CLASSICAL AND MOLECULAR CYTOGENETICS IN HETEROPTERA

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Abstract

Cytogenetic studies in Heteroptera began more than a hundred years ago and classical and molecular cytogenetic techniques have contributed to get a deeper insight into the organization, function and evolution of the holokinetic chromosomes in this insect group. In this article we describe the general cytogenetic features of Heteroptera and give some examples of evolutionary trends within some families. Changes in karyotype are intimately related to the evolutionary process, and karyotype analyses can give us valuable clues to phylogeny, evolution and taxonomic relationships.

Introduction

Cytogenetics developed from the originally separated sciences of genetics and cytology, and is concerned with the relationship between genetic and cytological (principally chromosomal) features characterizing the genetic system of the species. This field of investigation comprises the study of the organization of chromosomes, their behavior during mitosis and meiosis, and their relation to the transmission and recombination of genes. Chromosomal characters are relatively simple features, closely representing the organism's genotype that can give valuable clues to phylogeny, evolution and taxonomic relationships.

Cytogenetics comprises descriptive and functional researches. It is based on light and electronic microscopy, and along time it has made use of very different techniques that allowed precise chromosome visualization and contributed to a better knowledge of chromosome organization. Among standard cytogenetic techniques the use of specific stains favored the observation of chromatin through the cell cycle, the squash technique contributed to a better analysis of meiosis, the use of colchicine and hypotonic solutions helped to the description of karyotypes, and different chromosome banding techniques revealed a longitudinal differentiation of chromosomes [1-3]. In the field of molecular cytogenetics one of the more recent advances is the fluorescent *in situ* hybridization technique, which uses fluorescent labeled DNA probes on chromosome preparations and makes it possible to localize specific DNA sequences on particular chromosomes or chromosome segments [2,4].

The karyotype is the particular chromosome complement of an individual or of a related group of individuals. Karyotypes may differ in respect to the basic chromosome number, the shape and relative size of the chromosomes, the number and location of secondary constrictions, the absolute size of the chromosomes, the distribution, composition and size of hetero and euchromatic chromosome segments, and the total genomic DNA content. In most species the karyotype remains remarkably stable, and closely related species have generally more similar karyotypes than distantly related ones. Species evolve by multiple mechanisms, and changes in karyotype are characteristic of the evolutionary process [4-6].

Karyotype analysis may be limited by the techniques and the material itself. In general, the study of insect chromosomes is difficult due to the small number of cells available for karyotype analyses and the problem of obtaining good chromosome spreads. Although such cells do exist in many tissues, it is very difficult to determine the best time to carry out chromosome preparations. This time varies greatly within the same order, from one family to another, and even from one genus to another. In addition, cell development cycles can be interrupted by diapauses, during which all the cells are quiescent. Mitotic chromosomes are generally obtained from larval cerebral ganglia but they can also be obtained from spermatogonias and oogonias; however, it is difficult to determine the period of active gametogenesis which can occur either in the larval stages or in the imago. Male gonads are very frequently used because they are more easily observed than female gonads under the binocular microscope and, in addition, the testes are richer in dividing cells. For chromosome preparations besides the commonly used squash method, other procedures such as the spreading technique can be used. The longitudinal characterization of insect chromosomes has been achieved by C-banding in many different groups, and NOR-banding has been frequently used to reveal those nucleolus organizing regions that were active in the previous interphase. With the development of C-banding it was possible a better characterization of karyotypes. The original use of C-banding was to identify chromosomal sites of heterochromatin, to detect heterochromatin heteromorphisms as a marker to distinguish homologues, and to study chromosome evolution. Identification of heterochromatin sites, their size, range of variability and composition are all essential aspects of the characterization of a species karyotype [4]. Methods of banding using fluorochromes that are specific for particular DNA bases (such as DAPI or chromomycin A_3) are important since they provide extra information on insect chromosomes organization. Modern cytogenetic techniques such as fluorescent bandings and fluorescent *in situ* hybridization (FISH)

have only just begun to make an impact in insect cytogenetics [2].

