

Localization of rDNA sites in holocentric chromosomes of three species of triatomines (Heteroptera, Triatominae)

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ABSTRACT. Chromatin organization in the holocentric chromosomes of three triatomines species was cytologically studied by fluorescent *in situ* hybridization with a 45S rDNA probe of *Drosophila melanogaster* to localize ribosomal genes. In *Triatoma tibiamaculata*, metaphases I showed telomeric highlights in a single, larger bivalent. In *T. protacta*, hybridization was detected in one of the telomeres of an autosomal chromosome. In *T. platensis*, there were highlights in a single, smaller chromosome (X chromosome). The results obtained did not agree with the expected localization of rDNA genes in the sex chromosomes of triatomines, as demonstrated by silver impregnation, and suggest that the chromosome reorganization that occurred in this group during evolution may be a more important mechanism involved in rDNA distribution.

Key words: Fluorescent *in situ* hybridization, Holocentric chromosomes, Nucleolar organizer region, Heteroptera, Triatominae

INTRODUCTION

Triatomines are hematophagous insects that are of great medical and sanitation interest because they are vectors of Chagas' disease. In addition, they are excellent models for cytogenetic studies because they have holocentric chromosomes and sex chromosomes that are involved in an uncommon form of meiosis (Tavares and Azeredo-Oliveira, 1997; Tartarotti and Azeredo-Oliveira, 1999; Severi-Aguiar and Azeredo-Oliveira, 2002).

Thus, as in other eukaryotic organisms, their ribosomal genes are organized into two highly repeated gene families, one that codes for the major ribosomal subunit composed of 28S, 18S and 5.8S rRNA and the other that codes for the minor ribosomal subunit composed of 5S rRNA. The first gene family corresponds to nucleolar organizer regions (NORs) and is identified with silver staining. It is one of the most widely studied genomic portions and an important chromosomal marker even in phylogenetic analyses (Deiana et al., 2000).

Silver-stained NORs (Ag-NORs) are the chromosomal regions that contain a cluster of multiple copy ribosomal RNA genes (rDNAs; 18S, 5.8S and 28S) (*major*) that form active nucleoli. The 5S rDNA region (*minor*) contains another multiple copy ribosomal gene that is not involved in the formation of nucleoli and is not silver-stainable. Ag-NORs are not rDNAs themselves but a complex of acidic residual proteins associated with the nucleolar fibrillar center, and they are used to investigate rDNA expression. The determination of the number and localization of rDNA/Ag-NOR loci, which are species-specific, makes them an important karyotypical marker, especially in some organisms such as fish (Fujiwara et al., 1998). In other vertebrates, anurans, Lourenço et al. (1998) demonstrated that silver staining was able to detect all the genomic NORs of these individuals in agreement with the data obtained by fluorescent *in situ* hybridization (FISH). Hirai et al. (1996) inferred that kinetochores, like active NORs, are silver stained in many eukaryotes, but that this method is not always efficient in detecting rDNA sites in inactive NORs, making FISH thereby necessary.

In fish, FISH results are not consistent with those obtained with silver impregnation. Almeida-Toledo et al. (1996) hybridized *Eigenmannia* renal cells with an rDNA probe and verified that the regions where hybridization occurred coincided with Ag-NORs. Deiana et al. (2000) examined *Micropterus salmoides* lymphocytes and demonstrated that 45S and 5S ribosomal genes are localized in two different chromosome pairs but both in GC-rich regions. In *Epinephilus marginator*, Sola et al. (2000) found that 5S rDNA sites are CMA₃-negative and therefore do not contain GC-rich sequences. Woznick et al. (2000), using rDNA probes, demonstrated comparable NOR localization in geographically isolated populations of *Salmo trutta*.

In larvae of the tsetse fly (*Glossina*), Willhoeft (1997) observed that the rDNA probe hybridized with the long arm C-band of an autosome in all the species studied and that, in some lineages, hybridization occurred on the Y chromosome. In both mitotic and meiotic cells of the fly *Pantonella intermutans*, hybridization with an rDNA probe showed that NOR is localized in an autosomal pair where a large heterochromatin block is found (Parise-Maltempi and Avancini, 2000).

