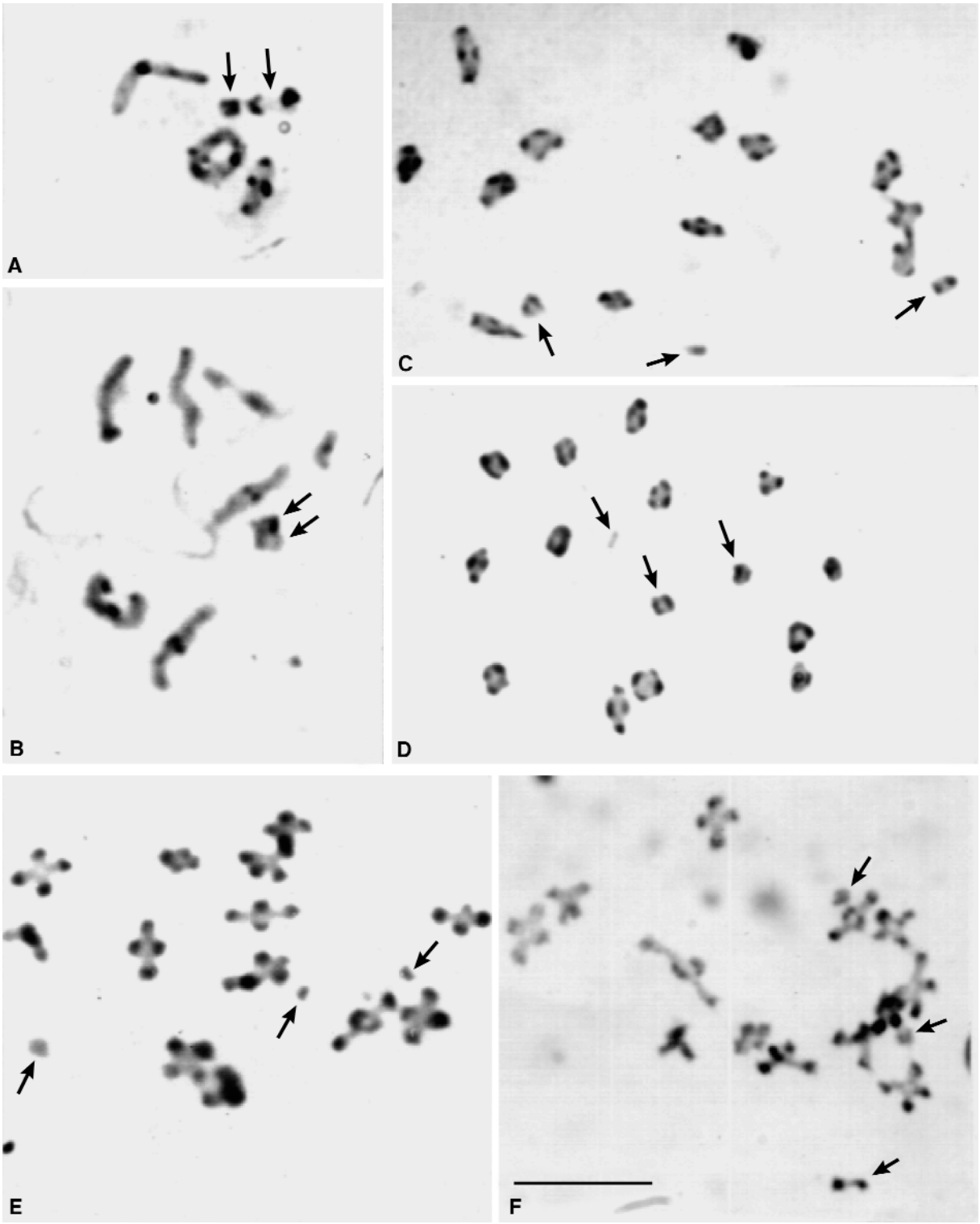

FIG. 3. C-banding of cells at diakinesis of *Belostoma oxyurum* (A), *B. micantulum* (B), *B. elegans* (C), *B. dilatatum* (D), *B. elongatum* (E), and *B. dentatum* (F). Arrows point sex chromosomes. Bar = 10 μm.

