Cytogenetic and Genome Research

Cytogenet Genome Res 2010;128:77–87
DOI: 10.1159/000298824

Published online: April 19, 2010

Cytogenetics and Genome Evolution in the Subfamily Triatominae (Hemiptera, Reduviidae)

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Key Words

Chagas disease vectors · Chromosome evolution · Cytogenetics · Flow cytometry · Genome size · Hemiptera · Holocentric chromosomes · Triatominae

Abstract

The subfamily Triatominae (Hemiptera, Reduviidae), vectors of Chagas disease, includes over 140 species. Karyotypic information is currently available for 80 of these species. This paper summarizes the chromosomal variability of the subfamily and how it may reveal aspects of genome evolution in this group. The Triatominae present a highly conserved chromosome number. All species, except 3, present 20 autosomes. The differences in chromosome number are mainly caused by variation in the number of sex chromosomes, due to the existence of 3 sex systems in males (XY, X_1X_2Y and $X_1X_2X_3Y$). However, inter- and intraspecific differences in the position, quantity and meiotic behavior of constitutive heterochromatin, in the total genome size, and in the location of ribosomal 45S rRNA clusters, have revealed considerable cytogenetic variability within the subfamily. This cytogenetic diversity offers the opportunity to perform cytotaxonomic and phylogenetic studies, as well as structural, evolutionary, and functional analyses of the genome. The imminent availability of the complete genome of *Rhodnius prolixus* also opens new perspectives for understanding the evolution and genome expression of triatomines. The application of fluorescence in situ hybridization for the mapping of genes and sequences, as well as comparative analyses of genome homology by comparative genomic hybridization will be useful tools for understanding the genomic changes in relation to evolutionary processes such as speciation and adaptation to different environments.

Triatominae are classified as a subfamily of the Reduviidae (Hemiptera, Heteroptera) customarily defined by their blood-sucking habit and morphological adaptations associated with host-finding and blood-feeding. The subfamily currently includes 140 species mainly distributed in the New World, occupying diverse habitats from southern Argentina to the North American Great Lakes [Schofield and Galvão, 2009]. These insects are considered an exceptional chromosome model because they have holocentric chromosomes characterized by the presence of a diffuse or non-localized centromere [Hughes-Schrader and Schrader, 1961]. They are also the vectors of Chagas disease or American trypanosomiasis, recognized as the

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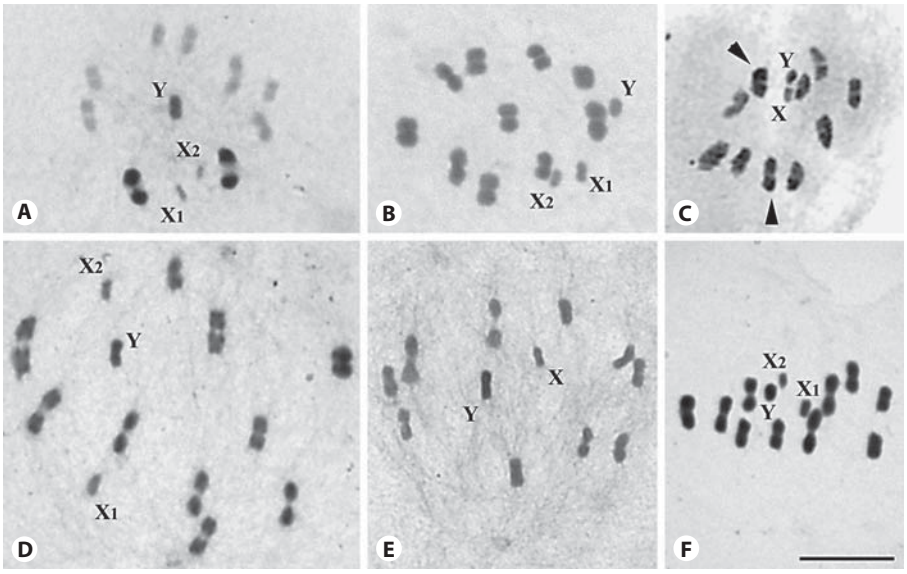

Fig. 1. Interspecific variation of chromosome number in the subfamily Triatominae. All photographs correspond to metaphase I of spermatogenesis, except **C** which is metaphase II. **A** *Triatoma nitida* (2n = 18A + X_1X_2Y), C-banding. Two autosomal bivalents and the Y chromosome are mainly heterochromatic. **B** *Belminus corredori* (2n = 20A + X_1X_2Y), Orcein staining. **C** *Rhodnius domesticus* (2n = 20A + XY), C-banding. The Y chromosome is heterochromatic and some autosomes exhibit C-blocks (arrowheads). **D** *Panstrongylus chinai* (2n = 20A + X_1X_2Y), Orcein staining. **E** *Triatoma guazu* (2n = 20A + XY), C-banding. **F** *T. phyllosoma* (2n = 20A + X_1X_2Y), Orcein staining. A = Autosomes. Scale bar = 10 μm.

most serious human parasitic disease of Latin America in terms of its social and economic impact, with around 7–11 million people infected and a further 60 million at risk of infection [Schofield et al., 2006]. Owing to their cytogenetic and medical importance, the Triatominae have been subject to many genetic studies. In this paper we review the karyological and genome size variability of triatomines and their contribution to understanding the genome evolution of the subfamily.