In ants, Hirai et al. (1994) demonstrated that in *Myrmecia pilosula*, 28S rDNA is repeated and localized in pericentromeric C-bands in the chromosomes of an ancestral eukaryote, which became involved in the saltatory increase of C-bands following centric fission. The excess copies of rDNA and the related C-bands were inactivated and eliminated from the genome (inversion or fusion) during species evolution. However, rDNAs remain dispersed and

tend to increase in the genome as chromosome number is increased by centric fission. Two years later, these authors were thus led to publish a study on rDNA dispersion in this genus, showing that the number of chromosomes carrying 28S rDNA increases from 2 in species with low chromosome number, to 19 in species with high chromosome numbers (Hirai et al., 1996). In *Tapynoma nigerrimum* Lorite et al. (1999), using FISH, found the presence of an Ag-NOR that was also CMA₃⁺, on the short arm of an autosome.

Mandrioli et al. (2000) studied the hybridization of 28S rDNA probes with the holocentric chromosomes of the aphid *Schizaphis graminum* and observed that rDNA genes were found in one of the telomeres on each X chromosome, which were the only CMA₃-positive regions while all the other heterochromatin bands were DAPI-positive. Similar behavior was observed in *Acyrtosiphon pisum*. The telomere of its X chromosome, which was CMA₃⁺ and Ag-NOR⁺, was strongly fluorescent after hybridization with 28S rDNA sequences, thus demonstrating that this region was the NOR that contained actively transcribed rDNA genes (Bizzaro et al., 2000). In the present study, we report the results of hybridization of *Drosophila melanogaster* 45S rDNA probe with the meiotic chromosomes of three triatomine species (kissing bugs).

MATERIAL AND METHODS

Young male specimens of the triatomine species *Triatoma protacta*, *T. platensis* and *T. tibiamaculata* were provided by the Insectary of the Araraquara Special Health Service (SESA), Araraquara (SP), organ of the Department of Epidemiology, São Paulo Public Health School, São Paulo University (SP, Brazil). Meiotic cells, obtained by crushing the testes, were fixed in 45% acetic acid. *T. platensis* and *T. protacta* present karyotype 2n = 20, XY and 2n = 20, X₁X₂Y, respectively (Schreider and Pellegrino, 1950; Ueshima, 1966), and *T. tibiamaculata* 2n = 20, X₁X₂Y (Severi-Aguiar and Azeredo-Oliveira, 2004).

In situ hybridization was performed with a 12-kb rDNA probe (pDm 238- *Drosophila melanogaster*, 15 ng/μL), biotin-labeled with bionik (Gibco BRL, nick translation) as described by Viégas-Péquino (1992). The chromosome preparation was pre-treated with RNase, dehydrated in an ethanol series, air-dried and denatured in 70% formamide (20% 10X SSC) at 70°C for 2 min and, immediately, dehydrated in an ethanol series: 50, 75 and 100%. Hybridization was carried out for 40 h in a moist chamber at 37°C.

The slides were washed twice in 50% formamide (2X SSC) and twice in 2X SSC for 5 min each. Subsequently, they were incubated with the first antibody (antibiotin) for 45 min in a moist chamber at 37°C. Following washing in PBT (PBS 0.1%, Tween 20 and 0.4% BSA 30%, w/v), the slides were incubated for 45 min with the second antibody (RAG-FITC) in a moist chamber at 37°C. The slides were then submitted to a final washing in PBT, stained with propidium iodide and mounted with an antifade (Vectashield). Photomicrographs were taken with an Olympus microscope, Model BX-FLA, FITC filter (BP450-480). Kodak 400 ASA ULTRA film was used for documentation.