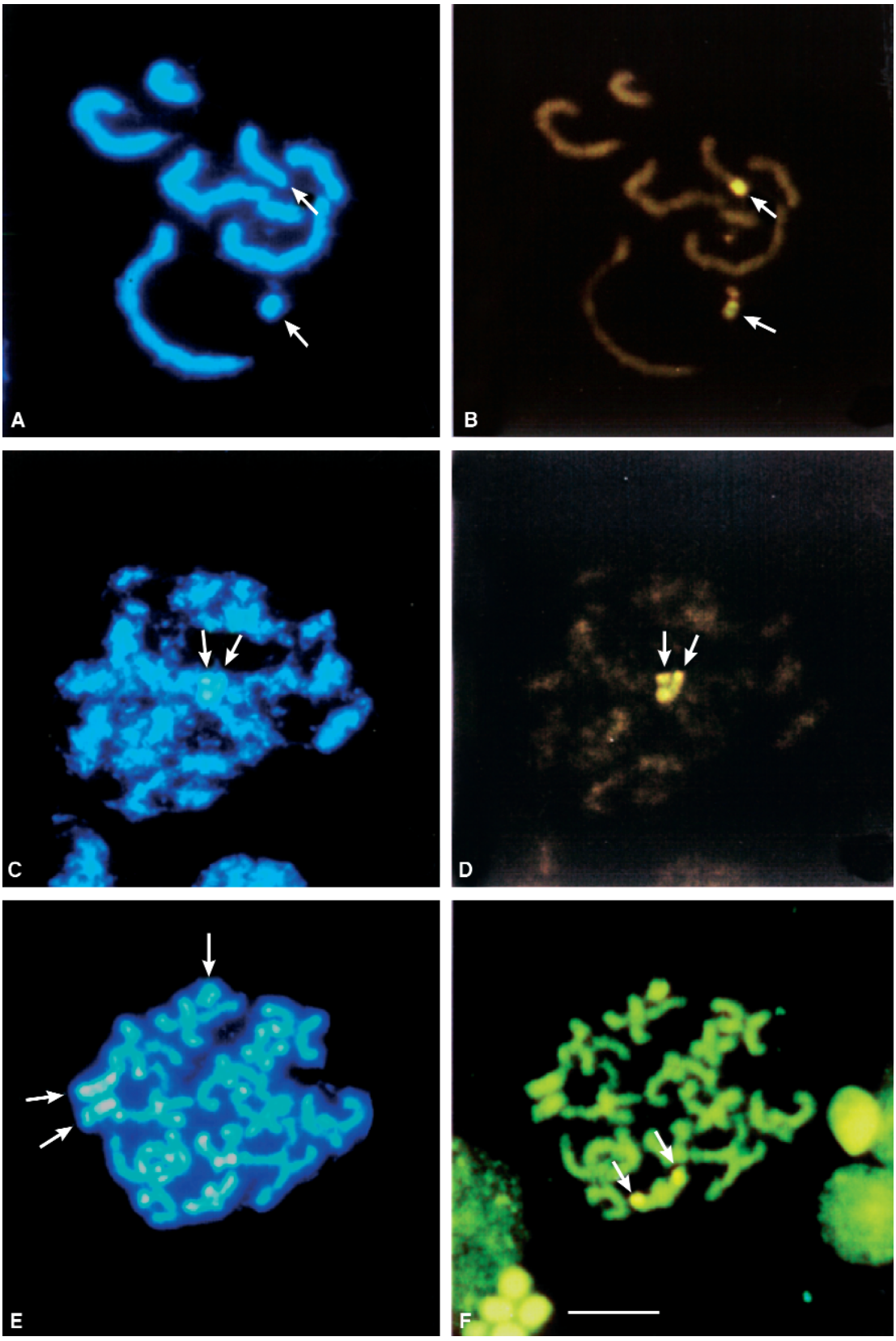

FIG. 4. DAPI (A, C, E) and CMA (B, D, F) banding in: spermatogonial prometaphase (A, B) of *Belostoma oxyurum*; diffuse stage (C, D) of *B. micantulum*; diakinesis (E, F) of *B. elegans*. Arrowheads point CMA_3-bright bands corresponding to NOR regions. Arrows point sex chromosomes. Bar = 10 μm.