Holocentric Nature of the Chromosomes of Triatomines: Behavior and Segregation

Holocentric chromosomes are restricted to certain scattered groups of animals and plants, being particularly well represented in insects. All heteropteran species have holocentric chromosomes that are characterized by a unique mitotic and meiotic behavior [Ueshima, 1979]. During mitosis, spindle fibers attach to the chromosome by means of a trilaminar kinetochore that occurs over a wide area of the poleward chromatid surface [Buck, 1967]. These holocentric interactions of microtubule fibers lead to a parallel segregation of sister chromatids during mitotic anaphase [Hughes-Schrader and Schrader, 1961].

During meiosis, microtubules attach directly to the chromatin without a kinetochore structure [Buck, 1967]. This attachment is restricted to the telomeres and kinetic activity in autosomes and sex chromosomes can be observed to either of the 2 chromatid ends during the first and second meiotic anaphase [Nokkala, 1985; Pérez et al., 1997]. Autosomal bivalents of Heteroptera were assumed to present complete chiasma terminalization and to be disposed with the long axes perpendicular to the equatorial plate (axial orientation) [White, 1973]. However, our studies in triatomines using banding techniques did not support this generalization [Pérez et al., 1997, 2000]. In *Triatoma infestans*, the single observed chiasma can occur in any part of the chromosome and it is not terminalized at first metaphase. Furthermore, as either chromatid end can show kinetic activity, 2 alternative orientations are possible for a given bivalent at first metaphase. We demonstrated that the chromosomal end that shows kinetic activity in the first meiotic division is inactive in the second division (reversal of kinetic activity) [Pérez et al., 2000].

Sex chromosomes follow a different mode of segregation than that observed in the autosomes. The sex chromosomes in heteropteran males are achiasmatic and behave as univalents during the first meiotic division [Solari, 1979]. At first metaphase, the sex chromosomes are usually disposed at the equatorial plate, occupying the center of a ring formed by the autosomal bivalents. Although they appear together, there is no visible physical connection between them ('associated at a distance') (figs. 1 and 4) [Ueshima, 1979]. During anaphase I, sister chromatids of the sex chromosomes segregate poleward resulting in equational division. In metaphase II, the chromosomes appear associated end-to-end to form a pseudobivalent, which is disposed in the center of the

Table 1. List of the Triatominae species cytogenetically described until now, discriminated by diploid chromosome number (2n) in males

2n	Genus: species
18A + X_1X_2Y = 21	***Panstrongylus:*** *megistus* ***Triatoma:*** *nitida*
20A + XY = 22	***Psammolestes:*** *coreodes, tertius* ***Rhodnius:*** *brethesi, colombiensis, domesticus, ecuadoriensis, milesi*, nasutus, neglectus, neiva, pallescens, pictipes, prolixus, robustus, stali* ***Dipetalogaster:*** *maximus* ***Paratriatoma:*** *hirsuta* ***Triatoma:*** *arthurneivai, brasiliensis, carcavalloi*, circummaculata, costalimai, garciabesi, delpontei, guasayana, guazu, infestans, juazeirensis, jurbergi, lecticularia, maculata, matogrossensis, melanica, patagonica, petrochiae, platensis, pseudomaculata, rubrovaria, sherlocki*, sordida, vandae*, williami*
20A + X_1X_2Y = 23	***Belminus:*** *herreri, corredori** ***Eratyrus:*** *cuspidatus, mucronatus* ***Mepraia:*** *gajardoi, spinolai* ***Panstrongylus:*** *chinai, geniculatus, howardi*, lignarius, rufotuberculatus, tupynambai* ***Triatoma:*** *barberi, bassolsae, bruneri*, dimidiata, sp. aff. dimidiata, flavida, gerstaeckeri, hegneri, longipennis, mazzottii, mexicana*, pallidipennis, peninsularis, phyllosoma, picturatus, protracta, rubida, ryckmani, sanguisuga, sinaloensis, tibiamaculata*
20A + $X_1X_2X_3Y$ = 24	***Triatoma:*** *eratyrusiformis, vitticeps*
22A + X_1X_2Y = 25	***Triatoma:*** *rubrofasciata*

A = Autosomes. The asterisks indicate the species reported here for the first time.

ring of autosomes (figs. 1 and 4). During anaphase II, these chromatids segregate to opposite poles showing reductional divisions. This reversion in the order of segregation of sex chromosomes is called inverted meiosis or post-reductional segregation [Hughes-Schrader and Schrader, 1961]. Our results on the segregation of these chromosomes have revealed that, as observed in the autosomes, there is a reversal in the kinetic activity of the chromosomal ends during both meiotic divisions [Pérez et al., 2000].

Chromosome Number: Interspecific Variation

Of the 140 recognized species of triatomines, included in 15–19 genera [Galvão et al., 2003; Schofield and Galvão, 2009], karyotypic information is currently available for 80 species of the following 9 genera: *Belminus*, *Dipetalogaster, Eratyrus, Mepraia*, *Panstrongylus*, *Paratriatoma, Psammolestes, Rhodnius* and *Triatoma* [Ueshima, 1966; Panzera et al., 1998; Dujardin et al., 2002]. Chromosome numbers for more than half of the species were reported by our group and 8 of them are described here for the first time (table 1, figs. 1–4). The diploid number in triatomines is very homogenous, ranging from 21 to 25 chromosomes in males and from 22 to 26 in females. The number of autosomes is remarkably constant. All species present 20 autosomes (A), with the exception of 2 *Triatoma* species (18A and 22A) and 1 species of *Panstrongylus* (18A) (table 1).

