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Cytogenetic Study of Holocentric Chromosomes in Three Species of Triatomines (Heteroptera, Reduvidae)

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Summary In the present work, spermatogenesis was analyzed in 3 species of the genus *Triatoma* (*T. platensis*, *T. proctata*, *T. tibiamaculata*). Lacto-acetic orcein staining was used in order to investigate chromosomal meiotic behavior of these species. It allowed the identification of the *T. tibiamaculata* karyotype (20, X_1X_2Y), the observation that in *T. protacta* doesn't occur late migration of sexual chromosomes and corroborated knowledgments about holocentric chromosome nature.
Key words Triatomines, Meiosis, Holocentric chromosomes.

The importance of triatomines in human parasitology, specially those of the genus *Triatoma*, lies on the fact that they are vectors of *Trypanosoma cruzi*, which causes Chagas' disease. This disease is a serious public health problem, not only for its high prevalence, but also for the harm it causes to the population in Brazil and other Latin American countries, where it is widely spread. Triatomines are also of genetic interest due to the fact that their chromosomes present diffuse kinetochores, distributed along the chromosome, and an unusual form of meiosis with postreductional segregation of sex chromosomes (Tartarotti and Azeredo-Oliveira 1999).

The great majority of triatomines have 10 autosome pairs and 1 pair of sex chromosomes. However, some species have multiple sex chromosomes derived from the fragmentation of the original X, while other species have 9 or 11 autosome pairs (Hughes-Schrader and Schrader 1961, Ueshima 1966).

In the present work, three species of the genus *Triatoma* (*T. platensis*, *T. proctata*, *T. tibiamaculata*) were compared with emphasis on the analysis of several spermatogenesis stages and the correlation of the differences and similarities found in order to achieve a better understanding of the evolution of this group of insects.

Materials and methods

The species studied were *T. platensis*, *T. protacta* and *T. tibiamaculata*. These hematophagous insects pertain to the order Heteroptera, family Reduviidae and sub-family Triatominae (Lent and Wygodzinsky 1979, Manna 1995). The testes of 6–8 young adult males (provided by the Insectary of the Special Health Service (SESA), Araraquara (SP, Brazil) were fixed in acetic acid and stained with lacto-acetic orcein (De Vaio *et al.* 1985). For documentation was used KODAK TMAX 100ASA film. Black-and-white photomicrographs were taken under an OLYMPUS BX40 microscope.

Results

The polyploid nuclei of the testicular tubule wall showed a single heteropycnotic corpuscle in

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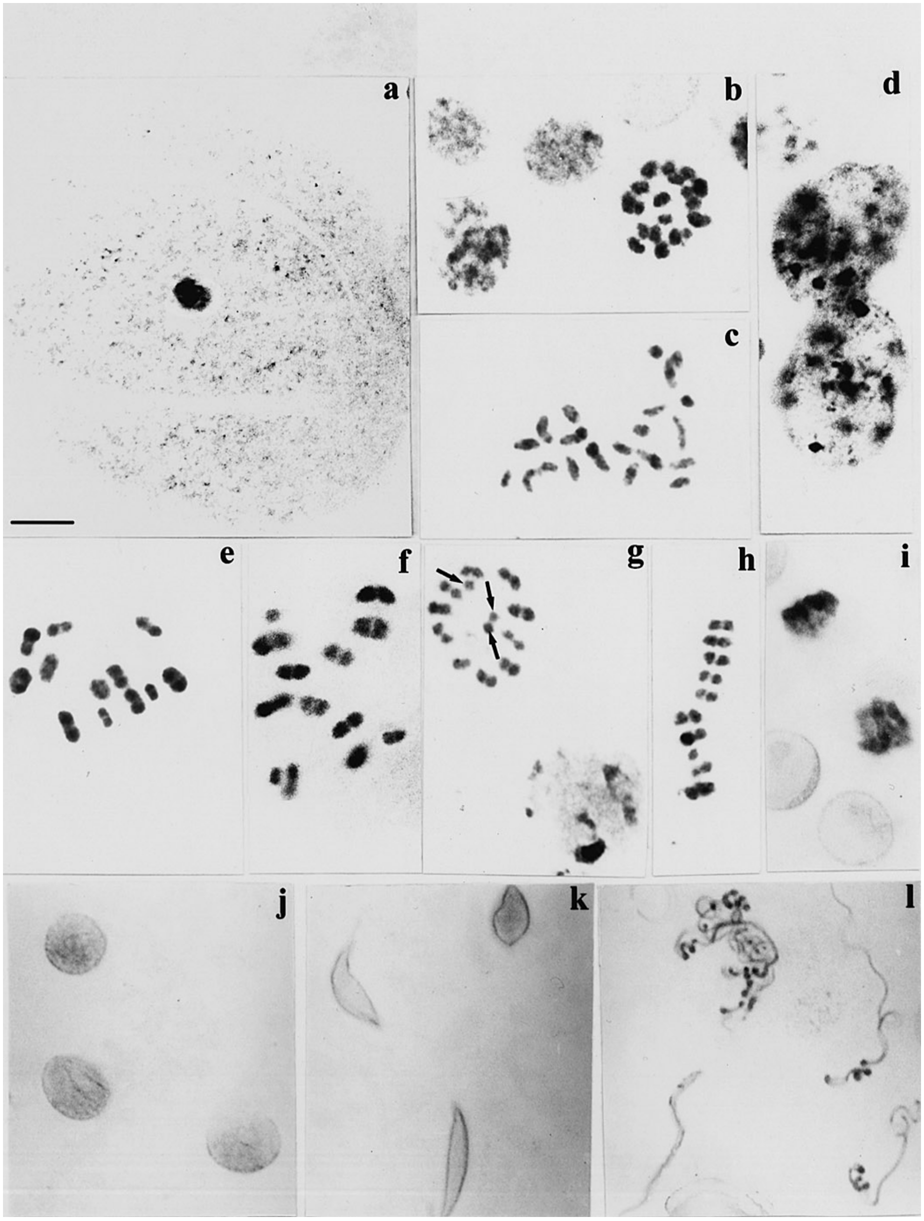