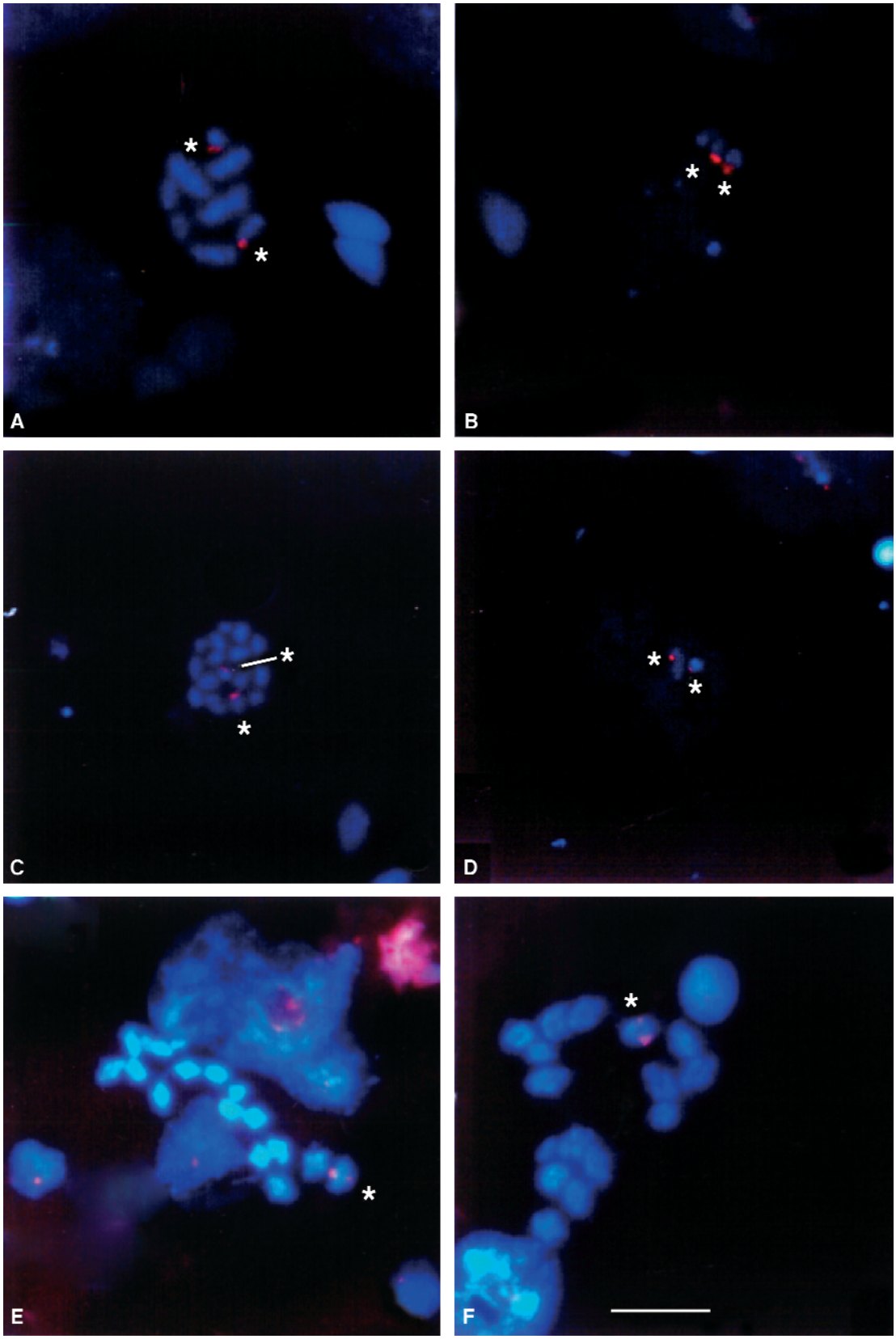

FIG. 5. Fluorescence *in situ* hybridization with an 808 bp 18S rDNA probe in: spermatogonial prometaphase (A) and diffuse stage (B) of *Belostoma oxyurum*; spermatogonial prometaphase (C) and diffuse stage (D) of *B. micantulum*; diakinesis (E, F) of *B. elegans*. Asterisks point rDNA regions. Bar = 10 μm.

NOR region is also detected as a CMA_3-bright/DAPI-dull band. These observations suggest that the reduction in the diploid number probably involved the fusion of the ancestral sex chromosome pair with the autosomes carrying the NOR. Alternatively, the rDNA gene cluster could have been translocated from an autosomal location (as in *B. elegans*) to the X and Y chromosomes (as detected in *B. micantulum* and *B. oxyurum*). Further studies in other species of *Belostoma* will shed light on the karyotype evolution within the genus.

CONCLUDING REMARKS

More than a hundred years have gone since Henking's first report on *Pyrrhocoris apterus*, but chromosome organization, function and evolution in Heteroptera is far from being fully understood. Molecular cytogenetic techniques are promising tools that will help uncover the mechanisms governing chromatin organization of holokinetic chromosomes, their behaviour during mitosis and meiosis, and the importance of chromosome change during the speciation of heteropterans.

Fusions and fragmentations, along with variations in the amount of heterochromatin, location and constitution are the principal mechanisms of karyotype evolution so far reported in Heteroptera. However, different genera and even families are karyotypically more variable than others, a fact that probably reflects differences in the biology of the species, and their environmental relationships. The coming years will probably outline some answers, but also bring about more new questions.

ACKNOWLEDGEMENTS

The authors wish to thank Dra. Lidia Poggio, Dr. Carlos Naranjo, and Dr. Axel Bachmann for their teachings and guidance. Different grants from CONICET, CIC, ANPCyT, Fundación Antorchas, UNLP and UBA made possible to carry out our investigations.

REFERENCES

Angus RB, Kemeny CK, Wood EL, 2004. The C-banded karyotypes of the four British species of *Notonecta* L. (Heteroptera: Notonectidae). *Hereditas*, 140: 134-138.

Bagga S, 1959. On the pre-reduction of the sex chromosomes during meiosis in a belostomatid *Lethocerus indicum*. *Proceedings of the zoological society of Calcutta*, 12: 19-22.

Banerjee MK, 1958. A study of the chromosomes during meiosis in twenty-eight species of Hemiptera (Heteroptera, Homoptera). *Proceedings of the zoological society of Calcutta*, 11: 9-37.

Banerjee MK, 1959. Chromosome elimination during meiosis in the males of *Macroceroea* (*Lohita*) *grandis* (Gray). *Proceedings of the zoological society of Calcutta*, 12: 1-8.