One important cytogenetic difference among insects is the kinetic nature of their chromosomes. While most insect groups possess chromosomes with localized centromeres (monocentric chromosomes), chromosomes with non-localized centromeres (holokinetic chromosomes) have been found in Dermaptera, Heteroptera, homopterans Sternorrhyncha and Auchenorrhyncha, Lepidoptera, Odonata, Phthiraptera (Mallophaga and Anoplura), Psocoptera, Trichoptera and Zoraptera [7]. The absence of localized centromeres limits basic karyotype descriptions to chromosome number and size; furthermore, this particular kinetic organization brings about differences in the mitotic and meiotic behaviour of the chromosomes as well as in the relative frequency of different chromosome rearrangements.

Cytogenetics in Heteroptera

Cytogenetic studies in Heteroptera date from 1891 with Henking's morphological study on the spermatogenesis of the bug *Pyrrhocoris apterus* Linnaeus (Pyrrhocoridae) [8]. The earliest reports that followed referred to the male chromosome number and sex chromosome system in different heteropteran species, and some incomplete descriptions of male meiosis (for revisions see [9,10]). At present approximately 1600 heteropteran species belonging to 46 families have been cytogenetically analyzed; however, they are by no means a representative sample of the group (4.2% of approximately 38000 described species) [11].

As mentioned before, some serious constraints limit comparative and evolutionary cytogenetics of this insect group. Since heteropterans possess holokinetic chromosomes which are generally relatively small and of similar size no detailed analysis of their morphology is possible. Karyotype descriptions had been limited to the chromosome number, their relative size and the sex chromosome system. The behavior of the holokinetic chromosomes of Heteroptera is different in mitosis and meiosis, and even during meiosis the behavior is also different for autosomes, sex chromosomes and m chromosomes. During mitosis holokinetic chromosomes attach to the spindle fibers along all their length and at mitotic anaphase sister chromatids segregate parallel to each other and perpendicular to the polar spindle [12]. During meiosis the kinetic activity is restricted to telomeric regions and the chromosomes can be regarded as telokinetic [13]. As a rule, autosomal bivalents are chiasmatic while the sex chromosomes and m chromosomes are achiasmatic. The autosomal bivalents segregate reductionally during meiosis I and equationally during meiosis II; the m chromosomes associate at first meiotic division forming a pseudo-bivalent and segregate reductionally at anaphase I. On the other hand, sex chromosomes 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", segregating reductionally at anaphase II [9,10].

One distinctive cytogenetic feature of some Heteroptera families is the presence of a pair of "m chromosomes" [9]. Wilson [14] introduced this term to describe the smallest chromosome pair in species of Coreidae, which behaves differently from both the autosomes and the sex chromosomes during male meiosis. Although the m chromosomes are generally of small size, they are actually defined by their meiotic behavior. The m chromosomes have been reported in species of Corixidae, Naucoridae, Notonectidae, Pleidae, Saldidae, Colobathristidae, Lygaeidae, Largidae, Alydidae, Coreidae, Rhopalidae and Stenocephalidae (Table 1).

Sex chromosome systems described so far in Heteroptera are simple systems of the types XY/XX (71.4%) 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-systems are very rare and until present they have only been reported in seven species and subspecies (0.5%) namely *Lethocerus indicus* Lep. et Servielle and *Lethocerus* sp Mayr (Belostomatidae), *Rhytidolomia senilis* (Say) (Pentatomidae), *Hebrus pusillus* Fallén (Hebridae), *Dundocoris nodulicarinus novena* Jacobs and *D. nodulicarinus septeni* Jacobs (Aradidae) and *Dysdercus albofasciatus* Berg (Pyrrhocoridae) [15]. It is generally accepted that the multiple systems probably originated from simple systems through the fragmentation of the atavic X or Y chromosome [16] while neo-systems can have a more complex origin [17,18].

Most cytologists agree that fusions and fragmentations are the principal mechanisms of karyotype evolution in Heteroptera [9,10,15]. Although DNA measurement is the best means of testing fusion/fragmentation hypothesis in karyotype evolution of holokinetic chromosomes, this technique has been seldom carried out in this group of insects [15,19,20]. Apart from the classical cytospectro-photometric procedure which is used to measure DNA content in spermatids stained with Shiff´s reagent, flow cytometry is an easy, rapid, accurate and convenient tool for estimating genome size, assessing DNA content and

analyzing the cell cycle [2]. However, the latter has not yet been applied in Heteroptera.

