Chromosomal evolution trends of the genus *Panstrongylus* (Hemiptera, Reduviidae), vectors of Chagas disease

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Abstract

The genus *Panstrongylus* includes 14 species widely distributed from Mexico to Argentina, some of them with great epidemiological significance as vectors of Chagas disease. We study the karyotype and the male meiotic process of *Panstrongylus chinai*, *P. geniculatus*, *P. herreri*, *P. lignarius*, *P. megistus*, *P. rufotuberculatus* and *P. tupynambai*. All species present the same sex mechanism (X1X2Y in males and X1X1X2X2 in females) and they also have 20 autosomes, with the exception of *P. megistus* that only presents 18 autosomes. The analysis of C-banding patterns and meiotic chromosome behaviour show a great level of variability allowing the identification of three clearly differentiated groups. In the first group, we only include *P. megistus* because of its unusual number of autosomes. The second group includes *P. chinai*, *P. herreri*, *P. lignarius* and *P. rufotuberculatus*. Their autosomes present terminal heterochromatic regions that appear scattered throughout the nucleus and associated with the sex chromosomes. Actually, *P. herreri* and *P. lignarius* can be considered cytogenetically identical. Our results are in agreement with morphological, ecological and molecular data indicating that they should be regarded as the same species. The third group only includes *P. tayronahai* that shows autosomes without C-positive regions. *Panstrongylus geniculatus* shares characters will all the three groups. Its karyotypic features are extremely polymorphic depending on their geographic origin. Some populations do not show any heterochromatic regions, while others exhibit few or several heterochromatic blocks. The chromosomal variability observed, together with its wide distribution and phenetic variability, suggest that *P. geniculatus* is a species complex comprising at least two distinct species. Considering the entire subfamily, the level of cytogenetic variation in *Panstrongylus* is lower than that observed in *Triatoma* but considerably more than that of *Rhodinus*, which is a very homogenous genus in terms of chromosome appearance and behaviour. This would endorse the closer relationship between *Panstrongylus* and *Triatoma*, and their divergence from *Rhodinus*, in accordance with current tribal classification.

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1. Introduction

The Triatominae subfamily (Hemiptera, Reduviidae) is constituted by haematophagous insects that are vectors for the parasitic agent of Chagas’ disease: *Trypanosoma cruzi*. Chagas’ disease is one of the most important diseases in the tropics and subtropics of the America, with about one million cases per year and more than 40,000 deaths (Schotfield and Ponce, 1999). According to the World Health Organisation (WHO, 1991), 25% of the total population of Central and South America is at risk.

The Triatominae are mainly distributed in the New World, their centre of diversity and likely site of origin. In the Americas, these insects occupy diverse habitats from southern Argentina to the Great Lakes of North America. The subfamily includes well over 100 species divided into
six tribes and 18 genera (Carcavallo et al., 2000). The genus *Panstrongylus* is the second most numerous of the tribe Triatominae with 14 species widely distributed from southern Mexico to Argentina, including *Panstrongylus sherlocki* recently described (Jurberg et al., 2001). Some, such as *P. megistus*, are important vectors of Chagas disease due to their close association with humans. Others, such as *P. chinai*, *P. geniculatus*, *P. herreri* and *P. rufotuberculatus* are considered secondary vectors because of their increasing ability to invade and colonise domestic habitats (Lent and Wygodzinsky, 1979). By contrast, Schofield (1988) has proposed that the Triatominae are monophyletic and that haematophagy arose from several predaceous ancestors. The genus *Panstrongylus* are considered a well-recognized monophyletic group (Lent and Wygodzinsky, 1979), but recently molecular data suggest a polyphyletic origin of this genus (Marcilla et al., 2002).

Genetic analysis of Triatominae phylogeny has mainly involved examination of isozyme variability, cytogentic features, random amplified polymorphic DNA profiles and some DNA sequences studies (Dujardin et al., 2000). How- ever, the analysis has been centred on the genera *Triatoma* and *Rhodnius*, with the genus *Panstrongylus* studied mainly on morphological characteristics. Cytogenetic studies have been performed in only three *Panstrongylus* species: *P. megistus* has been extensively studied (Schreiber and Pellegrino, 1950, 1951; Schreiber et al., 1972; Tartarotti and Azeredo-Oliveira, 1999a,b; Panzera et al., 1998). Cytogenetic data of *P. geniculatus*, *P. chinai*, *P. lignarius* and *P. rufotuberculatus* are here described for the first time.