Bawa SR, 1953. Studies on insect spermatogenesis. I. Hemiptera-Heteroptera. The sex chromosomes and cytoplasmic inclusions in the male germ cells of *Laccotrephes maculatus* Fabr. and *Sphaerodema rusticum* Fabr. *Research bulletin east of the Panjab university*, 39: 181-192.

Bizzaro D, Manicardi GC, Bianchi U, 1996. Chromosomal localization of a highly repeated EcoRI DNA fragment in *Megoura viciae* (Homoptera, Aphididae) by nick translation and fluorescence *in situ* hybridization. *Chromosome research*, 4: 392-396.

Blanchelot A, 1991. A *Musca domestica* satellite sequence detects individual polymorphic regions in insect genome. *Nucleic acids research*, 19: 929-932.

Bressa MJ, 2003. Citogenética básica y evolutiva de Heterópteros fitófagos de interés agroeconómico de la Argentina. Tesis Doctoral, Universidad Nacional de La Plata.

Bressa MJ, Papeschi AG, Mola LM, Larramendy ML, 1998. Meiotic studies in *Largus rufipennis* (Castelnau) (Largidae, Heteroptera). II. Reciprocal translocation heterozygosity. *Heredity*, 51: 253-264.

Bressa MJ, Papeschi AG, Mola LM, Larramendy ML, 1999. 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. *Chromosome research*, 7: 503-508.

Bressa MJ, Fumagalli E, Ituarte S, Frassa MV, Larramendy ML, 2002. 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. *Hereditas*, 137: 125-131.

Bressa MJ, Papeschi AG, Fumagalli E, van Doesburg PH, Larramendy M, 2003. Cytogenetic and nucleolar meiotic cycle analyses in *Dysdercus imitator* Blöte, 1931 (Heteroptera, Pyrrhocoridae) from Argentina. *Folia biologica*, 51: 135-141.

Bressa MJ, Larramendy M, Papeschi AG, 2005. Heterochromatin characterization in five species of Heteroptera. *Genetica*, 124: 307-317.

Brutlag DL, 1980. Molecular arrangement and evolution of heterochromatic DNA. *Annual review of genetics*, 14: 121-144.

Buck RC, 1968. Mitosis and meiosis in *Rhodnius prolixus*:

the fine structure of the spindle and diffuse kinetochore. *Journal of ultrastructure research*, 18: 489-501.

Camacho JPM, Belda J, Cabrero J, 1985. Meiotic behaviour of the holocentric chromosomes of *Nezara viridula* (Insecta, Heteroptera) analysed by C-banding and silver impregnation. *Canadian journal of genetics and cytology*, 27: 490-497.

Cattani MV, Papeschi AG, 2004. Nucleolus organizing regions and semi-persistent nucleolus during meiosis in *Spartocera fusca* (Thurnberg) (Coreidae, Heteroptera). *Hereditas*, 140: 105-111.

Cattani MV, Greizerstein EJ, Papeschi AG, 2004. Male meiotic behaviour and nucleolus organizing regions in *Camptischium clavipes* (Fabr.) (Coreidae, Heteroptera) analyzed by fluorescent banding and *in situ* hybridization. *Caryologia*, 57: 267-273.

Chickering AM, 1916. A preliminary study of the spermatogenesis of *Belostoma flumineum*. *Transactions of the american microscopy society*, 35: 45-53.

Chickering AM, 1927a. Spermatogenesis in the Belostomatidae. II. The chromosomes and cytoplasmic inclusions in the male germ cells of *Belostoma flumineum* Say, *Lethocerus americanus* Leidy, and *Benacus griseus* Say. *Journal of morphology and physiology*, 44: 541-607.

Chickering AM, 1927b. An unusual complex in *Lethocerus*. *Anatomical record*, 37: 156.

Chickering AM, 1932. Spermatogenesis in the Belostomatidae. III. The chromosomes in the male germ cells of a *Lethocerus* from New Orleans, Louisiana. *Papers of Michigan academy of science*, 15: 357-360.

Chickering AM, Bacorn B, 1933. Spermatogenesis in the Belostomatidae. IV. Multiple chromosomes in *Lethocerus*. *Papers of Michigan academy of science*, 17: 529-533.

Comings DE, Okada TA, 1972. Holocentric chromosomes in *Oncopeltus*: kinetochore plates are present in mitosis but absent in meiosis. *Chromosoma*, 37: 177-192.

Dey SK, Wangdi T, 1990. Banding patterns of the holocentric chromosomes in some species of Heteroptera. *Cytologia*, 55: 181-186.

Dutt MK, 1957. Cytology of three species of coreid bugs with special reference to multiple sex chromosome mechanism. *Genetica*, 29: 110-119.

Fossey A, Liebenberg H, 1995. Meiosis and nucleolar structures in the stink bug *Carlisis wahlbergi* Stål (Coreidae: Heteroptera). *Cytobios*, 81: 7-15.

Franco M, 2005. Caracterización citogenética de especies de Coreidae (Heteroptera) mediante técnicas de bandas cromosómicas. Tesis de Licenciatura en Ciencias Biológicas, Universidad de Buenos Aires.

