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Distribution of constitutive heterochromatin in species of triatomines with fragmentation of sex chromosomes X

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ABSTRACT. Cytogenetic analyses of triatomines are considered to be important taxonomic tools. Thus, we analyzed the pattern of constitutive heterochromatin in 7 species of triatomine with fragmentation of the sex chromosome X, focusing on the cytotaxonomy of these triatomines. The species analyzed included *Triatoma vitticeps*, *Triatoma melanocephala*, *Triatoma tibiamaculata*, *Triatoma protracta*, *Meccus pallidipennis*, *Panstrongylus megistus*, and *Panstrongylus lignarius*. The seminiferous tubules of the adult males were subjected to C-banding. *P. megistus* and *P. lignarius* showed differences in chromosome number and disposition of constitutive heterochromatin, as only *P. lignarius* showed C-blocks in autosomes. C-banding can differentiate these species, since one of the sex chromosome (X) is heterochromatic in *T. vitticeps*. *T. protracta* showed C-blocks in both ends of all autosomes, *T. tibiamaculata* showed terminal C-dots in some autosomal pairs and *M. pallidipennis* did not show constitutive heterochromatin in autosomes. Thus, we confirmed the heterochromatic pattern of 7 species of insects and emphasized the importance of cytogenetic techniques for C-banding for taxonomy studies of the triatomines, which are important vectors of Chagas disease.

Key words: C-banding; Cytotaxonomy; Triatominae

INTRODUCTION

The Triatominae subfamily is composed of 148 species of hematophagous insects (Abad-Franch et al., 2013; Alevi et al., 2013a; Jurberg et al., 2013; Poinar, 2013). Triatomines can become infected with the protozoan *Trypanosoma cruzi*; once contaminated, these organisms become vectors of Chagas disease (Noireau et al., 2009).

In addition to their epidemiological importance, triatomines are biological models of cell studies because they present some peculiarities such as holocentric chromosomes, meiosis inverted for the sex chromosomes, and nucleolar persistence during meiosis (Ueshima, 1966; Alevi et al., 2013b, 2014a).

Cytogenetic analyses of these vectors are considered to be important taxonomic tools (Ueshima, 1966; Pérez et al., 1992). Cytogenetic studies in subcomplex Brasiliensis proposed by Schofield and Galvão (2009) allowed exclusion of the 3 species by karyosystematic studies (Alevi et al., 2012a, 2014b,c), confirming the relationship between a species originally classified using only morphological data (Alevi et al., 2012b, 2013c), correlation of evolutionarily related species (Alevi et al., 2013d), and differentiation of morphologically related species (Alevi et al., 2013e,f).

Chromosomal evolution of triatomine restricted and 5 types of karyotypes (2n = 21, 22, 23, 24, 25 chromosomes) (Alevi et al., 2013a) and presence or absence of heterochromatic blocks of autosomes vary in position [end(s), whole chromosome] and quantity (Panzera et al., 2010). Based on this information, we hypothesized that chromosomes evolve at a lower rate than other insect genes.

Thus, we analyzed the pattern of constitutive heterochromatin in 7 species of triatomine with fragmentation of the sex chromosome X, focusing on the cytotaxonomy of these triatomines.

MATERIAL AND METHODS

The species analyzed included *Triatoma vitticeps*, *Triatoma melanocephala*, *Triatoma tibiamaculata*, *Triatoma protracta*, *Meccus pallidipennis*, *Panstrongylus megistus*, and *Panstrongylus lignarius*. For the study, 5 specimens of each species were used. The insects were provided by the Triatominae Insectarium of Universidade Estadual Paulista (UNESP, Araraquara, Brazil). The seminiferous tubules of the adult males were subjected to C-banding (Sumner, 1972). The slides were analyzed using a light microscope (Jenaval-Zeiss, Jena, Germany), which was coupled to a digital camera and an AxioVision LE 4.8 image analyzer (Copyright© 2006-2009 Carl Zeiss Imaging Solutions GmbH). The images were magnified by 1000X.

RESULTS

The analysis of meiotic metaphases I and II enabled the heterochromatic pattern of 7 species analyzed to be examined. These results are represented in an idiogram (Figures 1 and 2). The karyotype of *P. megistus* showed 21 chromosomes (2n = 18A + X_1X_2Y) and the absence of heterochromatin in the autosomes (Figure 1A). *T. vitticeps* (Figure 1B) and *T. melanocephala* (Figure 1C) showed 24 chromosomes (20A + $X_1X_2X_3Y$) and the absence of heterochromatin in the autosomes. *T. vitticeps* showed a heterochromatic block on a sex chromosome X. *P. lignarius*, *T. protracta*, *T. tibiamaculata*, and *M. pallidipennis* showed 23 chromosomes (2n = 20A + X_1X_2Y). However, differences in the disposition of constitutive heterochromatin in the autosomes of all species were observed: *P. lignarius* with C-blocks in 1 or both ends of almost autosomes (Figure 2A), *T. protracta* with C-blocks in both ends of all autosomes (Figure 2B), *T. tibiamaculata* with terminal C-dots in some autosomal pairs (Figure 2C), and *M. pallidipennis* without constitutive heterochromatin (Figure 2D). All species studied showed that the sex chromosome Y was heterochromatic.