RESULTS

FISH showed that rDNA genes were distributed differently in the species studied. In metaphase I, *T. platensis* showed a fluorescent spot on the X chromosomes (Figure 1a,b). In *T. protacta*, hybridization occurred in one of the telomeres of an autosomal chromosome (Figure

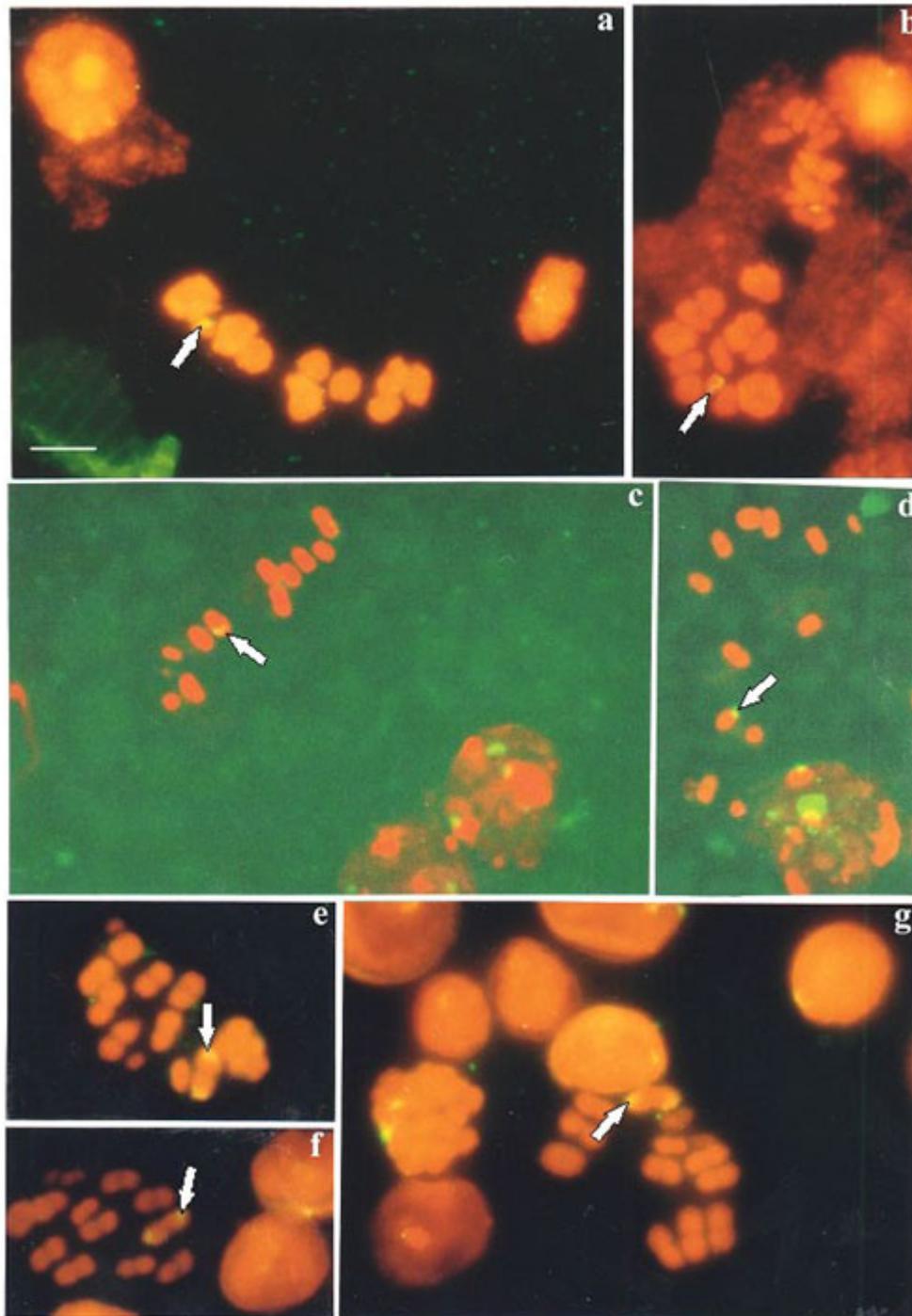


Figure 1. Meiotic metaphases and nuclei submitted to FISH with rDNA probe. **a,b**, Metaphases of the first meiotic division (MI) in *Triatoma platensis* that showed a fluorescent spot on X chromosome; **c,d**, MI in *T. protacta* showing a sign of hybridization at the border of one of the autosomes, and **e-g**, MI in *T. tibiamaculata* showing hybridization at the poles of one of the bivalents. Notice the presence of fluorescent spots on interphase nuclei. Arrows indicate fluorescent regions. Bar = 16 μ m.

1c,d) and *T. tibiamaculata* displayed telomeric staining in a single and larger bivalent (Figure 1e-g). Fluorescent regions were observed in interphase nuclei of all the species studied.

DISCUSSION

The holocentric nature of some chromosomes is a great barrier for cytogenetic studies. However, in the present work all of the species studied showed signs of hybridization with the 45S rDNA probe.

In *T. platensis*, a fluorescent spot was seen in one of the sex chromosomes, probably X chromosome. Considering that this same X chromosome was shown to be impregnated by silver ions (data not published), digested by endonuclease restriction treatment with *Hae*III (whose restriction site is a sequence of GG↓CC bases; data not published), and was CMA₃⁺ (a fluorochrome specific for CG bases; Severi-Aguiar GDC and Azeredo-Oliveira MTV, unpublished results), these findings support the hypothesis that the sex chromosomes in triatomines bear NORs.

