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Cytogenetic analysis in the spermatogenesis of *Triatoma melanosoma* (Reduviidae; Heteroptera)

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ABSTRACT. Triatomines are of great concern in public health because they are vectors of Chagas' disease. This study presents an analysis of the species *Triatoma melanosoma*. The cytogenetic characteristics of triatomines include holocentric chromosomes, post-reductional meiosis in the sex chromosomes and nucleolar fragmentation in the meiotic cycle. The methodology utilized consisted of the techniques of lacto-acetic orcein staining and silver ion impregnation. The organs analyzed were adult testicles. The results enabled to classify the chromosomes by number and size, being three large, eight medium and one small heterochromosome. The three largest chromosomes and the heterochromosomes showed heteropyknotic chromatin in meiosis. The heterochromosomes in 8.05% of the cells in metaphase I behaved as pseudobivalents, contrasting with 91.95% of the cells with individualized sex chromosomes, confirming the achiasmatic nature of these chromosomes. However, the pseudobivalents occurred prominently in metaphase II (78.38%), this fact probably is related to the post-reductional nature of the sex chromosomes. The nucleolus in *T. melanosoma* persisted until the diplotene phase after which it began to fragment. Nucleolar corpuscles were observed in metaphases I and II and during anaphases I and II, these characteristics being related to the phenomenon of nucleolar

persistence. In the initial spermatids, peripheral silver ion impregnation occurred, which could be analogous to the pre-nucleolar corpuscles observed after fragmentation. Thus, this study extends our knowledge of the characteristics of triatomines, in particular, heteropyknotic degree, kinetic activity, formation of sex chromosome achiasmatic pseudobivalency, confirmation of the fragmentation phenomenon, and post-meiotic nucleolar reactivation.

Key words: Triatomines; Cytogenetics; Meiosis; Nucleolus; Holocentric chromosomes; Spermatogenesis

INTRODUCTION

Chagas' disease is an endemic parasitosis with a pronounced importance among heart diseases in South America (Tartarotti et al., 2004). This disease affects more than 11 million people and occurs in Mexico and Central and South America (Dias et al., 2002). On the American continent, Chagas' disease is one of the main endemic diseases after malaria (Tartarotti et al., 2004).

The etiologic agent of Chagas' disease is the protozoan *Trypanosoma cruzi*, and the transmission of this disease occurs by contact with the feces of the triatomine contaminated with the protozoan (Coll-Cárdenas et al., 2004). However, the disease may also be transmitted congenitally and by blood infection (Tartarotti et al., 2004).

The triatomines are divided into six tribes: Alberproseniini, Bolboderini, Cavernicoli, Linshcosteini, Rhodniini and Triatomini, totaling 137 species (Galvão et al., 2003). Within the Triatomini tribe, in the *Triatoma* genus, there is the *Triatoma melanosoma* species. This species is distributed throughout the extreme north of Argentina, in the province of Misiones. Initially, it was described by Martinez and collaborators (1987) as a *T. infestans* subspecies, and as a result it was denominated *T. infestans melanosoma* (Lent et al., 1994). This classification originated from the erroneous conception of *T. melanosoma* as a dark form of *T. infestans*. *T. melanosoma* is now recognized to be a species (Lent et al., 1994).

In cytogenetic terms, male triatomines have a diploid number of chromosomes varying from 21 to 25, and the typical form of the group is 2n = 22 (Ueshima, 1966). The chromosomes of these insects are holocentric and have an unusual meiotic segregation (De Vaio et al., 1985): the autosomes are chiasmatic and pre-reductional, while the sex chromosomes are achiasmatic and post-reductional (Solari, 1979). The heterochromosomes, during metaphase II, alternatively behave as pseudobivalents. The chromosomal size is usually homogeneous in the triatomines, but there are some exceptions: *T. infestans*, *T. platensis*, *T. rubrovaria* and *T. pseudomaculata*. These species have from one to three chromosomes larger than the others and are heteropyknotic in conventional staining (De Vaio et al., 1985; Pérez et al., 1992).