Frydrychová R, Grossmann P, Trubac P, Vitková M, Marec F, 2004. Phylogenetic distribution of TTAGG telomeric repeats in insects. *Genome*, 47: 163-178.

González-García JM, Antonio C, Suja JA, Rufas JS, 1996. Meiosis in holocentric chromosomes: kinetic activity is randomly restricted to the chromatid ends of sex univalents in *Graphosoma italicum* (Heteroptera). *Chromosome research*, 4: 124-132.

Grozeva S, 1997. Cytogenetic analysis of some aradid species (Heteroptera: Aradidae). *European journal of entomology*, 94: 421-424.

Grozeva S, Nokkala S, 1996. Chromosomes and their meiotic behavior in two families of the primitive infraorder Dipsocoromorpha (Heteroptera). *Hereditas*, 125: 31-36.

Grozeva S, Nokkala S, 2001. Chromosome numbers, sex determining systems, and patterns of the C-heterochromatin distribution in 13 species of lace bugs (Heteroptera, Tingidae). *Folia biologica*, 49: 29-41.

Grozeva S, Nokkala S, 2003. C-heterochromatin and extra (B) chromosome distribution in six species of the *Nabis* (Heteroptera, Nabidae) with the modal male karyotype 2n = 16 + XY. *Folia biologica*, 51: 13-21.

Grozeva S, Kuznetsova VG, Nokkala S, 2004. Patterns of chromosome banding in four nabid species (Heteroptera, Cimicomorpha, Nabidae) with high chromosome number karyotypes. *Hereditas*, 140: 99-104.

Halkka O, 1959. Chromosome studies on the Hemiptera Homoptera Auchenorrhyncha. *Annales academiae scientiarum fennicae*, 43: 1-71.

Hebsgaard MB, Andersen NM, Damgaard J, 2004. Phylogeny of the true water bugs (Nepomorpha: Hemiptera-Heteroptera) based on 16S and 28S rDNA and morphology. *Systematic entomology*, 29: 488-508.

Heitz E, 1933. Die somatischen Heteropyknose bei *Drosophila melanogaster* und ihre genetische Bedeutung. *Zeitschrift für zellforschung*, 20: 237-287.

Heitz E, 1935. Die Herkunft der Chromozentren. *Planta*, 18: 571-635.

Henking H, 1891. Untersuchungen über die ersten Entwicklungsvorgänge in den Eiern der Insekten. II. Über Spermatogenese und deren Beziehung zur Eientwicklung bei *Pyrrhocoris apterus* L. *Zeitschrift für wissenschaftliche zoologie*, 51: 685-736.

Hughes-Schrader S, Schrader F, 1961. The kinetochore of the Hemiptera. *Chromosoma*, 12: 327-350.

Ituarte S, Papeschi AG, 2004. Achiasmatic male meiosis in *Tenagobia (Fuscagobia) fuscata* (Heteroptera, Corixoidea, Micronectidae). *Genetica*, 122: 199-206.

Jacobs DH, 2004. The evolution of a neo-XY_1Y_2 sex chromosome system by autosome-sex chromosome fusion in *Dundocoris nodulicarinus* Jacobs (Heteroptera: Aradidae: Carventinae). *Chromosome research*, 12: 175-191.

Jacobs DH, Liebenberg H, 2001. Cytogenetics of *Adamanotus uncotibialis* Jacobs (Heteroptera: Aradidae). *Caryologia*, 54: 83-96.

Jacobs DH, Groeneveld HT, 2002. Cytogenetics and karyotype evolution of the genus *Silvacoris* Jacobs (Heteroptera: Aradidae: Carventinae) with a description of a statistical method to compare karyotypes. *Cytologia*, 67: 159-168.

Jande SS, 1959. An analysis of the chromosomes in the four species of the family Belostomatidae (Heteroptera, Cryptocerata). *Research bulletin (N.S.) of the Panjab university*, 10: 25-34.

Kuznetsova VG, 1988. The unusual mechanism of sex-chromosome determination in the cotton bug *Dysdercus superstitiosus* F. (Pyrrhocoridae, Heteroptera). *Doklady akademii nauk*, 301: 456-458.

Lauck DR, Menke AS, 1961. The higher classification of the Belostomatidae. *Annals of the entomological society of America*, 54: 644-657.

Mandrioli M, Bizzaro D, Giusti M, Manicardi GC, Bianchi U, 1999a. The role of rDNA genes in X chromosomes association in the aphid *Acyrthosiphon pisum*. *Genome*, 42: 381-386.

Mandrioli M, Bizzaro D, Manicardi GC, Gionghi D, Bassoli L, Bianchi U, 1999b. Cytogenetic and molecular characterization of a highly repeated DNA sequence in the peach potato aphid *Myzus persicae*. *Chromosoma*, 108: 436-442.