In this paper, we address several aspects of the cytoge- netic of these *Panstrongylus* species, with the purpose of identifying the major trends in their karyological evolution.

2. Materials and methods

2.1. Material analysed

The origin and number of individuals studied for each species are shown in Table 1.

2.2. Cytogenetic studies

Gonads (testes and ovaries) were removed from freshly killed adults, fixed in an ethanol-acetic acid mixture (3:1) and stored at −20°. Gonadal squashes were made on slides in a drop of 50% acetic acid. Part of the material was used in squashes stained with laco-acetic orcein for mitotic descrip- tions. The remaining material was studied using C-banding technique, according to Pérez et al. (1997), to observed the distribution and behaviour of C-heterochromatin during mitosis and meiosis.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Collection sites and number of <em>Panstrongylus</em> specimens used in this report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Site and habitat of collection</td>
</tr>
<tr>
<td><em>P. chinai</em></td>
<td>Santa, Lambayeque, Peru Domestic</td>
</tr>
<tr>
<td><em>P. geniculatus</em></td>
<td>Belem, Para, Brazil Sylastic</td>
</tr>
<tr>
<td></td>
<td>Oritaga, Sucre, Colombia Sylastic</td>
</tr>
<tr>
<td></td>
<td>Bucaramanga, Santander, Colombia Sylatic</td>
</tr>
<tr>
<td></td>
<td>Anzáli, Asunción, Colombia Paragominic</td>
</tr>
<tr>
<td><em>P. herreri</em></td>
<td>Colony FIOCRUZ, Brazil</td>
</tr>
<tr>
<td></td>
<td>Cuatro, Cajamarca, Peru. Domiciliary</td>
</tr>
<tr>
<td></td>
<td>Nueva Union San Martín, Peru Domiciliary</td>
</tr>
<tr>
<td><em>P. lignarius</em></td>
<td>São Paulo, Brazil Sylastic</td>
</tr>
<tr>
<td></td>
<td>Belem, Brazil Sylastic</td>
</tr>
<tr>
<td><em>P. megistus</em></td>
<td>Pampulha, Minas Gerais, Brazil Domiciliary</td>
</tr>
<tr>
<td></td>
<td>Campo Formoso, Bahia, Brazil Paragominic</td>
</tr>
<tr>
<td></td>
<td>Cacapé, Santa Catarina, Brazil Paragominic</td>
</tr>
<tr>
<td></td>
<td>Uberaba, Minas Gerais, Brazil Paragominic</td>
</tr>
<tr>
<td><em>P. rufotuberculatus</em></td>
<td>Santander, Colombia Sylastic</td>
</tr>
<tr>
<td></td>
<td>Anzáli, Asunción, Colombia Sylatic</td>
</tr>
<tr>
<td><em>P. tupynambai</em></td>
<td>Artigas, Uruguay Sylastic</td>
</tr>
<tr>
<td></td>
<td>P. chinai</td>
</tr>
<tr>
<td>-------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Autosomal number</td>
<td>20</td>
</tr>
<tr>
<td>Constitution of meiotic chromosome blocks</td>
<td>X&lt;sub&gt;1&lt;/sub&gt;X&lt;sub&gt;1&lt;/sub&gt;Y + A and dispersed dots</td>
</tr>
<tr>
<td>Number of autosomal pairs with C-blocks</td>
<td>10</td>
</tr>
<tr>
<td>Amount (%) autosomal C-heterochromatin (internal)</td>
<td>28-33</td>
</tr>
<tr>
<td>Chromosome localization of autosomal C-blocks</td>
<td>In both ends: 10 pairs</td>
</tr>
</tbody>
</table>

*As automes.*
In order to compare the species and populations, we considered several cytogenetic markers previously used to differentiate triatomine species (Pérez et al., 1992; Panzera et al., 1995, 1997). Those traits based on relative size (autosomal size and autosomes versus sex chromosomes) were determined by observing first and second metaphases. The chromosomal traits used are shown in Table 2.