Fig. 1. *T. protacta* testicular tubules stained with lacto-acetic orcein. a) Polyploid nucleus of the tubular wall nutritive cells with a larger heterochromatic corpuscle. Notice the vesicles inside the corpuscle with a less stained halo around it. b, c) Spermatogonial metaphase. d) Nuclei at the beginning of prophase I (confused stage) with an heteropycnotic corpuscle formed by sex chromosomes. e, f) Meiotic metaphase I with 10 autosomal bivalents and 3 sex chromosomes. g, h) Meiotic metaphase II—frontal and lateral view—with 10 autosomes and 3 sex chromosomes (arrows). Notice the ring formed by the autosomes and the sex chromosomes in its center. i) Meiotic telophase. j) Spermatids at the beginning of spermiogenesis. k, l) Spermatids at more advanced stages of spermiogenesis. Bar=18 μm.

T. protacta (Fig. 1a) and *T. tibiamaculata* (Fig. 3a). However, in *T. platensis*, several corpuscles were observed (Fig. 2a). Spermatogonial metaphases presented 20 autosomes and 3 sex chromosomes in *T. protacta* (Fig. 1b, c) and *T. tibiamaculata* (Fig. 3b) whereas only 2 sex chromosomes were observed in *T. platensis* (Fig. 2b, c). During the "confused stage", the nuclei displayed a single