Mandrioli M, Ganassi S, Bizzaro D, Manicardi GC, 1999c. Cytogenetic analysis of the holocentric chromosomes of the aphid *Schizaphis graminum*. *Hereditas*, 131: 185-190.

Mandrioli M, Volpi N, 2003. The genome of the lepidopteran *Mamestra brassicae* has a vertebrate-like content of methyl-cytosine. *Genetica*, 119: 187-191.

Mandrioli M, Manicardi GC, Marec F, 2003. Cytogenetic and molecular characterization of the MBSAT1 satellite DNA in holokinetic chromosomes of the cabbage moth, *Mamestra brassicae* (Lepidoptera). *Chromosome research*, 11: 51-56.

Manicardi GC, Bizzaro D, Galli E, Bianchi U, 1996. Heterochromatin heterogeneity in the holocentric X chromatin of *Megoura viciae* (Homoptera:Aphididae). *Genome*, 39: 465-470.

Manna GK, 1951. A study of the chromosomes during meiosis in forty-three species of Indian Heteroptera. *Proceedings of the zoological society of Bengal*, 4: 1-116.

Manna GK, 1984. Chromosomes in evolution in Heteroptera. In: Sharma AK, Sharma A, eds. *Chromosomes in evolution of eukaryotic groups*. CRC Press, Boca Raton, Florida: 189-225.

Manna GK, Deb-Mallick S, 1981. Meiotic chromosome constitution in forty-one species of Heteroptera. *Chromosome information service*, 31: 9-11.

Manna GK, Ueshima N, Dey SK, Deb-Mallik S, 1985. Marked sex chromosomal variations between Indian and Japanese species of *Physopelta* (Largidae, Heteroptera). *Cytologia*, 50: 621-630.

Maudlin I, 1974. Giemsa banding of metaphase chromosomes in triatomine bugs. *Nature*, 252: 392-393.

Mc Clung CE, 1901. Notes on the accesory chromosome. *Anatomischer anzeiger*, 20: 220.

Mendes LOT, 1947. Sôbre a meiose de *Dysdercus mendesi* Bloete (1937) - Hemiptera, Pyrrhocoridae. *Bragantia*, 7: 243-256.

Mendes LOT, 1949. Observações citologicas em *Dysdercus*. Cadeias de cromossômios em tecido somático de *Dysdercus mendesi* Bloete *(Hemiptera-Pyrrhocoridae)*. *Bragantia*, 9: 53-57.

Mola LM, 1995. Post-reductional meiosis in *Aeshna* (Aeshnidae, Odonata). *Hereditas*, 122: 47-55.

Mola LM, Papeschi AG, 1993. Meiotic studies in *Largus rufipennis* (Castelnau) (Largidae, Heteroptera): frequency and behaviour of ring bivalents, univalents and B chromosomes. *Heredity*, 71: 33-40.

Mola LM, Papeschi AG, 1997. Citogenética de *Dysdercus chaquensis* (Heteroptera: Pyrrhocoridae). *Revista de la sociedad entomológica Argentina*, 56: 20.

Montgomery TH, 1901. Further studies on the chromosomes of the Hemiptera Heteroptera. *Proceedings of the academy of natural sciences of Philadelphia*, 53: 261-271.

Montgomery TH, 1906. Chromosomes in the spermatogenesis of the Hemiptera-Heteroptera. *Transactions of the American philosophical society*, 21: 97-173.

Motzko D, Ruthmann A, 1984. Spindle membranes in mitosis and meiosis of the heteropteran insect *Dysdercus intermedius*. A study of the interrelationship of spindle architecture and the kinetic organization of chromosomes. *European journal of cell biology*, 33: 205-216.

Nokkala C, 1985. Restriction of kinetic activity of holokinetic chromosomes in meiotic cells and its structural basis. *Hereditas*, 102: 85-88.

Nokkala S, 1986. The mechanisms behind the regular segregation of the m-chromosomes in *Coreus marginatus* L. (Coreidae, Hemiptera). *Hereditas*, 105: 73-85.

Nokkala S, Nokkala C, 1983. Achiasmatic male meiosis in two species of *Saldula* (Saldidae, Hemiptera). *Hereditas*, 99: 131-134.

Nokkala S, Nokkala C, 1984. The occurrence of the X0 sex chromosome system in *Dictyonota tricornis* (Schr.) (Tingidae, Hemiptera) and its significance for concepts of sex chromosome system evolution in Heteroptera. *Hereditas*, 100: 299-301.

Nokkala S, Nokkala C, 1999. Chromosomes in two bug species of *Hebrus* (Hebridae, Heteroptera). The occurrence of neo-XY sex chromosome system in Heteroptera. *Caryologia*, 52: 27-30.

Nordenskiöld H, 1962. Studies of meiosis in *Luzula purpurea*. *Hereditas*, 48: 503-519.

Panzera F, Alvarez F, Sanchez-Rufas J, Pérez R, Suja JA,

Scvortzoff E, Dujardin JP, Estramil E, Salvatella R, 1992. C-heterochromatin polymorphism in holocentric chromosomes of *Triatoma infestans* (Hemiptera: Reduviidae). *Genome*, 35: 1068-1074.