On the other hand, *T. tibiamaculata* and *T. protacta*, exhibited autosomes with stained spots. In *T. tibiamaculata*, such spots were telomeric and found in only one bivalent. These spots seem to coincide with the CMA₃⁺ regions that were observed in an autosome at the same location in a study previously conducted by our team (data not published). In *T. protacta* autosomes, no CMA₃⁺ region was detected (data not published). It has been suggested that in holocentric chromosomes the heterochromatic areas are dispersed along the chromosomal axis, in contrast to the high heterochromatin concentration found near the centromere in monocentric chromosomes (Mandrioli et al., 2000). This could partly explain why these areas are present in autosomal chromosome regions but not in the sex chromosomes of these two species. Nonetheless, further studies are necessary to confirm these findings.

rDNA sites were present in sex chromosomes when the species showed one X chromosome (*T. platensis*). However, in species where X chromosome fragmentation occurs as shown by chromomycin A₃ (*T. tibiamaculata* and *T. protacta*, data not published), these sites have been observed only on autosomes. Perhaps, this localization is the result of chromosome fission over time during evolution in this insect group.

Information about the composition of chromatin in triatomines is very scarce. Therefore, the quantification and localization of NORs by FISH together with the results of silver impregnation and affinity for CMA₃ and DAPI, reported herein, contribute to a better understanding of chromosomal structure, including the intra- and inter-specific relationships found in this group as well as the holokinetic nature of its chromosomes.