2.3. Quantification of autosomal C-heterochromatin

We estimated the relative length of the C-heterochromatin in the total length of the autosomal complement. At least three individuals for each species and one specimen per locality were analysed. For each specimen, three photographs of metaphase plate (gonial) were digitalized and quantified by appropriated software (IPP plus®).

3. Results

3.1. Comparative analysis of Panstrongylus species: common characteristics

In spite of specific chromosomal traits described below, there are some common characteristics shared by Panstrongylus species studied in this paper. All of them have 20 autosomes, with the exception of P. megistus that only presents 18 autosomes. These chromosomes do not show the striking size differences, being two or three pairs slightly larger. Sex chromosomes are very similar not only in number and mechanism (X₁X₂Y in males and X₁X₁X₂X₂ in females) but also in size and staining. The Y chromosome is always C-heterochromatic and it presents a medium size in relation to the autosomes. The X₁ chromosomes have an intermediate staining and they are the smallest of the complement. The X₂ is slightly larger than the X₃ chromosome. Species with autosomal heterochromatin always present C-bands in one or both chromosomal ends. We did not detect interstitial C-bands as observed in other triatomines species.

3.2. Panstrongylus chinai (Del Ponte, 1929), P. herreri (Wygodzinsky, 1948), P. lignarius (Walker, 1873) and P. rufotuberculatus (Champion, 1899)

Cytogenetic data of P. chinai, P. lignarius and P. rufotuberculatus are described here for the first time. Chromosome number and some cytogenetic characteristics of P. herreri, including C-banding pattern, were previously described by other authors (Ueshima, 1966; Schreiber et al., 1972; Tartarotti and Azeredo-Oliveira, 1999a,b). We confirm some of the previous descriptions and add new data about this species. These four species are described together because their cytogenetic characteristics are similar. P. herreri (Fig. 1) is used to point up the chromosomal features of this group of species. They present the same chromosome number constituted by 23 chromosomes in males (2n = 20 autosomes + X₁X₂Y) and 24 chromosomes in females (2n = 20A + X₁X₁X₂X₂). Meiotic phase is characterised by the presence of one main heterochromatic chromocentre constituted by the associated sex chromosomes and some autosomal C-positive or heterochromatic regions (arrow in Fig. 1A–D). Several heterochromatic dots or small chromocentres are also observed scattered throughout the nucleus (Fig. 1A and B). In diplotene, these C-positive regions can be localized in one or both chromosome ends of most autosomes (Fig. 1C and D).

The karyotype shows little variation in the autosomal size, although two or three autosomal pairs are slightly larger than the rest (Fig. 1E). All species show a large Y sex chromosome that is totally heterochromatic. The X₁ and X₂ sex chromosomes are the smallest of the complement and present an intermediate staining (Fig. 1E and F).

We detect variation in the content of autosomal C-heterochromatin among these four species: P. rufotuberculatus ranges from 16 to 20%, P. lignarius and P. herreri ranges from 22 to 24% meanwhile P. chinai show the greatest amount of heterochromatin (from 28 to 33%; Fig. 2). The localization and size of these heterochromatic regions in the karyotype can also vary. In P. chinai, all autosomes have C-bands in both chromosomal ends (Fig. 2D and E). In the other species, C-band size is smaller and some autosomes have only one band or are totally euchromatic (Table 2). In spite of these heterochromatin variations, the meiotic behaviour already described is maintained in the four species (compare Fig. 1A–C with Fig. 2A–C).

No cytogenetic differences were observed among individuals of the same species coming from different origins (Table 1).

3.3. Panstrongylus tapynumbai (Lent, 1942)

Chromosome number and meiotic process of this species were previously reported by Panzera et al. (1998). C-banding pattern is described here for the first time. This species presents the same chromosome number that the previous ones, but the 10 autosomal pairs do not have any heterochromatic regions. The staining of the sex chromosomes (X₁X₂ and Y) is similar to that observed in the previous species. During meiotic prophase there is one heteropycnotic chromocentre formed only by the association of the three sex chromosomes.