More than twenty years ago we started our studies in Heteroptera cytogenetics particularly in Belostomatidae, and later in species of Coreidae, Corixidae, Largidae, Lygaeidae, Naucoridae, Pentatomidae, Pyrrhocoridae, Reduviidae and Rhopalidae. We have focused our attention on different cytogenetic subjects such as the organization and behavior of holokinetic chromosomes, the mechanisms of karyotype evolution and their relation to the speciation processes.

Karyotype evolution

The diploid chromosome number of Heteroptera ranges from 2n= 4 (*Lethocerus* sp., Belostomatidae) to 80 (four species of *Lopidea* Uhler, Miridae). The most represented diploid number is 14 (460 species), but about 70% of the species have diploid numbers between 12 to 34 chromosomes (Table 1). Heteroptera comprises 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), within the Pentatomomorpha the families Coreidae, Lygaeidae and Pentatomidae are the best represented (108, 402 and 303 species, respectively) and within Nepomorpha, the families Belostomatidae and Corixidae (27 and 28 species, respectively) [9,10,15].

Among the aquatic heteropteran families, the **Belostomatidae (Nepoidea)** are often referred to as giant water bugs and are worldwide distributed, but their greatest diversity is in the tropics. According to Lauck and Menke [21] the 146 species of the family can be grouped in 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 in Belostomatidae describe the male chromosome complement and meiosis of seventeen *Belostoma* species, three *Dyplonychus* species and seven *Lethocerus* species. 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.) [15,22-24]. Our results with standard cytogenetic techniques, DNA content measurements, C and fluorescent bandings, and fluorescent *in situ* hybridization (FISH) with a rDNA probe led us to suggest that the atavic karyotype of the family is 2n= 26+XY with scarce C positive heterocromatin. This karyotype is still represented by *Lethocerus melloleitaoi* De Carlo and *Lethocerus annulipes* Herrich-Schaeffer. From this karyotype evolutionary changes involved a) the fragmentation of the atavic X chromosome to give rise to the multiple sex chromosome mechanism X_1X_2Y and b) the increase in heterochromatin content; these characteristics are observed in medium and large sized belostomatids such as *B. elegans* and *B. dentatum*. A different evolutionary trend led to the reduction in diploid numbers (probably through autosomal and/or sex chromosome fusions) and characterizes the small sized species of *Belostoma* [16,25,26].

The **Corixidae** (**Corixoidea**) occur worldwide in various types of both stable and temporary continental and insular, fresh and saline waters [11,27]. Cytogenetic reports on 28 species of this family describe a diploid chromosome number of 24 (except in *Cymatia borsdorfii* (Sahlberg), 2n= 26), with a pair of m chromosomes and an XY/XX sex chromosome system. Our recent results in *Sigara (Tropocorixa) denseconscripta* (Breddin), *S. (T.) rubyae* (Hungerford) and *S. (T.) chrostowskii* Jaczewski reveal similarities in karyotype and meiotic behavior to the species previously analyzed and suggest that this family is cytogenetically homogeneous (unpublished data).

The **Naucoridae** (**Naucoroidea**) live in a variety of aquatic environments. Although the group occurs worldwide, the greatest diversity is conspicuously in the tropics. Cytogenetic studies in the family are scarce, but all the species possess a pair of m chromosomes and an X0/XX sex chromosome system: *Ambrysus mormon* Montandon and *A. pulchellus* Montandon share a diploid number of 20+2m+X0, *Limnocoris* sp has 26+2m+X0 and *Pelocoris femoratus* Palisot de Beauvois possesses 2n= 20 [9,28]. The Argentinean species *Pelocoris lautus* Berg, *P. binotulatus binotulatus* (Stål) and *P. b. nigriculus* Berg have 2n= 18+2m+X0 and a similar meiotic behavior [22].