In triatomines, and in other insects, studies of nucleolar behavior have revealed the number of nucleoli in the spermatocytes and their fragmentation during the meiosis prophase I, as well as nucleolar reactivation at the end of spermatogenesis (Warchalowska-Śliwa and Maryanska-Nadachowska, 1992; González-García et al., 1995; Tavares and Azeredo-Oliveira, 1997; Tartarotti and Azeredo-Oliveira, 1999; Morielle and Azeredo-

Oliveira, 2004). Another objective of cytogenetic study is the location of nucleolar organizing regions (NORs), observed in some autosomes and/or heterochromosomes. In triatomines, nucleolar fragmentation occurs during prophase I between diplotene and diakinesis. Nucleolar corpuscles are also observed in the metaphases, together with the nucleolar reactivation that occurs in anaphase II, which are both phenomena typical of triatomines. Usually, these nucleolar corpuscles become smaller as the spermatids mature (Tavares and Azeredo-Oliveira, 1997; Tartarotti and Azeredo-Oliveira, 1999; Morielle and Azeredo-Oliveira, 2004).

The aim of the present study was the characterization of the meiotic cycle with an emphasis on nucleolar activity in *T. melanosoma*, in order to contribute to a better understanding of the chromosomal behavior and the phenomenon of nucleolar fragmentation in *Triatominae*.

MATERIAL AND METHODS

The species analyzed was *T. melanosoma* (Heteroptera: Reduviidae). For the study, 15 specimens were used. The insects were provided by the Special Health Service of Araraquara, State of São Paulo, Brazil, belonging to the Department of Epidemiology of University of São Paulo (USP). The seminiferous tubules of adult males were submitted to the cytochemical techniques of lacto-acetic orcein (De Vaio et al., 1985, with modifications) and silver staining (Howell and Black, 1980). The slides were examined with a Zeiss-Jenaval photomicroscope and the photomicrographs were made on a 400-ASA film.

RESULTS

Lacto-acetic orcein

The polyploidy nuclei of testicular tubule nutritive cells showed dispersed heteropyknotic corpuscles (Figure 1A). The spermatogonial metaphases showed chromatin bridges and heteropyknosis at the chromosome ends (Figure 1B). During the diffuse stage (prophase I), in progressive degrees of chromosome condensation, the heteropyknotic regions showed peripheral or central dispositions (Figure 1C,D). In the diplotene, the presence of terminal and interstitial chiasmata was observed in the bivalent autosomes and achiasmatic sex chromosomes. The heteropyknosis of three autosomes and at least one sexual chromosome was observed (Figure 1F,G). In the diakinesis, there was typical chiasmatic terminalization of the autosomes (Figure 1H). In metaphase I (Figure 1I), the sex chromosomes were visualized alternatively in the pseudobivalent (Figure 1J) or individualized form (Figure 1K). The pseudobivalent presence or absence was analyzed by means of cellular score. In metaphase I, 91.95% of the cells displayed individualized heterochromosomes, and in metaphase II, 78.38% of the observed cells were in the pseudobivalent configuration (Table 1). In metaphase II, the heterochromosomes were again observed to be either individualized (Figure 1L,M) or pseudobivalent (Figure 1N). During anaphase, parallel migration, typical of holocentric chromosomes, was observed and the late migration of chromosomes was verified (Figure 1O-Q). In spermiogenesis, dots were observed on the periphery of early spermatids (Figure 1R,S). During differentiation the dots reduced to the formation of small points at the beginning of the spermatozoids (Figure 1T).