3.4. Panstrongylus geniculatus (Latreille, 1811)

All cytogenetic features of this species are here described for the first time. It shows the same diploid chromosome number observed in the other species (23 chromosomes in males and 24 chromosomes in females) and the sex chromosomes exhibit the same characteristics. However, a marked variation in the number of heterochro-
Fig. 1. *P. herreri*, 2n = 20A + X₁X₂Y/X₁X₂X₂. Male meiosis, C-banding technique. (A and B) Early and late Pachytene. One main heterochromatic chromocentre is formed by the association of the three sex chromosomes with the heterochromatic regions of some autosomes (arrows). Several C-positive dots or small chromocentres are also observed scattered throughout the nucleus. (C) Early Diplotene stage. Autosomal heterochromatic dots are clearly observed in the terminal regions of most bivalents. (D) Diplotene stage. The associated sex chromosomes (X₁X₂Y) are attached to four autosomal bivalents (arrow). (E) Metaphase I: all autosomal bivalents and the three sex chromosomes appear clearly separated. The Y chromosome appears heterochromatic, while the X₁ and X₂ chromosomes show an intermediate staining. (F) Metaphase II: chromosomes adopt the typical configuration seen in other Triatominae species, that is with the sex chromosomes in the centre of a ring formed by the autosomes. The X₁ and X₂ chromatids segregate to the same pole, while the Y chromatid migrates to the opposite one. Bar = 10 μm.

3.4. Panstrongylus megistus (Burmeister, 1835)

*P. megistus* has been extensively studied cytogenetically (Schreiber and Pellegrino, 1950, 1951; Schreiber et al., 1972; Barth, 1956; Mello et al., 1986; Panzera et al., 1998; Tartarotti and Azeredo-Oliveira, 1999a,b). Some contradictions appear in this literature, and for this reason we re-describe this karyotype including new data.

We confirmed that this species has 21 chromosomes constituted by nine autosomal pairs and multiple sex chromosomes (2n = 18A + X₁X₂Y) in males (Fig. 4B and C). The individuals analysed here do not show autosomal pairs with C-bands (Fig. 4A). The Y sex chromosome is the only one that shows heterochromatic regions (Fig. 4C). The variation of autosomal size is more marked than in the other *Panstrongylus* species, particularly in three chromosomal pairs that appear substantially larger (Fig. 4C).
4. Discussion

Cytogenetic studies have been successfully used in several insects acting as disease vectors. Identification of *Simulium damnosum* species complex, vectors of onchocerciasis, demands adequate knowledge of the chromosomal features that distinguish human-biting members from nonanthropophilic forms (Maegga and Cupp, 1993). Cytogenetic identification of sibling species was also performed in phlebotomine sandflies that are involved in *Leishmania* transmission (Yin et al., 1999). Cytogenetic studies in *Anopheles* (Diptera, Culicidae) allowed the identification of genetic entities within morphologically uniform taxa of these malaria vectors (Coetzee et al., 1999; Suguna et al., 1988; Ramírez and Dessen, 2000).

During the past decade, our research group has carried out cytogenetic studies on triatomines, including intra- and inter-populations analysis (Panzera et al., 1992, 1997), comparison of species of the same and different genera (Pérez et al., 1992; Panzera et al., 1995, 1998) as well as chromosomal behaviour during meiosis (Pérez et al., 1997, 2000). These results give insights both in the chromosomal evolution of the subfamily and in the establishment of the taxonomic status of some taxa. It is clear that these insects are more variable cytogenetically that was initially thought, making cytogenetic studies a remarkable tool for population, evolutionary and systematic analysis. Most cytogenetic studies have included species of the genus *Triatoma* owing to the large number of available species and its epidemiological significance. Despite the importance of some *Panstrongylus* species as Chagas’ disease vectors, this genus has received relatively little attention not only in chromosomal but also in isoenzymatic and DNA studies. For this reason, in this paper, we try to summarise the cytogenetic data already published and complement it with our new results.