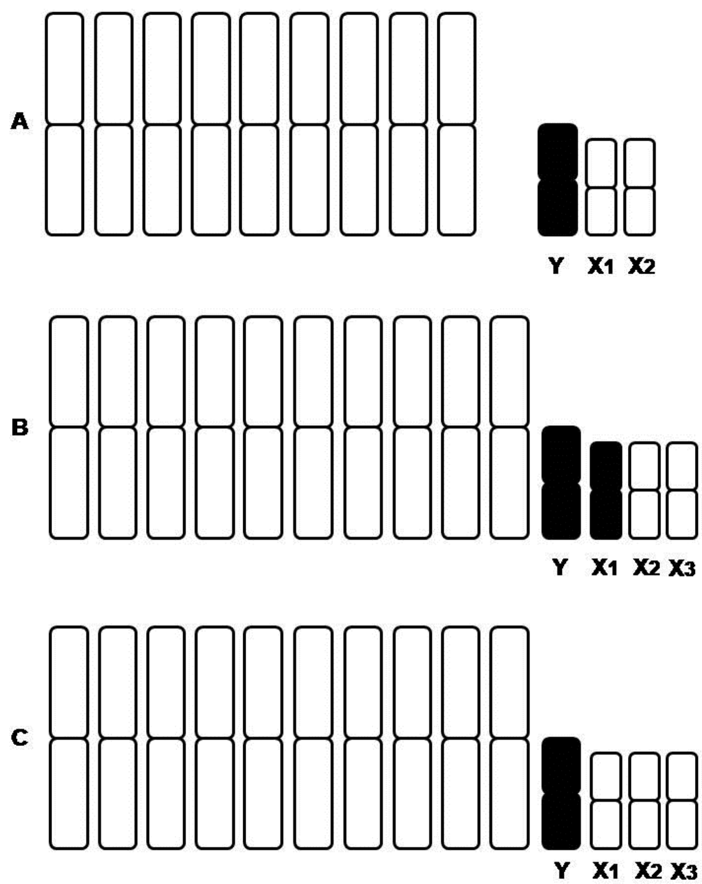

Figure 1. Idiogram representing the heterochromatic pattern in *Panstrongylus megistus* (**A**), *Triatoma vitticeps* (**B**) and *T. melanocephala* (**C**).