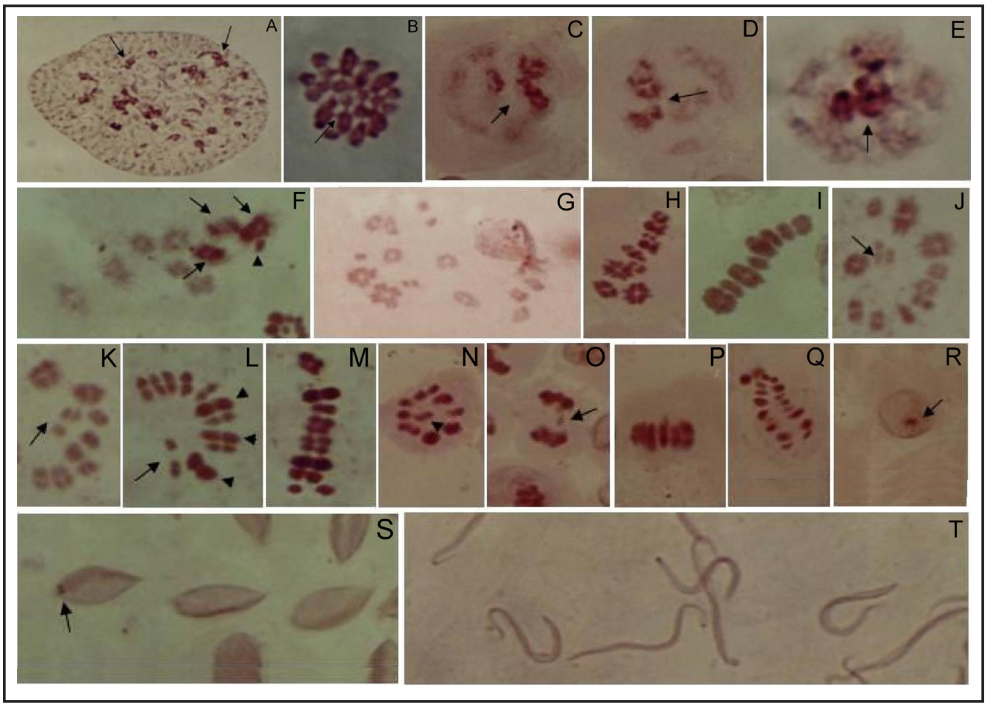

Figure 1. Seminiferous tubule of *Triatoma melanosoma* stained by lacto-acetic orcein. **A.** Polyploidy nucleus with dispersed heteropyknotic corpuscles (arrows). **B.** Spermatogonial metaphase with chromatin bridges joining the chromosomes (arrow). **C,D.** Diffuse stage with peripheral chromocenter, see arrows. **E.** Diffused stage showing chromocenter alternatively in central position (arrow). **F.** Diplotene, observe heteropyknosis in the three largest autosomes (arrows) and at least one heterochromosome (arrowhead). **G.** Final diplotene stage, note the typical chiasmatic figures, stressing the achiasmatic behavior of the sex chromosomes. **H,I.** Diakinesis, note the chiasmatic finalization, and above all pseudobivalent heterochromosome behavior. **J,K.** Circular metaphase I, note achiasmatic sex chromosomes in the center (arrows). **L,M.** Metaphase II, note the three largest autosomes with a greater degree of pyknosis (arrowheads) and individualized sex chromosomes (arrow). **N.** Circular metaphase II with sex pseudobivalent (arrowhead). **O.** Anaphase I, note late chromosome migration (arrow). **P,Q.** Anaphase II, note parallel chromosome migration. **R,S.** Spermatids, note peripheral heteropyknotic corpuscles (arrows). **T.** Spermatids in final elongation stage.

Table 1. Presence of sex chromosomes as pseudobivalents in meiotic metaphases.

	Presence of pseudobivalent	Absence of pseudobivalent
Metaphase I	7 (8.05%)	80 (91.95%)
Metaphase II	58 (78.38%)	16 (21.62%)

Silver ion impregnation

The nucleolar cycle was observed in the cells of the seminiferous tubules during spermatogenesis (Figures 2 and 3). The polyploidy nuclei of the nutritive cells of the testicular tubules showed several nucleolar corpuscles; in addition, the polyploidization phase of this cell was observed (Figure 2A-C). The spermatogonial cells displayed intense

nucleolar activity (Figure 2D). The cells in spermatogonial metaphase demonstrated no specific staining but showed chromatin bridges between the chromosomes (Figure 2E-G). The spermatogonial interphasic cells showed an evident nucleolus, reflecting the gene activity of these nuclei (Figure 2H). In the initial diffuse stage, dispersed nucleolar staining was observed throughout the nucleus, together with the presence of the nucleolus (Figure 2I-K). Still in the diffuse stage, it was possible to observe NORs associated with the nucleolus and the beginning of chromosome condensation (Figure 2L). In the diplotene-diakinesis, nucleolar staining was visualized (Figure 3A), and in the advanced diakinesis stages, it was possible to relate the NORs to three autosomes and one heterochromosome (Figure 3B,C). In diakinesis, nucleolar fragmentation was also observed (Figure 3D). In metaphase, as well as in anaphase, the chromosomes became intensely compacted which made it difficult to analyze the staining. However, it was possible to observe small nucleolar fragments inside the cell (Figure 3E-H). Still in metaphase II, the behavior of pseudobivalents in the heterochromosomes was observed (Figure 3G). The spermatids, at the beginning of spermiogenesis, contained one corpuscle, suggesting post-meiotic nucleolar activity, possibly related to the differentiation process (Figure 3I). During the differentiation process of the spermatids, the corpuscles decrease and fragment (Figure 3J), until the spermatozoids formed (Figure 3K).