The main differences in chromosome number are caused by variation in the number of sex chromosomes due to the existence of 3 sexual systems in males (XY, X_1X_2Y and $X_1X_2X_3Y$) (figs. 1 and 4). A fourth sex mechanism, $X_1X_2Y_1Y_2$, has been described [Frias et al., 1998], but may have resulted from a misinterpretation [Calleros et al., 2010]. All species of one genus have the same sex mechanism, with the exception of the genus *Triatoma* which exhibits all 3 sex mechanisms (table 1). Interestingly, all *Triatoma* species from North America have multiple sex chromosomes (except *T. lecticularia*), while most *Triatoma* species from South America exhibit an XY system.

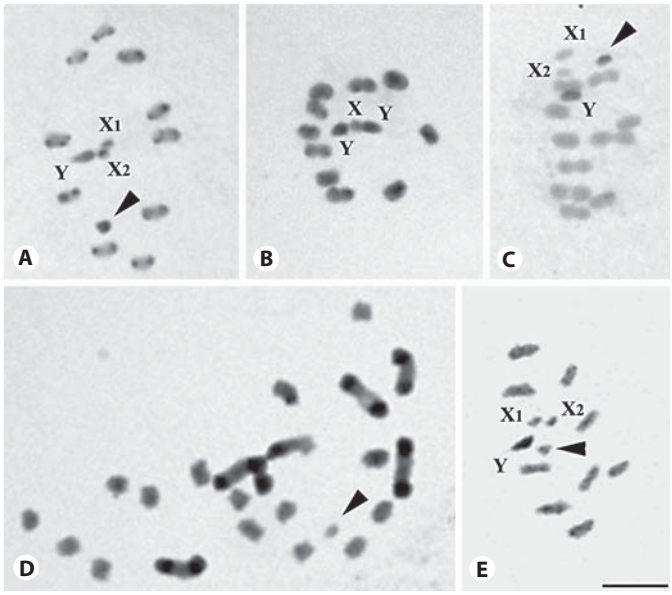

Fig. 2. Intraspecific variation due to supernumerary chromosomes in the chromosome complement of the subfamily Triatominae. C-banding. **A**, **B** *Mepraia spinolai* (2n = 20A + X_1X_2Y), second meiotic divisions of the same male individual. In **A** we observed a heterochromatic B chromosome (arrowhead) plus the normal complement (10 autosomal pairs and 3 sex chromosomes). In **B**, we observed an altered metaphase II plate with 2 Y chromosomes and only 1 X chromosome. **C** *Triatoma longipennis* (2n = 20A + X_1X_2Y), first meiotic division. The supernumerary chromosome appears heterochromatic (arrowhead), similar to the Y chromosome. **D** *Triatoma infestans* (2n = 20A + XY), the spermatogonial mitotic prometaphase shows an additional or supernumerary euchromatic chromosome (arrowhead). **E** *Mepraia gajardoi* (2n = 20A + X_1X_2Y), second meiotic division in a mutant individual (male). A euchromatic chromosome fragment (arrowhead) that possibly originated as a by-product of an autosomal translocation appears associated with the normal sex chromosomes. Besides, 9 autosomal pairs are observed instead of the 10 pairs usually found in normal individuals (without translocation). A = Autosomes. Scale bar = 10 μm.

Chromosome Number: Intraspecific Variability

The intraspecific variation in the chromosome number of Triatominae is mainly restricted to the occurrence of B chromosomes. Supernumerary chromosomes, accessory or B chromosomes, appear in the cells additionally to the normal complement (A chromosomes) [Camacho, 2005]. They are found in a great diversity of organisms, generally segregate irregularly and often are present in varying numbers, both between individuals of the same or different populations, and sometimes even between different cells of the same individual. They are considered by many authors as genomic parasites, since in most cases they apparently do not provide any benefit to the individuals carrying them [White, 1973]. In Triatominae, we detected these B chromosomes in 3 species: *Mepraia spinolai*, *Triatoma longipennis* and *T. infestans* (fig. 2) [Panzera et al., 2007a; this paper].

In *M. spinolai* we observed, in mitosis and meiosis, both in males and females, the occurrence of 1 to 3 supernumerary chromosomes (euchromatic and/or heterochromatic) at a very variable frequency even within the same individual (fig. 2A). Due to their spatial proximity to the sex chromosomes, these chromosomes can affect the normal segregation of the sex chromosomes during both meiotic divisions. Individuals with these chromosomes have a very small percentage of cells with a variable number of sex chromosomes (both X and/or Y) (fig. 2B).

In few individuals (3 out of 40) of *T. longipennis,* we detected a single heterochromatic B chromosome being present in all mitotic and meiotic cells. In meiosis, it is situated close to the sex chromosomes (similar to that observed in *M. spinolai*) (fig. 2C). However, in *T. longipennis* the additional chromosomes do not seem to affect the segregation of sex chromosomes as observed in *M. spinolai*.

In *T. infestans*, putative B chromosomes are observed in mitosis (fig. 2D). They are small, mainly heterochromatic, and their frequency varies from 1 to 3 according to the geographical origin of the individual studied. These chromosomes are not detected in meiotic divisions, and there is no evidence of alteration in the meiotic segregation of carrier individuals.