The species of *Panstrongylus* so far studied (7 out of 14) are quite homogeneous in their chromosome number. All species present the same sex mechanism (*X*1*X*2*Y* in males and *X*1*X*1*X*2*X*2 in females) and they also have 20 autosomes, with the exception of *P. megistus* that only presents 18 autosomes. Multiple sex chromosomes are characteristic and very common in North American *Triatoma* species. On the other hand, almost all South American species present an XY system that is considered primitive in the Triatominae (Ueshima, 1966). This result suggests that *Panstrongylus* species are far away from South American *Triatoma* species and that they could be related to the ancestors giving rise to...
Fig. 3. *P. geniculatus* from different populations, $2n = 20A + X_1X_2Y_1^*/X_1X_2X_1X_2$. Male meiosis (A–E) with C-banding technique and oogonial prometaphase (F) with orcein stain. (A) Diffuse: the association of the three sex chromosomes constitutes one heterochromatic chromocentre (arrow). No autosomal heterochromatic regions are observed. Brazilian and Colombian (Antioquia) populations. (B) Diffuse: the associated sex chromosomes form one heterochromatic chromocentre (arrow). Two heterochromatic dots, corresponding to one autosomal pair, are observed (arrowheads). Colombian (Sucre) population. (C) Diffuse: one heterochromatic chromocentre is formed by the association of sex chromosomes with the heterochromatic regions of some autosomes (arrows). Several C-positive dots are also observed scattered through the nucleus. Colombian (Santander) population. (D) Metaphase I: the sex chromosomes appear as univalents (they do not pair each other). The chromatids of the sex chromosomes will separate to opposite poles (equational division) during first anaphase. This “inverted” type of segregation is characteristic of hemipteran insects. (E) Metaphase II: the $X_1$, $X_2$ and $Y$ chromatids are situated in the centre of a ring formed by the autosomes. The two $X_1$ and $Y$ chromatids segregate to opposite poles. Therefore, the second division is reductional for the sex chromosomes, as in other Hemiptera. The $Y$ chromatid appears totally heterochromatic. (F) Oogonial prometaphase (mitosis): orcein stain. The female has 24 chromosomes constituted by 20 autosomes and four sex chromosomes ($X_1X_1X_2X_2$). Bar = 10 μm.

North American species. However, phylogenetic relationships based on similar sex mechanisms have to be taken with caution. Multiple sex chromosomes observed in different Triatominae species are not necessary homologous. The size variation and banding patterns found among $X_1$ chromosomes from several species suggest that they were independently originated by different fission events. Nevertheless, the identical size and C-banding characteristics presented

Fig. 4. *P. megistus*, $2n = 18A + X_1Y_1^*/X_1X_2X_1X_2$. Male meiosis, C-banding technique. (A) Diffuse stage: one heterochromatic chromocentre is formed only by the association of the three sex chromosomes (arrow). (B) Diploctene stage: the associated sex chromosomes are separated from the rest of the chromosomes (arrow). (C) Metaphase I: the nine autosomal bivalents and the three sex chromosomes appear clearly separated. The $Y$ chromosome appears heterochromatic. Bar = 10 μm.
in Panstrongylus sex chromosomes suggest a common origin.

The analysis of C-banding patterns and chromosome behaviour during the male meiotic process show a great level of variability among Panstrongylus species. We can identify three clearly differentiated groups.

In the first group, we only include *P. megistus* because of the unusual number of 18 autosomes. *Triatoma nitida* is the only other triatomine species, out of the 54 already studied, that shows this particular number of autosomes (Dujardin et al., 2000). Considering that 20 autosomes is the most frequent and probably ancestral autosomal number in the subfamily Triatominae (Ueshima, 1966), we believe that a reduction in the number of autosomes (from 20 to 18) is due to the fusion of two autosomes. However, it is likely that more rearrangement was involved to produce the observable karyotype since there are three large pairs and not only one as expected by a simple fusion event. We did not observe conspicuous heterochromatic regions in the autosomes of this species, irrespective of their origin, but these regions have been described by Tartarotti and Azevedo-Oliveira (1999a). This apparent discrepancy could indicate a polymorphism, or may be a misleading observation because the euchromatic bivalents of *P. megistus* are so heavily condensed during the meiotic prophase that they can resemble heterochromatic zones (Fig. 4).