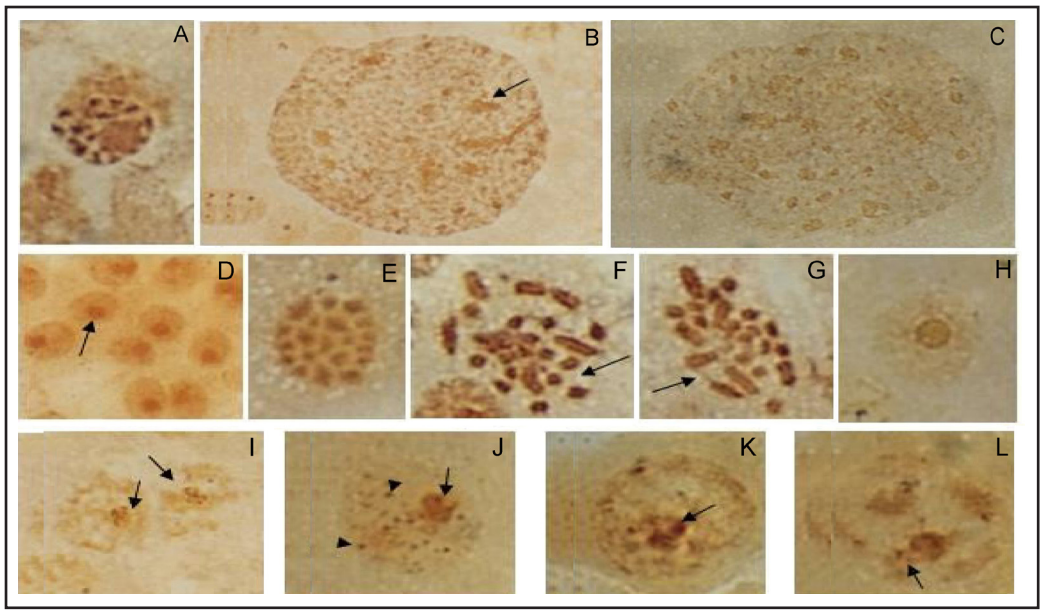

Figure 2. Seminiferous tubules of *Triatoma melanosoma* stained by silver ion impregnation. **A.** Polyploid nuclei in early polyploidization. **B,C.** Polyploid nuclei in progressive ploidy stages, note the great number of nucleolar corpuscles (arrow). **D.** Spermatogonial nuclei with nucleolar activity (arrow). **E-G.** Spermatogonial metaphases with chromatin bridges (arrows). **H.** Primary spermatocyte with evident nucleolus. **I-K.** Initial diffused prophase stage, note nucleoli (arrows) and nucleolar corpuscles (arrowheads). **L.** Final diffused stage, note chromatin associated with nucleolar region (arrow).