These examples illustrate the variability of supernumerary chromosomes in Triatominae, both in their number, frequency, size, and staining, and especially in their genetic consequences. These features also suggest the existence of different mechanisms of formation of B chromosomes in this group of insects. It has been suggested that B chromosomes originated as chromosomal remnants of structural rearrangements of the autosomes; in a mutant male of *M. gajardoi,* chromosomal fragments resembling B chromosomes originated as by-products of an autosomal translocation [Pérez et al., 2004]. The presence of these fragments dramatically interferes with the normal segregation of the regular chromosomal complement (both autosomes and sex chromosomes), producing non-viable gametes and affecting the fecundity of the individual with the autosomal rearrangement (fig. 2E) [Pérez et al., 2004].

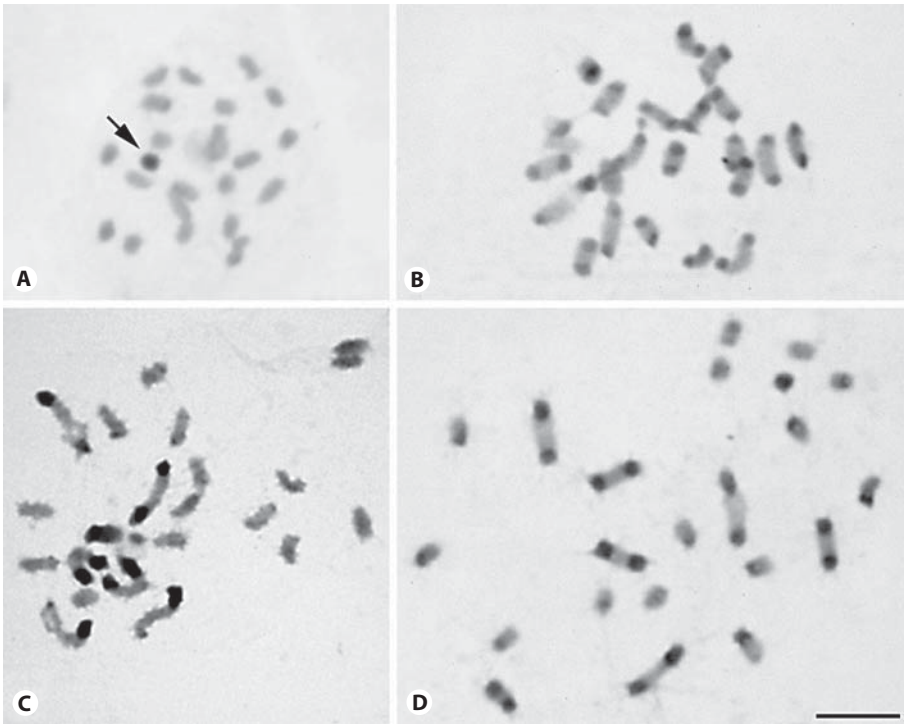

Fig. 3. Inter- and intraspecific variability of autosomal C-heterochromatin in Triatominae. Spermatogonial prometaphases, C-banding. **A** *Triatoma costalimai* (2n = 20A + XY). All autosomes are euchromatic. Only the Y chromosome appears heterochromatic (arrow). **B** *Triatoma protracta* (2n = 20A + X_1X_2Y). All autosomes present a heterochromatic block in each chromosomal end. **C**, **D** *Triatoma infestans* (2n = 20A + XY), intraspecific variability. **C** Non-Andean group. 4 to 7 autosomes have C-heterochromatin. **D** Andean group. Most autosomes (10 pairs) have one or both chromosomal ends with C-heterochromatin blocks. A = Autosomes. Scale bar = 10 μm.

Changes in the Distribution and Behavior of the C-Heterochromatin

In Heteroptera, the C-banding technique [Sumner, 1972] is the main tool for longitudinal differentiation of the holocentric chromosomes [Papeschi and Bressa, 2007]. This technique detects chromosomal regions of constitutive heterochromatin mainly composed of tandemly repeated (satellite) and dispersed (transposable elements) DNA [Gregory, 2005]. As the Triatominae are characterized by a high homogeneity in the chromosome number, the application of C-banding has proved very useful to characterize and differentiate species, especially within the highly variable genus *Triatoma* [Pérez et al., 1992, 2002; Panzera et al., 1995, 1997, 2004, 2006].

Our studies in Triatominae indicate that the autosomal heterochromatin is characterized by high interspecific variability, including changes in its quantity, size, location and behavior during meiosis (figs. 1–5). Heterochromatin quantity can vary between 0 and 45% of the total autosomal complement [Panzera et al., 1995, 1998]. The size of the heterochromatic blocks is also highly variable. In several species of *Rhodnius*, the C-bands are minute (fig. 1C), whereas in *T. nitida* the C-block represents over 80% of the whole chromosome (fig. 1A). The autosomal heterochromatin can be found in one or more, even all, of the autosomal pairs (fig. 3). It is generally located at the telomeres, in one or both chromosomal ends. Exceptionally, interstitial bands are also observed [Panzera et al., 1992, 1997]. These types of heterochromatin differences between triatomine species have been used to infer the existence of several cryptic species within the genus *Triatoma* [Panzera et al., 1997, 2006; Jurberg et al., 1998].