Taking in account the phylogenetic relationships of families and superfamilies of Nepomorpha proposed by Rieger [29] and Mahner [30]

the most primitive superfamilies Nepoidea and Gelastocoroidea show simple and multiple sex chromosome systems (XY and X_nY), and lack m chromosomes. The Corixoidea and the more recent superfamilies Naucoroidea and Notonectoidea present a pair of m chromosomes and they also show different sex chromosome systems (X0/XX and $X_1X_20/X_1X_1X_2X_2$). The available cytogenetic data suggest that the absence of m chromosomes and the presence of X and Y sex chromosomes could be considered plesiomorphic characters for Nepomorpha. The sex chromosome systems X0 and X_1X_20 should have originated during evolution through the loss of the Y chromosomes and a later fragmentation of the atavic X chromosome; these sex chromosome systems together with the presence of a pair of m chromosomes could be considered as derived characters for this group.

Almost no work has been done on phylogenetic relationships among the 29 families recognized in the Pentatomomorpha [11]. **Coreidae** includes at least 250 genera and 1800 species that are worldwide in distribution, but are more abundant in the tropics and subtropics [11]. The diploid number of the family ranges from 13 to 28 with a mode in 21, which is present in 47 out of the 108 species cytogenetically analyzed (43.5%). Coreidae is also characterized by the possession of a pair of m chromosomes that has been described in 81.9% of the species [9,31,32]. The most common sex chromosome systems of the family are X0/XX (male/female) (64.3%) and the multiple system $X_1X_20/X_1X_1X_2X_2$ (32.1%).

Most reports on C positive heterochromatin in Heteroptera show that C bands are terminally located [19,33-35]. However, recent reports describe different distribution patterns. In *Tenagobia fuscata* (Stål) (Corixidae), *Triatoma patagonica* Del Ponte (Reduviidae) and *Nezara viridula* (Linnaeus) (Pentatomidae) most bands are terminally located, but one autosomal pair presents a conspicuous C positive band in an interstitial position (corresponding at least in the latter to a nucleolus organizer region) [36-40]. Another distribution pattern was observed in 13 species of Tingidae and four species of *Nabis* (Nabidae) in which very scarce C positive heterochromatin was located either at telomeric positions or, in some chromosomes, at interstitial ones [41,42]. Available cytogenetic data in coreid species suggest that this family presents a great diversity in heterochromatin composition, amount and localization. *Athaumastus haematicus* (Stål) and *Eubule sculpta* (Perty) karyotypes are completely devoid of C positive heterochromatin; *Camptischium clavipes* (Fabricius) and *Leptoglossus impictus* (Stål) present very scarce heterochromatin terminally located; *Phthia picta* (Drury) has conspicuous heterochromatic C positive bands at telomeric regions in all the autosomes and the sex chromosome, in *Petillia patullicollis* Walker (Coreidae) most bands are terminally located, but one autosomal pair presents a conspicuous C positive band in an interstitial position [37], and *Spartocera batatas* (Fabricius) [31] and *Holhymenia rubiginosa* Breddin (Coreidae) are completely different because all or almost all autosomes have large C-positive bands interstitially located,[32,33] (unpublished results).

Heterochromatin seems to have important functions in the genetic system of Heteroptera and in view of the different and diverse patterns of C bands in coreid species we suggest that heterochromatin is probably an active component in the karyotype evolution of this family. Furthermore, since very different heterochromatin distribution patterns are observed some constraints would regulate the acquisition and/or accumulation of heterochromatin in the karyotype of each species. Further studies are necessary to understand the role of heterochromatin in the karyotype evolution of coreids.

The **Rhopalidae** are closely related to Coreidae and Alydidae. Although Rhopalidae has often been considered to be a subfamily of an inclusive Coreidae modern workers treat it as a distinct family. The subfamilies Rhopalinae and Serinethinae comprise 18 genera and 209 recognized species and are distributed in all major faunal regions in both the Old and the New Worlds [11]. Within this family, 24 species from 12 genera have been cytogenetically analyzed so far and all species are characterized by a constant male/female diploid number of 13/14 (10+2m+X0/XX), the possession of a pair of m chromosomes and an X0/XX (male/female) sex chromosome system [9]. Among the Serinethinae, cytogenetic data on the genus *Jadera* Stål with 18 species described is scarce [11,43]. Our results of *Jadera haematoloma* (Herrich-Schaeffer) and *J. sanguinolenta* (Fabricius) demonstrate that both species share the cytogenetic characteristics of the family, with males carrying 13 (10+2m+X0) chromosomes [44].