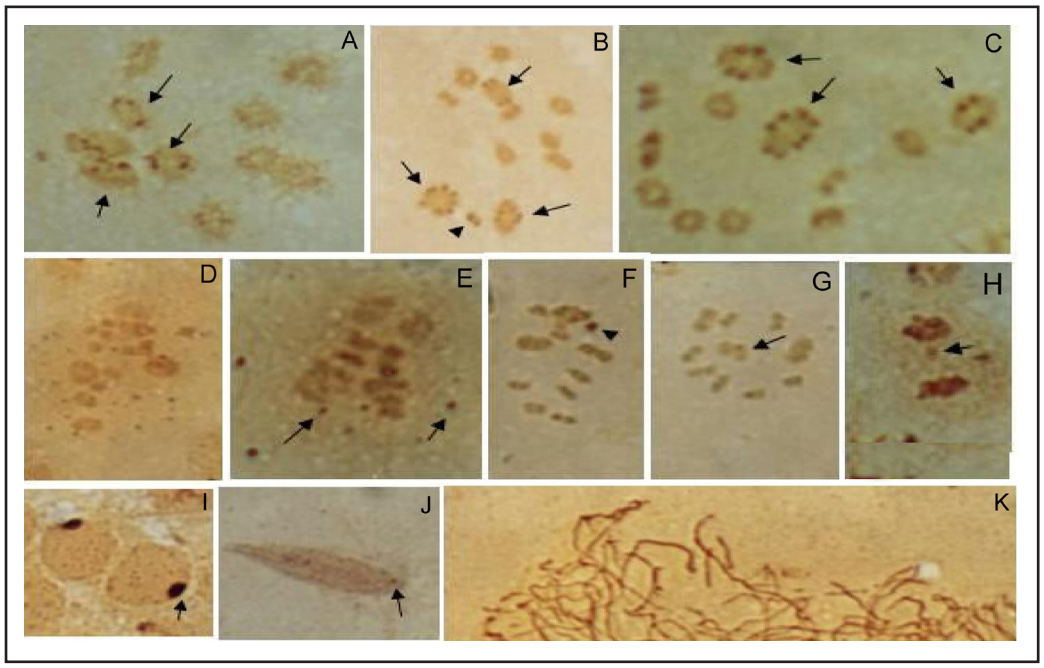

Figure 3. Seminiferous tubule of *Triatoma melanosoma* stained by silver ion impregnation. **A-C.** Diplotene-diakinesis with nucleolar organizing regions evidenced in at least three autosomal bivalents (arrows) and one heterochromosome (arrowhead). **D.** Diakinesis, note the complete nuclelolar fragmentation. **E.** Early anaphase I, note the persistence of nucleolar corpuscles (arrows). **F,G.** Ring metaphase II with sex pseudobivalent at the center (arrow), note the nucleolar persistence (arrowhead). **H.** Anaphase II, note chromosome showing late migration (arrow). **I.** Spermatid in early differentiation, notice silver ion impregnation in the periphery (arrow). **J.** Spermatids in elongation phase, note peripheral nuclear stain in opposition to the area of flagellum formation. **K.** Spermatozoid agglomeration.

DISCUSSION

The study of spermatogenesis in *T. melanosoma*, in association with the information in the literature, has made it possible to observe chromosome characteristics and meiotic behavior in Heteroptera. The polyploidy nuclei of *T. melanosoma* displayed several dispersed heterochromatic corpuscles. The study of polyploidy nuclei in other triatomines has revealed the existence of only one heterochromatic corpuscle as a frequent occurrence in these insects (Tavares and Azeredo-Oliveira, 1997; Tartarotti and Azeredo-Oliveira, 1999; Morielle and Azeredo-Oliveira, 2004).

The adult males of *T. melanosoma* showed a diploid number of chromosomes 2n = 22 (20A, XY), corroborating the study of Panzera and collaborators (1996). In the species analyzed, the chromosomes were classified in the metaphase II according to size; thus, they were denominated as three large, eight medium and one small, the last corresponding to one of the heterochromosomes. As mentioned above, in male triatomines the diploid number of chromosomes varies from 21 to 25 chromosomes (Ueshima, 1966); however, it is believed that 60% of the species analyzed possess a diploid number of chromosomes 2n = 20A + XY (Tavares and Azeredo-Oliveira, 1997).

In *T. melanosoma* the three largest autosomes of the diploid complement, together with the heterochromosomes, were heteropyknotic. In the triatomines, little variation in chromosome size is usually observed (De Vaio et al., 1985). However, the species *T. infestans*, *T. platensis*, *T. rubrovaria*, and *T. pseudomaculata* have between 1 and 3 chromosomes larger than the others, appearing heteropyknotic with conventional staining (De Vaio et al., 1985; Pérez et al., 1992).