This chromosomal marker has also been the most effective tool for the detection of intraspecific variation or polymorphisms in several species of the genera *Triatoma* [Panzera et al., 1992, 2004], *Panstrongylus* [Pérez et al., 2002] and *Rhodnius* [Gómez-Palacio et al., 2008]. Undoubtedly, the most striking example was reported in *T. infestans*, the main vector of Chagas disease in the Southern Cone countries, responsible for more than half of the people infected with the parasite *Trypanosoma cruzi*. The first reference to intraspecific chromosomal polymorphism in Heteroptera was observed in natural populations of *T. infestans* from Uruguay [Panzera et al., 1992]. Further analysis of specimens from Argentina, Brazil, Bolivia, Paraguay and Peru, identified 2 allopatric chromosomal groups called 'Andean' and 'non-Andean' (fig. 4C, D). The 'Andean' group shows 50% more hetero-

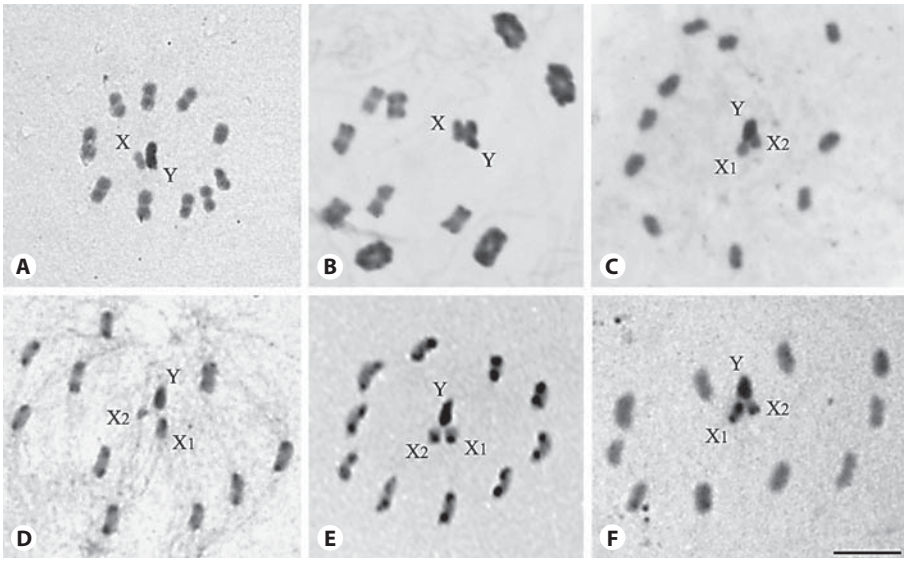

Fig. 4. C-banding variations of the X chromosomes in several species of Triatominae. First (**A**, **B**) and second (**C–F**) meiotic metaphases of spermatogenesis. In all triatomine species the Y chromosome is heterochromatic. **A** *Triatoma carcavalloi* (2n = 20A + XY). The X chromosome and all autosomes do not have C-heterochromatin. **B** *Triatoma infestans* (2n = 20A + XY), Andean group. The X chromosome and most autosomes are heterochromatic. **C** *Eratyrus cuspidatus* (2n = 20 + X_1X_2Y). Both X chromosomes are euchromatic. **D** *Triatoma barberi* (2n = 20A + X_1X_2Y). The largest X chromosome shows a C-block in one chromosomal end (X_1), while the other X chromosome does not have a C-block (X_2). **E** *Mepraia spinolai* (2n = 20A + X_1X_2Y). Both X chromosomes have similar size and show C-blocks. **F** *T. sp. affin dimidiata* (2n = 20A + X_1X_2Y) from Peten-Guatemala [Panzera et al., 2006; Bargues et al., 2008]. Both X chromosomes appear with C-blocks: the X_1 chromosome has 2 blocks and the X_2 has only one. In this species, one X chromosome has always a C-block, while the other appears polymorphic for the presence of heterochromatin. Scale bar = 10 μm.

chromatin than the 'non-Andean' group [Panzera et al., 2004]. The differences between both groups involve the number of heterochromatic autosomes, the location in the chromosome (in one or both ends) and the size of the C-blocks. It is likely that these cytogenetic changes are reflecting adaptive genomic changes that contribute to the ability of *T. infestans* to survive and reproduce in different environments [Panzera et al., 2004].

In relation to the sex chromosomes, all species of Triatominae have heterochromatin in the Y chromosome, but only some of them show heterochromatin in the X chromosomes (fig. 4). It is noticeable that the same sex mechanism can show different patterns with respect to the presence of a C-heterochromatic block on the X chromosome/s, even in species belonging to the same genus (compare fig. 4A with B and fig. 4D with F).

Fluorescent banding techniques to detect differences in base composition of heterochromatic regions have only recently been applied in Triatominae. Results not yet published by our group have revealed that the heterochromatin of *T. infestans* is subdivided into 2 regions: a telomeric DAPI-positive region (presumably AT-rich) and a subtelomeric chromomycin A3-positive region (probably GC-rich). Analysis of heterochromatin in other species with such fluorochromes shows that the composition and structure of the heterochromatic regions are very diverse within the genus *Triatoma*, so it appears as a potential marker for establishing evolutionary relationships.