Panzera F, Perez R, Panzera Y, Alvarez F, Scvortzoff E, Salvatella R, 1995. Karyotype evolution in holocentric chromosomes of three related species of triatomines (Hemiptera-Reduviidae). *Chromosome research*, 3: 143-150.

Panzera F, Hornos S, Pereira J, Cestau R, Canale D, Diotaiuti L, Dujardin JP, Perez R, 1997. Genetic variability and geographic differentiation among three species of triatominae bugs (Hemiptera-Reduviidae). *American journal of tropical medicine and hygiene*, 57: 732-739.

Papeschi AG, 1988. C-banding and DNA content in three species of *Belostoma* (Heteroptera) with large differences in chromosome size and number. *Genetica*, 76: 43-51.

Papeschi AG, 1991. DNA content and heterochromatin variation in species of *Belostoma* (Heteroptera, Belostomatidae). *Hereditas*, 115: 109-114.

Papeschi AG, 1992. Estudios citogenéticos y evolutivos en Heteroptera. Tesis Doctoral, Universidad de Buenos Aires.

Papeschi AG, 1994. Chromosome rearrangements in *Belostoma plebejum* (Belostomatidae, Heteroptera). *Caryologia*, 47: 223-230.

Papeschi AG, 1996. Sex chromosome polymorphism in species of *Belostoma* (Belostomatidae, Heteroptera). *Hereditas*, 124: 269-274.

Papeschi AG, Bidau CJ, 1985. Chromosome complement and male meiosis in four species of *Belostoma* Latreille (Heteroptera-Belostomatidae). *Brazilian journal of genetics*, 8: 249-261.

Papeschi AG, Mola LM, 1990. Meiotic studies in *Acanonicus hahni* (Stål) (Coreidae, Heteroptera). II. Male chromosome behaviour in a spontaneous inversion mutant. *Genetica*, 81: 59-66.

Papeschi AG, Bressa MJ, 2002. Cytogenetic studies in Belostomatidae from Argentina. *Second quadrennial meeting of the international heteropterists society, St. Petersburg, Russia*.

Papeschi AG, Bressa MJ, 2004. Nuevos aportes al estudio citogenético de la familia Belostomatidae (Insecta, Heteroptera). XXXIII *Congreso Argentino de genética. Sociedad Argentina de genética, Malargüe, Mendoza*.

Papeschi AG, Mola LM, Rebagliati P, Rodriguez Gil S, Bressa MJ, 2001. Heterochromatin characterization in the holokinetic chromosomes of some Heteroptera, Odonata and Araneae with DAPI - CMA. Abstracts 14th Chromosome Conference, *Chromosome research*, 9: 75.

Papeschi AG, Mola LM, Bressa MJ, Greizerstein EJ, Lia V, Poggio L, 2003. Behaviour of ring bivalents in holokinetic systems: alternative sites of spindle attachment in *Pachylis argentinus* and *Nezara viridula* (Heteroptera). *Chromosome research*, 11: 725-733.

Parshad R, 1957. Cytological studies in Heteroptera II. Chromosome complement and meiosis in the males of three pyrrhocorid bugs. *Cytologia*, 22: 127-135.

Pérez R, Panzera Y, Scaffiezzo S, Mazzella MC, Panzera F, Dujardin JP, Scvortzoff E, 1992. Cytogenetics as a tool for Triatomine species distinction (Hemiptera-Reduviidae). *Memorias do instituto Oswaldo Cruz*, 87: 353-361.

Pérez R, Panzera F, Page J, Suja JA, Rufas JS, 1997. Meiotic behaviour of holocentric chromosomes: orientation and segregation of autosomes in *Triatoma infestans* (Heteroptera). *Chromosome research*, 5: 47-56.

Pérez R, Calleros L, Rose V, Lorca M, Panzera F, 2004. Cytogenetic studies on *Mepraia gajardoi* (Heteroptera: Reduviidae). Chromosome behaviour in a spontaneous translocation mutant. *European journal of entomology*, 101: 211-218.

Piza SdT, 1946. Comportamento dos cromossômios na meiose de *Euryophthalmus rufipennis* Laporte (Hemiptera-Pyrrhocoridae). *Anais da escola superior de agricultura "Luiz de Queiroz"*, 3: 27-54.

Piza SdT, 1947a. Comportamento dos cromossômios sexuais de *Dysdercus mendesi* na segunda divisão dos espermatócitos. *Bragantia*, 7: 269-271.

Piza SdT, 1947b. Cromossômios de *Dysdercus* (Hemiptera-Pyrrhocoridae). *Anais da escola superior de agricultura "Luiz de Queiroz"*, 4: 209-216.

Piza SdT, 1951. Interpretacão do typo sexual de "*Dysdercus mendesi*" Blöete (Hemiptera Pyrrhocoridae). *Genética ibérica*, 3: 107-112.

Piza SdT, 1953. The crucial proofs of the dicentricity of Hemiptera chromosomes. *Anais da escola superior de agricultura "Luiz de Queiroz"*, 10: 156-186.