The chromosomes of *T. melanosoma* are characterized as holocentric based on migration pattern, late migration and lack of primary constriction. These characteristics are typical of the Heteroptera and of the Homoptera, Lepdoptera and Trichoptera orders (Wolf, 1996). In Hemiptera after high dosages of radiation, a series of chromosome structural rearrangements occur; however, the complete elimination of the chromosomes or fragments, due to a faulty inclusion in the telophase, has rarely been observed (Hughes-Schrader and Schrader, 1961). In holocentric chromosomes, the microtubules of the spindle interact with the whole extent of the chromatin and do not have a localized centromere (Pérez et al., 1997). Atomic force microscopic analyses of insects with holocentric chromosomes have shown, in a 3-D reconstruction, that the junction between the two chromatids is totally homogeneous over the whole chromosome (Mandrioli and Manicardi, 2003).

In triatomines, meiosis is inverted for the heterochromosomes. During anaphase I, the sex chromosomes are pre-equational, and in anaphase II they become post-reductional. However, the autosomes follow a standard meiotic segregation (Pérez et al., 2000). In addition, the meiosis of *T. melanosoma* exhibited patterns usually found in triatomines in metaphases I and II. In metaphase I, the sex chromosomes were positioned in the center of the ring formed by autosomes, as occurs in *T. infestans*, *T. pseudomaculata* and *Rhodnius pictipes* (De Vaio et al., 1985; Pérez et al., 1992). In addition, the heterochromosomes in 8.05% of the cells analyzed in metaphase I behaved as pseudobivalents, in contrast to the remaining cells, in which the sex chromosomes were individualized, confirming the achiasmatic nature of these chromosomes (Solari, 1979). The heterochromosomes also behaved as pseudobivalents during metaphase II, this being constituted of a chromatid from chromosome X and another from chromosome Y. These observations have also been made by other researchers (De Vaio et al., 1985; Tartarotti and Azeredo-Oliveira, 1999; Morielle and Azeredo-Oliveira, 2004). Interestingly, in the present study, the pseudobivalent formation occurs to a large extent in metaphase II (78.38%); this fact may have a possible relationship with the post-reductional nature of the sex chromosomes.

With regard to the chiasmatic presence in diplotene stages, four bivalents with two chiasmata, one terminal and the other interstitial were observed in *T. melanosoma*. Six bivalents exhibited terminal chiasmata, with a characteristic ring format. The heterochromosomes in this phase are completely individualized and achiasmatic, although in metaphase I they have a side by side association. Usually, holocentric chromosomes show 1 or 2 chiasms; however, this number may be higher in larger chromosomes. This is also the case with the Homoptera *Psylla foersteri*, which has three chiasmata in an autosomal pair originating in the fusion between 5 or 6 small chromosomes. However, these chromosomes migrate slowly, and the medium chiasmata are not usually segregated in anaphase I, resulting in cell loss during spermatogenesis (Nokkala et al., 2004).

A characteristic phenomenon in *T. melanosoma* was the anaphase with late migration, suggesting that the chromosomes with late migration may be the sexual ones (Morielle and Azeredo-Oliveira, 2004). The anaphasic figures observed in the present study indicate that the

kinetic activity of the chromosomes is located preferentially in terminal regions and its segregation mechanism is entirely equidistant throughout the extent of the chromosome, and that the orientation of the bivalent was uniform and parallel to the polar axis of the spindle. These observations are in agreement with that proposed in the literature regarding the chromosome pair in Hemiptera (Hughes-Schrader and Schrader, 1961).

In *T. infestans*, in anaphases I and II, the kinetic activity was restricted to the chromosome end, irrespective of whether this was euchromatic or heterochromatic. It was also observed that the kinetic activity at the euchromatic end was greater than that at the heterochromatic end (Pérez et al., 1997). However, studies with specific centromeric probes in holocentric chromosomes of Cyperaceae detected the presence of centromeric scattering which, interestingly, was restricted to heterochromatic areas (Guerra et al., 2006).

With regard to silver ion impregnation, the testicular tubules of *T. melanosoma* analyzed in the present study, revealed only one nucleolus in the initial nuclei of prophase I, differing in this way from some heteroptera that possess more than one nucleolus (Fossey and Liebenberg, 1995). However, at the beginning of the diffuse stage, in addition to the nucleolus, small arginophilic corpuscles dispersed throughout the nuclear area were observed. This observation suggests a transcriptional activity not restricted to the nucleolar structure at the beginning of the meiotic prophase.