The behavior of the heterochromatin during first meiotic prophase is an important variable for the differentiation of species. One of the most distinctive features of the meiotic system of triatomines is the existence of heteropycnotic associations during prophase named chromocenters. The species without autosomal C-bands present 1 chromocenter constituted only by the associated sex chromosomes (fig. 5). However, in the species with autosomal heterochromatin, a great diversity of chromocenters can be observed, both in number and in composition, involving different types of association between homologous and non-homologous autosomes [Panzera et al., 1997, 1998; Pérez et al., 2002]. The most extreme case is described in *T. delpontei*, where the heteropycnotic re-

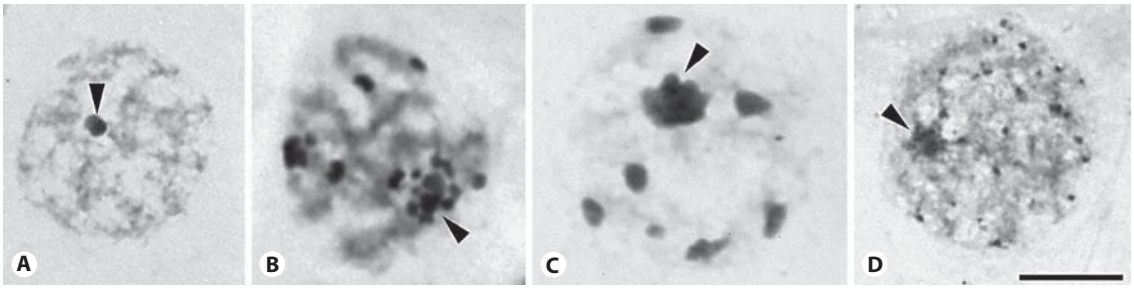

Fig. 5. Interspecific variability of chromosome behavior during first meiotic prophase in Triatominae. C-banding. **A** *Triatoma vandae* (2n = 20A + XY). A meiotic chromocenter appears, constituted only by the association of the XY sex chromosomes (arrowhead). This species does not have autosomal heterochromatin. **B** *Mepraia spinolai* (2n = 20A + X_1X_2Y). The associated sex chromosomes are surrounded by telomeric C-bands from several autosomes, forming a chromocenter that is characteristic of this species (arrowhead). Other heterochromatic regions outside this chromocenter are observed. **C** *Triatoma protracta* (2n = 20A + X_1X_2Y). A principal chromocenter, constituted by association of the sex chromosomes and several autosomes, is observed (arrowhead). The other chromocenters are constituted by the joining of different numbers of homologous and heterologous autosomes. **D** *Triatoma sherlocki* (2n = 20A + XY). A principal chromocenter (arrowhead) is formed by the sex chromosomes and 2 autosomes, while the heterochromatin of the other autosomes appears dispersed in the nuclei without heterologous association. The species in **B**, **C** and **D** present the same number of autosomes with heterochromatin (10 pairs), but their behavior during meiotic prophase is quite different. Scale bar = 10 μm.

gions of all autosomal bivalents and both sex chromosomes appear associated in a single chromocenter [Panzera et al., 1995]. The meiotic associations are species-specific and mainly depend on the number of autosomal pairs with C-blocks. However, even species with the same number of heterochromatic chromosomes can exhibit distinctive patterns of association and, as a consequence, different numbers of chromocenters (fig. 5B–D).

Localization of Specific Sequences by Fluorescence in situ Hybridization

The fluorescence in situ hybridization (FISH) technique is a valuable tool to determine the chromosomal localization of DNA sequences, contributing to our understanding of genome organization and evolution. Recently, several authors have established the chromosomal location of the 45S ribosomal cluster in several species of Triatominae. The location of this ribosomal cluster can vary between species, since it can be located on the sex chromosomes (X and/or Y), in the autosomes or in both [Severi-Aguiar and Azeredo-Oliveira, 2005; Severi-Aguiar et al., 2006; Morielle-Souza and Azeredo-Oliveira, 2007]. Even more striking was the detection of intraspecific variability in *T. infestans*, where the cluster can be located on the sex chromosomes or autosomes, depending on the geographical origin of the insects analyzed (unpublished data). These data strongly suggest that the processes of chromosomal exchanges between the autosomes and sex chromosomes are much more common than originally thought, possibly playing an important role in the speciation of this group of insects.

Variability of Genome Size

Knowledge of the total genome size of a species can be useful to make inferences about evolution of their DNA sequences [Gregory, 2005]. The amount of DNA (C-value) in Triatominae was initially analyzed using densitometry techniques but this methodology was very laborious and highly variable [Schreiber et al., 1972; Panzera et al., 1995]. In recent years, we have applied laser flow cytometry to measure genome size in several species of Triatominae [Panzera et al., 2004, 2006; Bargues et al., 2006]. Comparative analysis of 20 species by flow cytometry indicates that the haploid genome size of Triatominae species varies 4-fold, from 0.72 pg in *Rhodnius* species to 2.90 pg in *Triatoma delpontei* (table 2) [Panzera et al., 2007b]. The mean haploid genome size for the Triatominae (1.25 ± 0.09 pg) is very similar to the value proposed for all Heteroptera (1.18 ± 0.18 pg) [Gregory, 2005]. Despite the small number of species and genera analyzed, it appears that the genus *Triatoma* is the most variable while the genus *Rhodnius* appears as the most constant in genome size (table 2).