The role of the m chromosomes in Heteroptera cytotaxonomy has been shown to be quite significant. The m chromosomes are absent in Pentatomoidea and in many other families, while they are present in some primitive taxa (Table 1) [10]. According to Manna [10], this pair of chromosomes could have originated later by degradation of a pair of autosomes. Nothing is known about the genetic information that the m chromosomes

carry and there is still no information about their function in the genetic system of the species possessing them [15]. Since they are generally small sized, largely heteropycnotic during male meiosis and achiasmatic it is possible that they represent some kind of selfish DNA.

Up to the present, **Lygaeidae** (Lygaeoidea) consist of 16 subfamilies comprising at least 500 genera and approximately 4000 valid species that are distributed in all faunal regions both in the Old and in the New Worlds. This family is probably paraphyletic, consequently taxonomically difficult to characterize and the complex relationships among its members are far to be determined [11]. The subfamily Lygaeinae represents a large taxon with fifty-eight genera and about 500 species currently recognized. So far, the cytogenetic analysis of 26 species belonging to the Lygaeinae reveal a chromosome diploid number that varies from 12 to 22, with a sex chromosome system XY/XX, except *Arocatus suboeneus* Montandon which shows a multiple system $X_1X_2Y/X_1X_1X_2X_2$ [45]. Moreover, only five species of *Lygaeus* Fabricius have been cytogenetically analysed, namely *L. equestris* (Linnaeus) [46,47], *L. kalmii kalmii* Stål [45], *L. turcicus* Fabricius [48], *L. simla* Distant [49] and *L. alboornatus* Blanchard [50]. With the exception of *L. alboornatus* (2n= 12= 10+XY) and *L. simla* (2n= 22= 20+XY), the remaining three species share a diploid chromosome number of 14, and chromosomes do not differ much in their size. Our results on *L. alboornatus* reveal that this species shows the lowest chromosome number for members of Lygaeinae. Taking in account the presence of a remarkable big-sized pair of autosomes in this species, it seems highly probable that its karyotype originated from the ancestral complement through one autosomal fusion [50].

Within the superfamily Coreoidea, Alydidae (23 species cytogenetically analyzed) and Rhopalidae share a modal karyotype of 2n= 10+2m+X0, while Stenocephalidae (3 species) possess 2n= 10+2m+XY and could be envisaged as a bridge between Coreoidea and the Lygaeoidea. Furthermore, the Coreidae are unique among the Coreoidea for the high diploid number, which almost doubles the plesiomorphic one (2n= 12+XY) [10]. Considering the holokinetic nature of heteropteran chromosomes, the apomorphic karyotype of the Coreidae could have originated through chromosome fragmentations.

From a cytogenetic point of view eleven species of **Largidae** and 21 species of **Pyrrhocoridae** are known (**Pyrrhocoroidea**). The six species of the subfamily Larginae lack m chromosomes and have an X0/XX sex chromosome mechanism and the number of autosomes varies between 10 and 14 [51-53]. 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) [9].

Cytologically, **Pyrrhocoridae** are a heterogeneous family and 21 species belonging to eight genera have been analyzed. Chromosome numbers range from 12 to 33 with a modal number of 16. *Dysdercus* Guérin-Méneville, the most studied genus within this family, is present both in the Old and the New Worlds. So far, from the 13 cytogenetically analized species, seven belong to the Old World while the remaining six are American species. 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 [9,54-59]. The six neotropical species (*Dysdercus albofasciatus* Berg, *D. chaquensis* Freiberg, *D. peruvianus* Guérin Méneville, *D. ruficollis* (Linnaeus), *D. honestus* Bloete and *D. imitator* Bloete) 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) [9,17,59-67]. From these reports we suggest that 2n= 15= 14+X0 (male) should be the ancestral chromosome complement from *Dysdercus*. The multiple sex chromosome system should have originated through fragmentation of the atavic X chromosome (2n= 16= $14+X_1X_20$ present in *D. honestus* and *D. peruvianus*). 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* and *D. ruficollis* as well as in *D. imitator*. Finally, a later fusion between the original X chromosome and an autosome originated the neo-XY sex chromosome system present in *D. albofasciatus*, which possesses the lowest diploid chromosome number of the genus reported so far (2n= 12= 10+neo-XY) [17]. The fluorescent *in situ* hybridization (FISH) experiments with 18S rDNA probes in *Dysdercus albofasciatus* revealed two clusters of rDNA genes located on the sex chromosome neo-X. Since no hybridization signals were obtained in the neo-Y chromosome, the rDNA genes are located in the ancestral X-parts of the neo-X chromosome (unpublished data).