The nucleolus in *T. melanosoma* persisted until prophase I, specifically in the diplotene; after this phase the nucleolar fragmentation phenomenon began to occur. Nucleolar corpuscles were observed in metaphases I and II, as well as during anaphases I and II. These characteristics relate to the phenomenon of nucleolar persistence, in which the nucleolus does not disappear totaly in triatomines, but is preserved as pre-nucleolar corpuscles until the beginning of the next meiotic cycle (Tartarotti and Azeredo-Oliveira, 1999; Morielle and Azeredo-Oliveira, 2004). These pre-nucleolar corpuscles are composed of proteins involved in pre-RNA processing, which leave the nucleolus in the prophase and are mainly located in the periphery of the chromosomes during the cell cycle; these proteins originate from the dense fibrillar center and granular center of the active nucleolus (Hernandez-Verdum, 2006).

In *T. melanosoma*, between the diplotene and diakinetic phases, silver ion impregnation was observed in at least three autosomes and one heterochromosome, suggesting the existence of NORs in those chromosomes. Studies of the *Panstrongylus* genus (*P. megistus* and *P. herreri*) have demonstrated the existence of NORs in sexual chromosomes, in prophase I of the meiosis and in chromosomal associations of NOR-carrying autosomes in the nucleolus (Tartarotti and Azeredo-Oliveira, 1999). The association between the nucleolus and the sexual chromosomes and autosomes is a common characteristic in insects observed in Heteroptera and also in Psocoptera (Golub et al., 2004).

In the *T. melanosoma* diplotene-diakinesis stage, the positive Ag-NOR staining on one of the heterochromosomes persists until the meiotic metaphases. However, the silver ion impregnations do not persist during the meiotic stage; for example, in the Heteroptera *Belostoma oxyurum*, the sex chromosomes continue to be Ag-NOR positive until the diffuse stage, after which no staining was observed on these chromosomes (Papeschi, 1995).

At the beginning of cell differentiation, that is, in the initial spermatids, a prominent peripheral silver ion staining occurs. This may be analogous to the pre-nucleolar corpuscles observed in the early nucleolar fragmentation. In other words, after the reorganization of the nuclear envelope, silver-positive proteins that were previously small corpuscles in the cell, are

destined for the nucleus for future nucleolar reorganization.

In conclusion, the present study contributes to our knowledge of the cytogenetics of triatomines through the analysis of heteropyknotic degree in *T. melanosoma* spermatogenesis, chromosome size arrangement and their correlation at the different pyknotic levels. This study also corroborates the behavior of holocentric chromosomes in Heteroptera, terminal anaphase kinetic activity and the formation of achiasmatic pseudobivalents in the sex chromosomes. Finally, the data of this study confirm the phenomena of nucleolar fragmentation in triatomines and post-meiotic nucleolar reactivation at the beginning of cell differentiation.

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REFERENCES

Coll-Cardenas R, Espinoza-Gomez F, Maldonado-Rodriguez A, Reyes-Lopez PA, et al. (2004). Active transmission of human Chagas disease in Colima Mexico. *Mem. Inst. Oswaldo Cruz* 99: 363-368.

De Vaio ES, Grucci B, Castagnino AM, Franca ME, et al. (1985). Meiotic differences between three Triatomine species (Hemiptera, Reduviidae). *Genetica* 67: 185-191.

Dias JC, Silveira AC and Schofield CJ (2002). The impact of Chagas disease control in Latin America: a review. *Mem. Inst. Oswaldo Cruz* 97: 603-612.

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

Galvão C, Carvalho RU, Rocha DS and Jurberg J (2003). A checklist of the current valid species of the subfamily Triatominae Jeannel, 1919 (Hemiptera, Reduviidae) and their geographical distribution, with nomenclatural and taxonomic notes. *Zootaxa* 202: 1-36.

Golub NV, Nokkala S and Kuznetsova VG (2004). Holocentric chromosomes of psocids (Insecta, Psocoptera) analysed by C-banding, silver impregnation and sequence specific fluorochromes CMA3 and DAPI. *Folia Biol.* 52: 143-149.

Gonzalez-Garcia JM, Rufas JS, Antonio C and Suja JA (1995). Nucleolar cycle and localization of NORs in early embryos of *Parascaris univalens*. *Chromosoma* 104: 287-297.