Table 2. Summary of the haploid DNA content (C-value) reported for Triatominae species, expressed in picograms

Species	C-value (pg)	Chromosome number	Method[a]	Standard species for C-value[b]	Reference
Rhodnius ecuadoriensis	0.72	2n = 22	FCM	*Homo sapiens*	Panzera et al., 2007b
Rhodnius prolixus	0.75	2n = 22	FCM	*H. sapiens*	Panzera et al., 2007b
Rhodnius prolixus	0.26	2n = 22	NS	NS	Petitpierre, 1996
Dipetalogaster maximus	1.29	2n = 22	FCM	*H. sapiens*	Panzera et al., 2007b
Eratyrus cuspidatus	1.26	2n = 23/24	FCM	*H. sapiens*	Panzera et al., 2007b
Panstrongylus herreri	0.53	2n = 23/24	FD	*Bufo paracnemis*	Schreiber et al., 1972
Panstrongylus geniculatus	1.42	2n = 23/24	FCM	*H. sapiens*	Panzera et al., 2007b
Panstrongylus megistus	0.51	2n = 23/24	FD	*B. paracnemis*	Schreiber et al., 1972
Panstrongylus rufotuberculatus	1.44	2n = 23/24	FCM	*H. sapiens*	Panzera et al., 2007b
Triatoma barberi	1.23	2n = 23/24	FCM	*H. sapiens*	Panzera et al., 2007b
Triatoma brasiliensis	1.09	2n = 22	FCM	*H. sapiens*	Panzera et al., 2007b
Triatoma delpontei	2.90	2n = 22	FCM	*H. sapiens*	Bargues et al., 2006
Triatoma delpontei	1.80	2n = 22	FD	*Allium cepa*	Panzera et al., 1995
Triatoma dimidiata (cytotype 1)	1.07	2n = 23/24	FCM	*H. sapiens*	Panzera et al., 2006
Triatoma dimidiata (cytotype 2)	0.92	2n = 23/24	FCM	*H. sapiens*	Panzera et al., 2006
Triatoma dimidiata (cytotype 3)	0.98	2n = 23/24	FCM	*H. sapiens*	Panzera et al., 2006
Triatoma infestans	0.71	2n = 22	FD	*B. paracnemis*	Schreiber et al., 1972
Triatoma infestans ('Non-Andean group')	1.09	2n = 22	FD	*A. cepa*	Panzera et al., 1995
Triatoma infestans ('Non-Andean group')	1.52	2n = 22	FCM	*H. sapiens*	Panzera et al., 2004; Bargues et al., 2006
Triatoma infestans melanosoma (before *T. melanosoma*)	1.53	2n = 22	FCM	*H. sapiens*	Bargues et al., 2006
Triatoma infestans ('dark morph')	1.44	2n = 22	FCM	*H. sapiens*	Panzera et al., 2004; Bargues et al., 2006
Triatoma infestans ('Andean group')	1.98	2n = 22	FCM	*H. sapiens*	Panzera et al., 2004; Bargues et al., 2006
Triatoma longipennis	0.91	2n = 23/24	FCM	*H. sapiens*	Panzera et al., 2007b
Triatoma maculata	1.09	2n = 22	FCM	*H. sapiens*	Panzera et al., 2007b
Triatoma nitida	1.35	2n = 21/22	FCM	*H. sapiens*	Panzera et al., 2007b
Triatoma platensis	1.33	2n = 22	FCM	*H. sapiens*	Bargues et al., 2006
Triatoma platensis	0.61	2n = 22	FD	*B. paracnemis*	Schreiber et al., 1972
Triatoma platensis	0.87	2n = 22	FD	*A. cepa*	Panzera et al., 1995
Triatoma pseudomaculata	1.13	2n = 22	FCM	*H. sapiens*	Panzera et al., 2007b
Triatoma pseudomaculata	0.50	2n = 22	FD	*B. paracnemis*	Schreiber et al., 1972
Triatoma rubrovaria	1.17	2n = 22	FCM	*H. sapiens*	Bargues et al., 2006
Triatoma rubrovaria	0.54	2n = 22	FD	*A. cepa*	Panzera et al., 1995
Triatoma ryckmani	1.10	2n = 23/24	FCM	*H. sapiens*	Panzera et al., 2007b
Triatoma sordida	1.12	2n = 22	FCM	*H. sapiens*	Bargues et al., 2006
Triatoma vitticeps	0.77	2n = 24/26	FD	*B. paracnemis*	Schreiber et al., 1972

DNA values in this table differ from those previously reported in Panzera et al. [2007b] because the reference values of *Homo sapiens* and *Bufo paracnemis* were updated.

[a] FCM = Flow cytometry; FD = Feulgen densitometry; NS = not specified.

[b] *Allium cepa:* 1C = 16.80 pg; *Homo sapiens:* 1C = 3.5 pg; *Bufo paracnemis:* 1C = 3.29 pg.

Genome Evolution in Triatominae

The principal mechanisms of changes in chromosome number in Heteroptera are autosomal fusions or fragmentations and sex chromosome fragmentations (which originate multiple sex chromosome systems) [Ueshima, 1979; Papeschi and Bressa, 2007]. The homogeneity in the autosomal number of Triatominae indicates that the processes of fragmentation and fusion do not occur frequently in this subfamily. Ueshima [1966] suggested that the most frequent number of 20 autosomes is the primitive or ancestral number and that fission and fusions have resulted in the triatomine species with 22 *(T. rubrofasciata)* and 18 autosomes *(T. nitida, P. megistus)*, respectively.