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REFERENCES

- Almeida-Toledo LF, Stocker AJ, Foresti F and Toledo-Filho SA (1996). Fluorescence *in situ* hybridization with rDNA probes on chromosomes of two nucleolus organizer region phenotypes of a species of *Eigenmannia* (Pisces, Gymnotoidei, Sternopygidae). *Chromosome Res.* 4: 301-305.
- Bizzaro M, Mandrioli M, Zanotti M, Giusti M et al. (2000). Chromosome analysis and molecular characterization of highly repeated DNAs in the aphid *Acyrtosiphon pisum* (Aphididae, Hemiptera). *Genetica* 108: 197-202.
- Deiana AM, Cau A, Salvadori S, Coluccia E et al. (2000). Major and 5S ribosomal sequences of the largemouth bass *Micropterus salmoides* (Perciformes, Centrarchidae) are localized in GC-rich regions of the genome. *Chromosome Res.* 8: 213-218.
- Fujiwara A, Abe S, Yamaha E, Yamazaki F et al. (1998). Chromosomal localization and heterochromatin association of ribosomal RNA gene loci and silver-stained nucleolar organizer regions in salmonid fishes. *Chromosome Res.* 6: 463-471.
- Hirai H, Yamamoto MT, Ogura K, Satta Y et al. (1994). Multiplication of 28S rDNA and NOR activity in chromosome evolution among ants of the *Myrmecia pilosula* species complex. *Chromosoma* 103: 171-178.
- Hirai H, Yamamoto MT, Taylor RW and Imai HT (1996). Genomic dispersion of 28S rDNA during karyotypic evolution in the ant genus *Myrmecia* (Formicidae). *Chromosoma* 105: 190-196.
- Lorite P, García MF, Carrillo JA and Palomeque T (1999). Restriction endonuclease chromosome banding in *Tapinoma nigerrimum* (Hymenoptera, Formicidae). *Hereditas* 131: 197-201.
- Lourenço LB, Recco-Pimentel SM and Cardoso AJ (1998). Polymorphism of the nucleolus organizer regions (NORs) in *Physalaemus petersi* (Amphibia, Anura, Leptodactylidae) detected by silver staining and fluorescence *in situ* hybridization. *Chromosome Res.* 6: 621-628.
- Mandrioli M, Ganassi S, Bizzaro D and Manicardi GC (2000). Cytogenetic analysis of the holocentric chromosomes of the aphid *Schizaphis graminum*. *Hereditas* 131: 185-190.
- Parise-Maltempi PP and Avancini RMP (2000). Cytogenetics of the Neotropical flesh fly *Pattonella intermutans* (Diptera, Sarcophagidae). *Gen. Mol. Biol.* 23: 563-567.
- Schreider G and Pellegrino J (1950). Eteropicnosi di autosomi come possibile meccanismo di speciazione. *Sci. Genet.* 3: 215-226.
- Severi-Aguiar GDC and Azeredo-Oliveira MTV (2002). Cromossomos holocêntricos. *Naturalia* 27: 11-26.
- Severi-Aguiar GDC and Azeredo-Oliveira MTV (2004). Cytogenetic study of holocentric chromosomes in three species of Triatomines (Heteroptera, Reduviidae). *Cytologia* 69: 53-57.
- Sola L, De Innocentiis S, Gornung E, Papalia S et al. (2000). Cytogenetic analysis of *Epinephelus marginatus* (Pisces: Serranidae) with the chromosome localization of the 18S and 5S rRNA genes and of the (TTAGGG)_n telomeric sequence. *Mar. Biol.* 137: 47-51.
- Tartarotti E and Azeredo-Oliveira MTV (1999). Meiosis patterns of holocentric chromosomes in triatomines genus *Panstrongylus*. *Cytologia* 64: 235-240.
- Tavares MG and Azeredo-Oliveira MTV (1997). Cytogenetics study on holocentric chromosomes of five species of triatomines (Heteroptera:Reduviidae). *Cytobios* 89: 51-61.
- Ueshima N (1966). Cytotaxonomy of the Triatominae (Reduviidae, Hemiptera). *Chromosoma* 18: 97-122.
- Viégas-Péquino E (1992). *In situ* hybridization to chromosomes with biotinylated probes. In: *In situ* hybridization: A practical approach (Willman D, ed.). Oxford University Press, IRL Press, Oxon, England, pp. 137-158.
- Willhoeft U (1997). Fluorescence *in situ* hybridization of ribosomal DNA to mitotic chromosomes of tsetse flies (Diptera: Glossinidae: *Glossina*). *Chromosome Res.* 5: 262-267.
- Woznick P, Sanchez L, Martinez P, Pardo BG et al. (2000). A population analysis of the structure and variability of NOR in *Salmo trutta* by Ag, CMA3 and ISH. *Genetica* 108: 113-118.