Guerra M, Brasileiro-Vidal AC, Arana P and Puertas MJ (2006). Mitotic microtubule development and histone H3 phosphorylation in the holocentric chromosomes of *Rhynchospora tenuis* (Cyperaceae). *Genetica* 126: 33-41.

Hernandez-Verdum D (2006). Nucleolus: from structure to dynamics. *Histochem. Cell Biol.* 125: 127-137.

Howell WM and Black DA (1980). Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* 36: 1014-1015.

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

Lent H, Jurberg J, Galvão C and Carcavallo RU (1994). *Triatoma melanosoma* novo status para *Triatoma infestans melanosoma* Martinez, Olmedo & Carcavallo, 1987 (Hemiptera, Reduviidae). *Mem. Inst. Oswaldo Cruz* 89: 363-368.

Mandrioli M and Manicardi GC (2003). Analysis of insect holocentric chromosomes by atomic force microscopy. *Hereditas* 138: 129-132.

Martinez A, Olmedo RA and Carcavallo RU (1987). Una nueva subespecie argentina de *Triatoma infestans. Chagas* 4: 7-8.

Morielle A and Azeredo-Oliveira MTV de (2004). Description of the nucleolar activity and karyotype in germinative cell lines of *Rhodnius domesticus* (Triatominae, Heteroptera). *Caryologia* 57: 31-37.

Nokkala S, Kuznetsova VG, Maryanska-Nadachowska A and Nokkala C (2004). Holocentric chromosomes in meiosis. I. Restriction of the number of chiasmata in bivalents. *Chromosome Res.* 12: 733-739.

Panzera F, Perez R, Hornos S, Panzera Y, et al. (1996). Chromosome numbers in the Triatominae (Hemiptera-Reduviidae):

a review. *Mem. Inst. Oswaldo Cruz* 91: 515-518.
Papeschi AG (1995). Correspondence between C-banding and Ag-NOR in the sex chromosomes of *Belostoma oxyurum* (Belostomatidae, Heteroptera). *Cytologia* 60: 291-295.
Perez R, Panzera Y, Scafiezzo S, Mazzella MC, et al. (1992). Cytogenetics as a tool for triatomine species distinction (Hemiptera-Reduviidae). *Mem. Inst. Oswaldo Cruz* 87: 353-361.
Perez R, Panzera F, Page J, Suja JA, et al. (1997). Meiotic behaviour of holocentric chromosomes: orientation and segregation of autosomes in *Triatoma infestans* (Heteroptera). *Chromosome Res.* 5: 47-56.
Perez R, Rufas JS, Suja JA, Page J, et al. (2000). Meiosis in holocentric chromosomes: orientation and segregation of an autosome and sex chromosomes in *Triatoma infestans* (Heteroptera). *Chromosome Res.* 8: 17-25.
Solari AJ (1979). Autosomal synaptonemal complex and sex chromosomes without axes in *Triatoma infestans* (Reduviidae, Hemiptera). *Chromosoma* 72: 225-240.
Tartarotti E and Azeredo-Oliveira MTV (1999). Patterns of nucleolar activity during spermatogenesis of two triatomines, *Panstrongylus megistus* and *P. herreri*. *Caryologia* 52: 117-184.
Tartarotti E, Azeredo-Oliveira MTV and Ceron CR (2004). Problemática vetorial da doença de Chagas. *Arq. Ciênc. Saúde* 11: 44-47.
Tavares MG and Azeredo-Oliveira MTV (1997). Pattern of nucleolar activity during spermatogenesis in triatomines (Heteroptera, Reduviidae) as analyzed by silver staining. *Cytobios* 89: 93-103.
Ueshima N (1966). Cytotaxonomy of the Triatominae (Reduviidae: Hemiptera). *Chromosoma* 18: 97-122.
Warchalowska-Śliwa E and Maryanska-Nadachowska A (1992). Karyotypes, C-bands, NORs location in spermatogenesis of *Isophya brevipennis* Brunner (Orthoptera: Phaneropteridae). *Caryologia* 45: 83-89.
Wolf KW (1996). The structure of condensed chromosomes in mitosis and meiosis of insects. *Inst. J. Insect Morphol. Embryol.* 25: 37-62.