Chromosome rearrangements such as inversions and reciprocal translocations are exceptional in Heteroptera [Papeschi and Mola, 1990; Bressa et al., 1998]. In Triatominae, our group has described only 1 case of a reciprocal translocation in the genus *Mepraia* [Pérez et al., 2004]. It has been suggested that these types of rearrangements have greater chances of establishing in species with holokinetic chromosomes, since all the products of the alteration are transmissible at meiosis [White, 1973]. However, considering the extremely rare occurrence of translocations in Triatominae, it would appear that, at least for this group of insects, they are strongly negatively selected [Pérez et al., 2004]. The occurrence of chromosomal rearrangements seems to have an effect on meiosis much greater than that observed in chromosomes with a localized centromere. Univalents and fragments related with these chromosomal alterations used to associate with sex chromosomes, strongly affecting their behavior. We suggest that the constancy of chromosome number is not a reflection of 'genome stability' but is the result of strong selective pressure acting on the chromosome complement. This pressure would prevent the fixation of chromosomal rearrangements that alter meiotic segregation, leading to a consequent reduction in fitness of the individual [Pérez et al., 2004].

In relation to the sex chromosomes, and similar to the Heteroptera, the XY mechanism would be the ancestral sex system of Triatominae. For this reason, it is usually considered that multiple sex mechanisms have arisen by fragmentation of the original X chromosome [Ueshima, 1966]. Our analyses on the sex chromosomes show a great variability in size and C-banding patterns of X chromosomes (fig. 4), which suggests the occurrence of other chromosomal rearrangements involved in the formation of the multiple sex mechanisms. For example the behavior of the chromosomal fragments described here (fig. 2E) and previously published [Pérez et al., 2004] strongly suggest that autosomal rearrangements may also be involved in the origin of multiple sex chromosomes

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 triatomine species, we suggest that heterochromatin is probably an active component in the karyotype evolution of this subfamily [Panzera et al., 1995, 1997, 2006; Pérez et al., 2002]. Changes in the amount, composition and distribution of C-heterochromatin represent the main source of karyological differentiation between triatomine species. The spatial proximity of autosomal pairs with heterochromatin during meiotic and mitotic divisions in the chromocenters would facilitate the exchange of C-heterochromatic material between homologous and non-homologous chromosomes, similar to that suggested for chromosomes with a localized centromere [Schweizer and Loidl, 1987]. The different types of chromocenters observed in triatomines (fig. 5) may also affect the acquisition and/or accumulation of heterochromatin in the karyotype, leading to distinctive evolutionary trends. However, the existence of a striking C-polymorphism in *T. infestans* (figs. 3C, D) [Panzera et al., 2004], as well as fertile hybrid individuals from closely related species with different amounts and distribution of C-heterochromatin [Pérez et al., 2005], suggests that – at least for closely related species – heterochromatin does not play a direct role in speciation [Panzera et al., 1995; Pérez et al., 2005]. Further studies, involving detailed analyses of the repetitive sequences of heterochromatin, are necessary to understand the role of heterochromatin in the karyotype evolution of triatomines.

Considering that the Triatominae have very similar chromosome complements (between 21 and 25 chromosomes), the wide variation in genome size of this group (table 2) should reflect differences in the number and type of sequences they contain. In general, variations in the amount of DNA of related species are due to differences in the repeated fraction of the genome [Gregory, 2005]. This hypothesis seems to hold true for the Triatominae, where we found a positive correlation between genome size and amount of repeated DNA sequences reflected by the presence of C-heterochromatic blocks. The relationship between heterochromatin and genome size explains most of the differences observed both at the intraspecific level and amongst closely related species. The intraspecific variation of DNA from *T. infestans* [Panzera et al., 2004] and interspecific differences within the *infes-*

tans subcomplex *(T. platensis*, *T. infestans* and *T. delpontei)* [Bargues et al., 2006] are strongly associated with variations in the amount of heterochromatin. However, between evolutionarily distant species there are clear differences in DNA content mainly due to the euchromatic fraction. For example, *Rhodnius* species show significant differences of DNA content compared to species from other genera. These differences coincide with the smaller size of the chromosomes in the genus *Rhodnius*. Furthermore, species with similar amounts of heterochromatin, but evolutionarily distant, also show major differences in genome size attributable to the amount of euchromatin [Panzera et al., 2007b].

Future Perspectives

In holocentric systems, the Triatominae subfamily is one of the groups of insects with greater variability and chromosomal diversification. There is still little knowledge about the mechanisms of chromosomal evolution in this particular type of chromosomes. Future analyses of the variability of heterochromatin by fluorescent techniques and molecular characterization of their sequences will provide new elements for understanding chromosome diversification in this group. The imminent availability of the complete genome of *Rhodnius prolixus* (http://genome.wustl.edu/genomes/view/rhodnius_prolixus/) opens new perspectives for tracing the genome evolution at a sequence level. The application of FISH for the mapping of genes and sequences will provide new markers for understanding the genomic changes present in this group of insects. The application of whole genome in situ hybridization and comparative genome hybridization might provide new approaches to clarify the chromatin organization of holokinetic chromosomes as well as taxonomic uncertainties and phylogenetic relationships within several species of triatomines. All these techniques, together with the classical cytogenetic approaches presented here, will help to understand the importance of chromosome change during the speciation of the triatomines.

Acknowledgements

This work was partially supported by several projects from 'Comisión Sectorial de Investigación Científica' (CSIC), PEDECIBA and ANII from Uruguay, and international collaboration supported by Commission of the European Communities: EUSAPH network and ECLAT (IC18-CT98-0366), CDIA (ICA4-CT-2003-10049) and ATU (SSA-CT-2004-515942) projects. We are particularly grateful for specific comments from Christopher John Schofield (London School of Hygiene and Tropical Medicine). The observations and photographs were mostly made on NIKON photomicroscopes donated by the Government of Japan and another one recently acquired by CSIC.

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