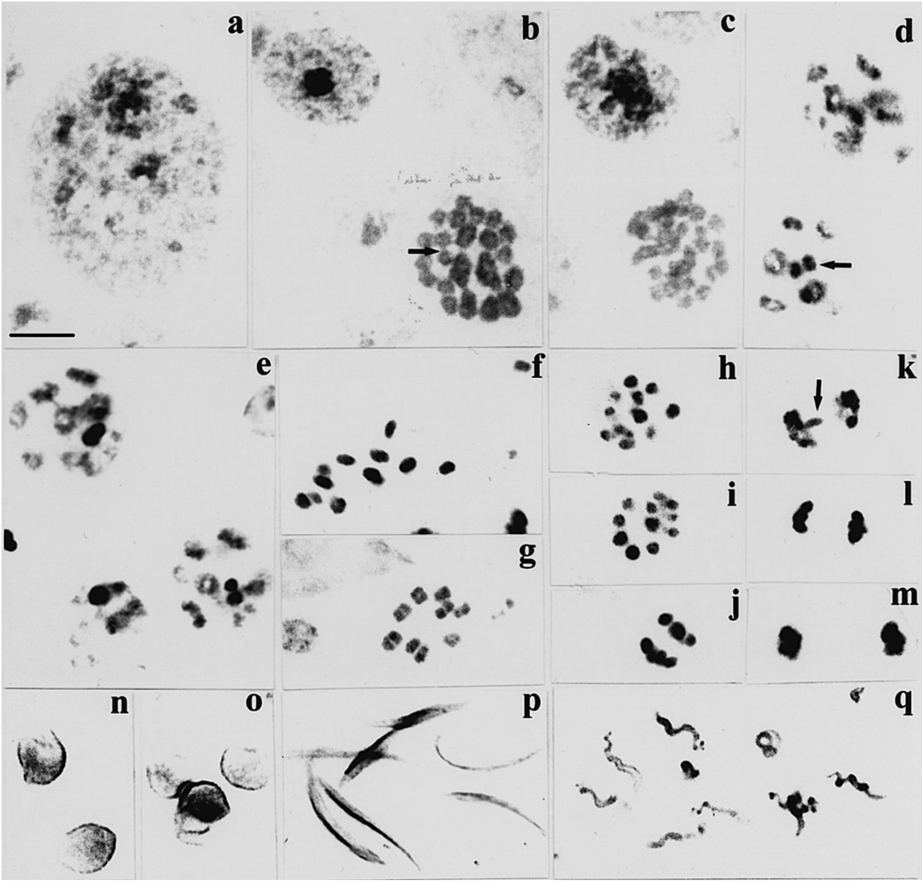

Fig. 2. *T. platensis* testicular tubules stained with lacto-acetic orcein. a) Polyploid nucleus of the tubular wall nutritive cells with several heterochromatic corpuscle. b, c) Spermatogonial metaphase. Arrow indicates chromatin filaments connecting chromosomes. d, e) Nuclei at the beginning of prophase I (confused stage). Arrow indicates the sex chromosomes. f, g) Meiotic metaphase I with 10 autosomal bivalents and 2 sex chromosomes. h, i) Meiotic metaphase II with 10 autosomes and 2 sex chromosomes. Notice the ring formed by the autosomes and the sex chromosomes in its center. j–l) Meiotic anaphase II. The arrow indicates chromosomes with late migration. m) Meiotic telophase II. n, o) Spermatids at the beginning of spermiogenesis. p, q) Spermatids at more advanced stages of spermiogenesis. Bar=18 μm.

corpuscle but in *T. platensis*, which exhibited a bipartite corpuscle (Fig. 2d, e). At metaphase I, 10 autosomal bivalents and 3 sex chromosomes (X_1X_2Y) were seen in *T. protacta* (Fig. 1e, f) and *T. tibiamaculata* (Fig. 3e, f) whereas *T. platensis* showed only 2 sex chromosomes (XY) (Fig. 2f, g). At metaphase II, an autosomal ring was formed and sex chromosomes were located in its center in *T. protacta* (Fig. 1g) and *T. tibiamaculata* (Fig. 3g) whereas in *T. platensis* they could be either center-positioned or not (Fig. 2h, i). At anaphase II, late migration was not observed only in *T. protacta*.

Discussion

In the 3 species investigated, chromosomes behaved as holocentric as they showed no primary constriction neither the traditional V-shaped figure during metaphase. Instead, they resembled small, round structures. Autosomes were radially arranged at the periphery of the metaphase plate (MII) and sex chromosomes could be located or not in the ring center. Chromosomes, as whole blocks,