The second karyotypic group includes *P. chinai*, *P. herreri*, *P. lignarius* and *P. rufotuberculatus*, with very similar chromosome features (Figs. 1 and 2). In the four species, most autosomes present terminal heterochromatic regions that, together with the sex chromosomes, exhibit a typical pattern during meiotic prophase. Actually, *P. herreri* and *P. lignarius* can be considered cytogenetically identical. Our chromosome data is in agreement with morphological, ecological and molecular data indicating that they should be regarded as the same species. In the cladogram constructed by Lent and Wygodzinsky (1979), *P. lignarius* and *P. herreri* are placed together, without pleio- or apomorphic traits to differentiate them. *P. herreri* and *P. lignarius* are morphologically similar so that it is often difficult to distinguish them (Carcavallo et al., 1999), and morphologically intermediate forms are common in regions of northern Peru (Schaffeld, personal communication). Moreover, Marcilla et al. (2002) show that their nucleotide composition of the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA is identical.

The gradient in the amount of heterochromatin detected in this group together with their geographic distribution seems to point out a speciation trend, with *P. rufotuberculatus* at one end (with broader distribution and less C-heterochromatin) and *P. chinai* at the other (with limited geographic range and more C-heterochromatin).

The third group includes only *P. tupaianus* that shows autosomes without C-positive regions. This species is exclusively sylvatic with a geographic distribution restricted to southern Brazil and Uruguay. *P. geniculatus* shares characters will all the above three groups. Its karyotypic features are extremely polymorphic (Fig. 3). Populations from Brazil and Antioquia in Colombia do not show any autosomal heterochromatic regions (similar to the third group), while populations from elsewhere in Colombia exhibit few (Sucre) or several (Santander) heterochromatic dots (similar to the second group). *P. geniculatus* is also highly variable in terms of size and markings, leading Lent and Wygodzinsky (1979) to suggest the possible existence of geographic races in this species. DNA sequence analysis of the ITS-2 (rDNA) also shows intraspecific variability (Marcilla et al., 2002). Considering the wide distribution of this species (from Argentina to Mexico) the chromosome polymorphism detected could indicate different levels of adaptation to variable environments. However, we have never observed this level of variation, from populations without autosomal heterochromatin to populations with several heterochromatic regions, in any other triatomine species. The only exception would be *T. sordida*, which exhibited substantial variation of heterochromatin (Panzer et al., 1997) forming two morphologically similar forms distinguishable by isoenzymes and subsequently described as cryptic species (Jorberg et al., 1998). The chromosomal variability observed here for *P. geniculatus*, coupled with its wide distribution and phenetic variability, suggest that *P. geniculatus* is a species complex comprising at least two distinct species. Colombian population (Santander type) is clearly more similar with *P. rufotuberculatus*, *P. lignarius*, *P. herreri* and *P. chinai*, while Brazilian population is clearly more related with *P. tupaianus*. *P. megistus*, with its reduced chromosome number, is most likely a derived species.

Overall, the level of cytogenetic variation in Panstrongylus is lower than that observed in Triatoma but considerably more than that of Rhodnius. In Triatoma, the heterochromatic regions show different behaviour depending on the species analysed, possibly reflecting the polyphyletic nature of this large genus (Dujardin et al., 2000). In some *Triatoma* species, particularly those of the *infestans* sub-complex (*T. melanosoma*, *T. infestans*, *T. platensis* and *T. delpontei*), the heterochromatic regions are generally associated to form a single chromosome centre (Panzer et al., 1995). In others, the heterochromatic regions appear scattered through the nucleolus and multiple chromocentres are formed, such as in *T. barberi*, *T. rubrofasciata*, *T. tibiamaculata*, *T. brasiliensis* (Panzer et al., 2000) and in some species of *sordida* sub-complex (Panzer et al., 1997). Only this latter kind of heterochromatin behaviour is observed in Panstrongylus, suggesting a common origin of this chromatin for all species of this genus so far analysed. By contrast, the cytogenetic variation observed in Panstrongylus is substantially more than seen in Rhodnius, which is a very homogenous genus in terms of chromosome appearance and behaviour. This would endorse the closer relationship between Panstrongylus and Triatoma, and their divergence from Rhodnius, in accordance with current tribal classification (Dujardin et al., 2000).
Acknowledgements

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References