Ray-Chaudhuri SP, Manna GK, 1955. Evidence of a multiple sex-chromosome mechanism in a pyrrhocorid bug, *Physopelta schlanbuschi* Fabr. *Proceedings of the zoological society of Calcutta*, 8: 65-77.

Ray-Chaudhuri SP, Banerjee MR, 1959. On the problem of sex-determination in the cotton stainer *Dysdercus koenigii* Fabr. (Pyrrhocoridae, Heteroptera). *The nucleus*, 2: 209-214.

Rebagliati P, Papeschi AG, Mola LM, 2003. Meiosis and fluorescent banding in *Edessa meditabunda* and *E. rufomarginata* (Heteroptera: Pentatomidae: Edessinae). *European journal of entomology*, 100: 11-18.

Rebagliati P, Mola LM, Papeschi AG, Grazia J, 2005. Cytogenetic studies in Pentatomidae (Heteroptera): A review. *Journal of zoological systematics and evolutionary research*, 43: 199-213.

Ruthmann A, Dahlberg R, 1976. Pairing and segregation of the sex chromosomes in X_1X_2-males of *Dysdercus*

intermedius with a note on the kinetic organization of heteropteran chromosomes. *Chromosoma*, 54: 89-97.

Sahara K, Marec F, Traut W, 1999. TTAGG telomeric repeats in chromosomes of some insects and other arthropods. *Chromosome research*, 7: 449-460.

Schrader F, 1940. The formation of tetrads and the meiotic mitoses in the male of *Rhytidolomia senilis* Say (Hemiptera Heteroptera). *Journal of morphology*, 67: 123-141.

Schrader F, Hughes-Schrader S, 1956. Polyploidy and fragmentation in the chromosomal evolution of various species of *Thyanta* (Hemiptera). *Chromosoma*, 7: 469-496.

Schrader F, Hughes-Schrader S, 1958. Chromatid autonomy in *Banasa* (Hemiptera:Pentatomidae). *Chromosoma*, 9: 193-215.

Schuh RT, Slater J, 1995. *True bugs of the world (Hemiptera: Heteroptera): classification and natural history*. Cornell University Press, New York.

Sharma GP, Sud GC, Parshad R, 1957. Chromosome complement and the sex mechanism in *Dysdercus cingulatus* (Pyrrhocoridae-Heteroptera). *Caryologia*, 9: 278-285.

Solari AJ, Agopian S, 1987. Recombination nodules, synaptonemal complexes and heterochromatin in the hemipteran *Triatoma infestans*. *Microscopía electrónica y biología celular*, 11: 179-195.

Spence JM, Blackman RL, Testa JM, Ready PD, 1998. A 169 bp tandem repeat DNA marker for subtelomeric heterochromatin and chromosomal re-arrangement in aphids of the *Myzus persicae* group. *Chromosome research*, 6: 167-175.

Suja JA, del Cerro AL, Page J, Rufas JS, Santos JL, 2000. Meiotic sister chromatid cohesion in holocentric sex chromosomes of three heteropteran species is maintained in absence of axial elements. *Chromosoma*, 109: 35-43.

Sumner AT, 2003. *Chromosomes: organization and function*. Blackwell Science, Malden, USA.

Takenouchi Y, Muramoto N, 1967. A survey of the chromosomes in twenty species of Heteroptera insects. *Journal of Hokkaido university (Educ. IIB)*, 18: 1-15.

Thomas DBJ, 1987. Chromosome evolution in the Heteroptera (Hemiptera): agmatoploidy versus aneuploidy. *Annals of the entomological society of America*, 80: 720-730.

Ueshima N, 1963. Chromosome behavior of the *Cimex pilosellus* complex (Cimicidae: Hemiptera). *Chromosoma*, 14: 511-521.

Ueshima N, 1979. Hemiptera II: Heteroptera. In: John B, ed. *Animal cytogenetics*. Gebrüder Borntraeger, Berlin-Stuttgart: Vt117.

Vidal OR, Lacau IM, 1985. Los cromosomas de *Euryophtalmus fasciatus* Blanchard (Heteroptera: Pyrrhocoridae). *Physis secc. c*, 43: 13-17.

Vítková M, Král J, Traut W, Zrzavý J, Marec F, 2005. The evolutionary origin of insect telomeric repeats, $(TTAGG)_n$. *Chromosome research*, 13: 145-156.

Wilson EB, 1905. Studies on chromosomes. II. The paired microchromosomes, idiochromosomes and heterotropic chromosomes in Hemiptera. *Journal of experimental zoology*, 2: 507-545.

Wilson EB, 1909a. The female chromosome groups in *Syromastes* and *Pyrrhocoris*. *Biological bulletin*, 16: 199-204.

Wilson EB, 1909b. Studies on chromosomes. IV The "accessory" chromosome in *Syromastes* and *Pyrrhocoris* with a comparative review of the types of sexual differences of the chromosome groups. *Journal of experimental zoology*, 6: 69-99.