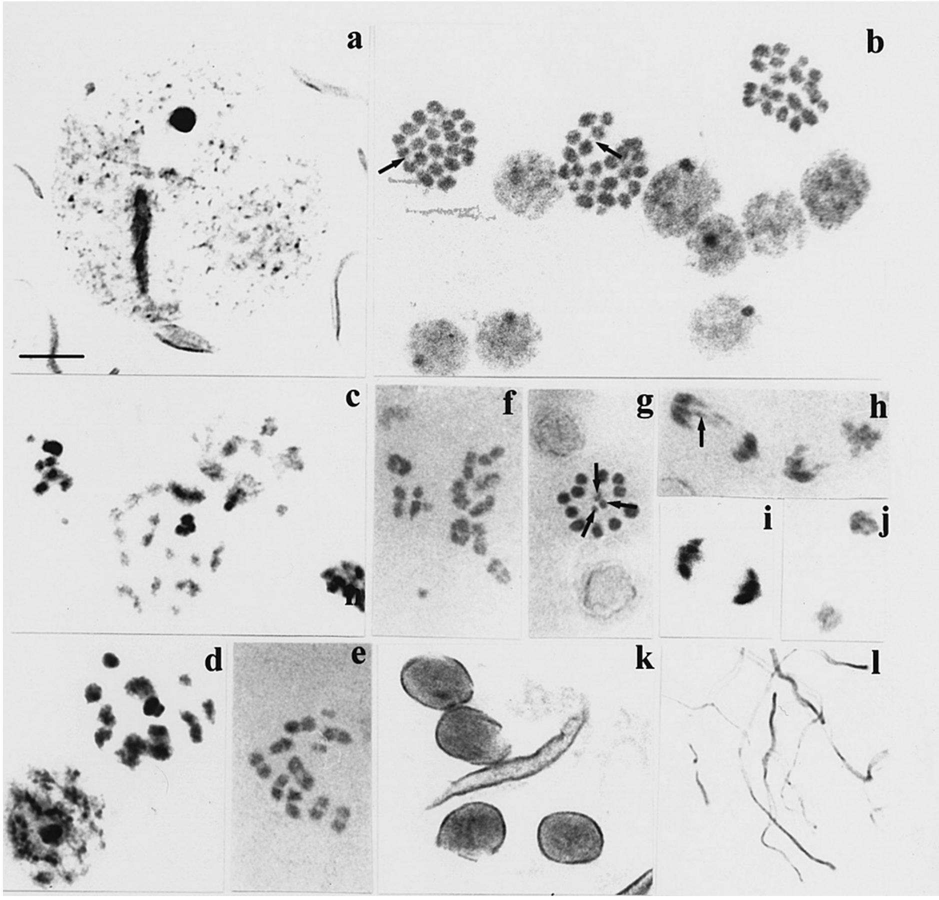

Fig. 3. *T. tibiamaculata* testicular tubules stained with lacto-acetic orcein. a) Polyploid nucleus of the tubular wall nutritive cells with a larger heterochromatic corpuscle. Notice the corpuscle with a less stained halo around it. b) Spermatogonial metaphases. Arrows indicate chromatin filaments connecting chromosomes. c, d) Nuclei at the beginning of prophase I (confused stage) with an heteropycnotic corpuscle formed by the sex chromosomes. e, f) Meiotic metaphase I with 10 autosomal bivalents and 3 sex chromosomes. g) Meiotic metaphase II with 10 autosomes and 3 sex chromosomes. Notice that autosomes formed a ring and sex chromosomes are located in its center (indicated by arrows). h, i) Meiotic anaphase II. Arrow indicates chromosomes with late migration. j) Meiotic telophase II. k) Spermatids at the beginning of spermiogenesis. l) Spermatids at spermiogenesis final stage. Bar=18 μm.

migrated to the cell poles during anaphase.

The 3 species studied (*T. protacta*, *T. platensis*, *T. tibiamaculata*) showed polyploid nuclei of the testicular tubule wall with 1 or several heteropycnotic corpuscles that might represent the heterochromatin of the sex chromosomes. Such nuclei seem to reflect the high metabolic rate necessary to keep cell division (De Vaio *et al.* 1985). In *T. protacta*, *T. platensis* and *T. tibiamaculata*, chromosomal behavior was similar to that in the remaining Heteroptera. They were connected by chromatin filaments at spermatogonial metaphase. Sex chromosomes formed a corpuscle, that could be bipartite, and characterized the nuclei at the "confused stage". During meiosis II, autosomes were radially located at the periphery of the metaphase plate and sex chromosomes could be found, or not, in the autosomal ring center. And, finally, during anaphase, 2 species (*T. platensis*, *T. tibiamaculata*) showed late chromosomal migration, *i.e.*, sexual chromosomes did not migrate synchronically with autosomes (Banergee 1958, Ueshima 1966, Manna 1995).

Conventional analysis with lacto-acetic orcein staining allowed the identification of the *T. tib-*

iamaculata karyotype $20+X_1X_2Y$, which had not been previously cytogenetically described in the literature. It corroborated, too, those reported for the other 2 species (*T. protacta*, *T. platensis*), whose karyotypes were described in mitotic cells by Ueshima (1966) and Schreiber and Pellegrino (1950), that are equivalent to the modal number of chromosomes in the sub-family Triatominae. In 2 of the species investigated (*T. protacta*, *T. tibiamaculata*), fragmentation of the X chromosome was very likely the mechanism responsible for chromosomal derivation (Ueshima 1979, Manna 1995).

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