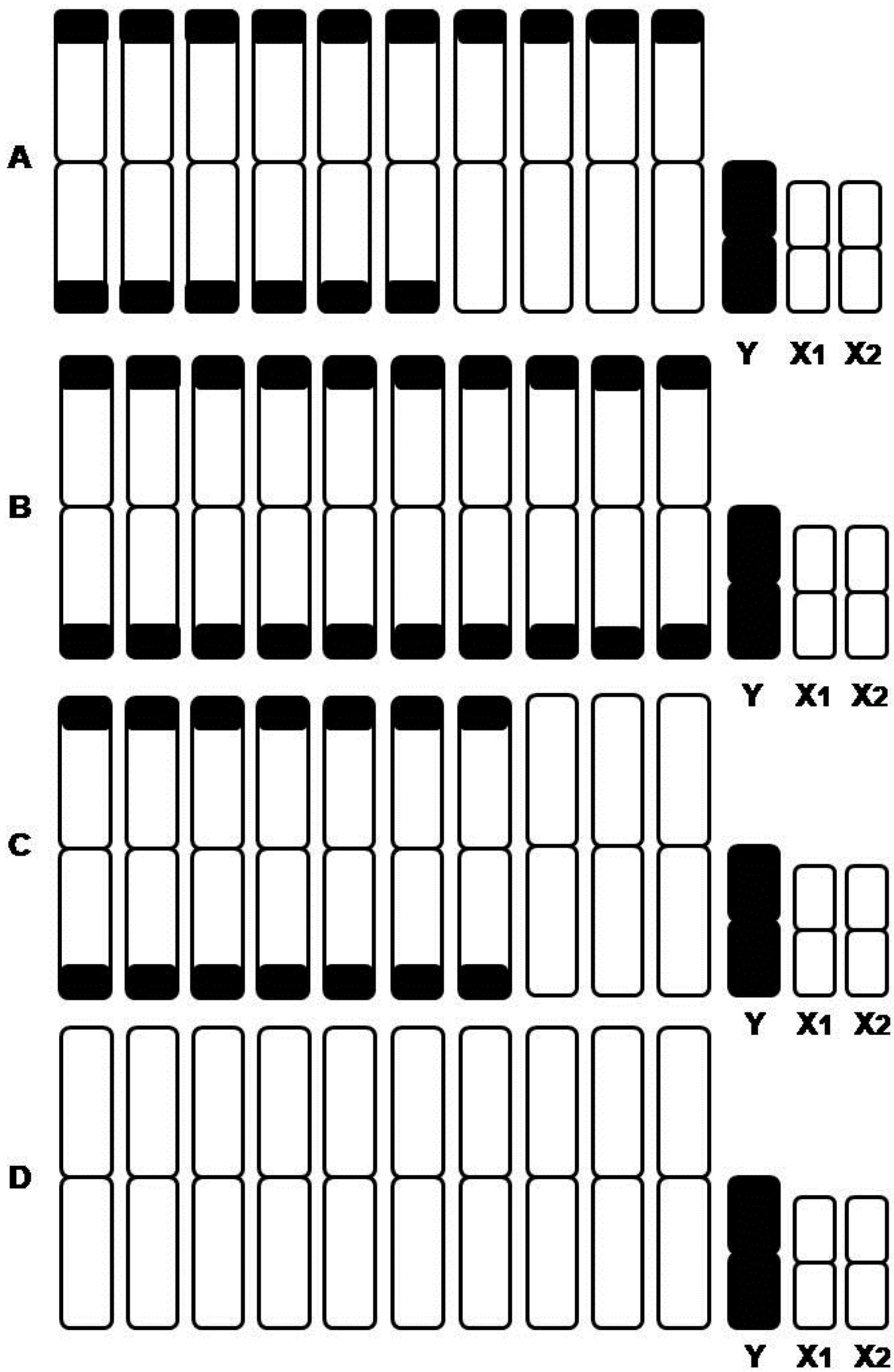

Figure 2. Idiogram representing the heterochromatic pattern in *Panstrongylus lignarius* (**A**), *Triatoma protracta* (**B**), *T. tibiamaculata* (**C**), and *Meccus pallidipennis* (**D**).

DISCUSSION

Karyotypic evolution in the Triatominae subfamily is considered to be related to two events: fission and fusion (Panzera et al., 2010). There are currently 86 karyotypes described in the literature and 40 present chromosome fragmentation (Alevi et al., 2013a).

The disposition of constitutive heterochromatin in the chromosomes is considered to be a tool for grouping evolutionarily related species; for example, species of the Brasiliensis subcomplex present heterochromatic blocks at 1 or both chromosomal ends of all autosomal pairs (Panzera et al., 2000; Alevi et al., 2012b), which can be used to differentiate morphologically related species, such as *Rhodnius neglectus* and *Rhodnius nasutus*, and assist in the description and revalidation of species such as *Triatoma garciabesi* (Jurberg et al., 1998).

The seven species analyzed showed differences in the disposition of heterochromatic blocks in chromosomes, confirming the importance of the C-banding technique for studying the taxonomy of the triatomines.

P. megistus and *P. lignarius* presented differences in chromosome number and disposition of constitutive heterochromatin, as only *P. lignarius* presented C-blocks in autosomes, confirming the results of Crossa et al. (2002).

T. vitticeps and *T. melanocephala* are species that are morphologically (Sherlocki and Guitton, 1980), cytogenetically (Alevi et al., 2013d), and molecularly (Gardim et al., 2014) related. However, C-banding can differentiate these species, as *T. vitticeps* contains a heterochromatic X chromosome, confirming the results of Severi-Aguiar et al. (2006) and Panzera et al. (2012). This is a peculiarity within the triatomine because typically only the sex chromosome Y is heterochromatic.

T. protracta, *T. tibiamaculata*, and *M. pallidipennis* showed no evolutionary relationship and were differentiated based on the arrangement of constitutive heterochromatin in the autosomes. *T. protracta* showed C-blocks in both ends of all autosomes, confirming the results of Ueshima (1966). *T. tibiamaculata* showed terminal C-dots in some autosomal pairs, confirming the results of Panzera et al. (2012), and *M. pallidipennis* did not show constitutive heterochromatin in autosomes, confirming the results of Panzera et al. (2012).

Thus, this study confirms the heterochromatic pattern of seven species of insects and emphasizes the importance of cytogenetic technique of C-banding for the taxonomy of the triatomines, important vectors of Chagas disease.

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