The reports on the number and location of nucleolus organizing regions (NORs) 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 [1]. An accurate localization of active and total number of NORs can only be achieved through silver impregnation techniques and fluorescent *in situ* hybridization (FISH) with rDNA probes, respectively.

In most heteropteran species a single NOR has been detected, but its location varies among species. In *Edessa meditabunda* (Fabricius) (Pentatomidae) and in *Carlisis wahlbergi* Stål (Coreidae) the Ag-NOR technique revealed the NOR at the telomeric position of one autosomal pair [68,69]. FISH with rDNA probes, however, gave diverse results concerning the NOR chromosome location. In *Belostoma oxyurum* (Dufour) and *B. micantulum* (Stål) (Belostomatidae) the NOR is located at the telomeric region of both the X and Y chromosomes; in *Largus rufipennis* Laporte (Largidae), *Graphosoma italicum* (Müller) (Pentatomidae) and *Triatoma platensis* Neiva (Reduviidae) it is found at the telomeric region of the X chromosome, while in *Dysdercus albofasciatus* Berg (Pyrrhocoridae) the signals are detected in the neo-X chromosome [15,70]. Conversely, the rDNA genes cluster is located in one autosomal pair at interstitial position in *Pachylis argentinus* Berg and *Nezara viridula* (Linneus) (Pentatomidae), and at one autosomal telomeric region in *B. elegans* (Mayr) (Belostomatidae), *Phthia picta* (Drury), *Spartocera fusca* Thunberg, *Camptischium clavipes* (Fabricius) (Coreidae), *Triatoma tibiamaculata* (Pinto) and *T. protacta* (Uhler) (Reduviidae) [15,36,39,70-73]. With the exception of *C. wahlbergi* all these NORs are associated with CMA bright bands indicating that the whole rDNA repeating unit is rich in GC. These results suggest that rDNA genes are excellent markers for the study of karyotype evolution and, in particular, sex chromosome evolution in true bugs.

Among the principal mechanisms of karyotype evolution in Heteroptera, autosomal fusions/fragmentations and sex chromosome fragmentations (which originate multiple sex chromosome systems) are to be mentioned [9,10,16,26,74]. Other chromosome rearrangements such as inversions and reciprocal translocations have been rarely reported [53,75]. It has been suggested that reciprocal translocations have greater chances of establishing in species with holokinetic chromosomes, since all the products of the rearrangement are transmissible at meiosis [5]. However, taking in consideration the frequency of reciprocal translocations in the different groups of organisms with holokinetic chromosomes, and its cytological and genetic consequences, it can be assumed that in general they are strongly negatively selected.

Future perspectives

Karyotypes are species–specific characters that have evolved through natural selection and can contribute to understand evolution and taxonomic relationships. In recent years the development of molecular cytogenetic tools such as chromosome painting, whole genome *in situ* hybridization (GISH) and comparative genome hybridization (CGH) have enabled us to get an insight into the evolutionary relationships in vertebrates principally mammals. It will not be long before these approaches are also used in insects and will help to clarify the phylogenetic relationships within Heteroptera. Furthermore, these molecular techniques will contribute to uncover the mechanisms governing chromatin organization of holokinetic chromosomes, their behavior during mitosis and meiosis, and the importance of chromosome change during the speciation of heteropterans.

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Table 1. Cytogenetics features of heteropteran families

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