Genetics and Molecular Research

Cytogenetic analysis of *Triatoma pseudomaculata* Corrêa and Espínola, 1964 (Hemiptera, Triatominae) from different Brazilian states

C.H.L. Imperador[1], K.C.C. Alevi[1], J. Oliveira[2], J.A. Rosa[2] and M.T.V. Azeredo-Oliveira[1]

[1]Laboratório de Biologia Celular, Departamento de Biologia,
Instituto de Biociências, Letras e Ciências Exatas,
Universidade Estadual Paulista "Júlio de Mesquita Filho",
São José do Rio Preto, SP, Brasil
[2]Laboratório de Parasitologia, Departamento de Ciências Biológicas,
Faculdade de Ciências Farmacêuticas,
Universidade Estadual Paulista "Júlio de Mesquita Filho", Araraquara, SP, Brasil

Corresponding author: K.C.C. Alevi
E-mail: kaiochaboli@hotmail.com

Genet. Mol. Res. 15 (1): gmr.15016937
Received June 3, 2015
Accepted November 13, 2015
Published March 31, 2016
DOI http://dx.doi.org/10.4238/gmr.15016937

ABSTRACT. *Triatoma maculata* and *T. pseudomaculata* are allopatric; however, it is believed that *T. maculata* was introduced into the Brazilian northeast by passive transportation of the nymphs between the feathers of migratory birds, followed by the speciation of *T. pseudomaculata*. *T. pseudomaculata* is the second most epidemiologically important species in the northeast of Brazil after *T. brasiliensis*. Therefore, given the broad range of *T. pseudomaculata*, the objective of the present study was to cytogenetically analyze different populations of *T. pseudomaculata* from different Brazilian states in order to investigate possible intraspecific chromosomal variation between them. Three adult *T. pseudomaculata* males from each population (Pernambuco, Ceará, Paraíba, Bahia, Rio Grande do Norte, and Piauí) were analyzed by lacto-acetic orcein and C-banding. All of

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the specimens analyzed exhibited the same cytogenetic characteristics, i.e., 22 chromosomes (20 autosomes and XY), a chromocenter formed by the X and Y sex chromosomes and one pair of autosomes, and heterochromatin blocks in three or four pairs of autosomes. These data confirm that all of the populations analyzed were *T. pseudomaculata*, and although they may be subject to different selection pressures they have maintained the genetic integrity that characterizes the species.

Key words: Cytogenetics; Chromosomal homogeneity; Triatominae subfamily

INTRODUCTION

The triatomines are insects that belong to the Hemiptera order, Heteroptera suborder, Reduviidae family, and Triatominae subfamily (Lent and Wygodzinsky, 1979). These organisms are characterized by one pair of hemelytra wings, one pair of membranous hind wings, and a buccal sucking apparatus (Schofield, 1994). The Triatominae is composed of 150 species, grouped in 18 genera and six tribes (Alevi et al., 2015a). All of the species of the subfamily are bloodsucking and potential vectors of the *Trypanosoma cruzi* protozoan, which is an etiological agent of Chagas disease. These vectors have been grouped in complexes and subcomplexes, based mainly on their morphological characteristics and geographical distributions (Schofield and Galvão, 2009).

The Maculata subcomplex is composed of the species *Triatoma arthurneivai* Lent and Martins 1940, *T. maculata* Erichson 1848, *T. pseudomaculata* Corrêa and Espínola 1964, and *T. wygodzinskyi* Lent 1951. *T. maculata* and *T. pseudomaculata* are allopatric, because the former has the following wide geographical distribution: Colombia, Guyana, Aruba, Bonaire, Curação, Suriname, Venezuela, and Brazil (Lent and Wygodzinsky, 1979; Carcavallo et al., 1997), whereas the latter is only found in Brazil, the Brazilian northeast being the probable center of dispersion for this species (Lent and Wygodzinsky, 1979; Freitas et al., 2005). Schofield (1988) proposed that *T. maculata* was introduced into the Brazilian northeast by passive transportation of the nymphs between the feathers of migratory birds, followed by the speciation of *T. pseudomaculata*. These species are morphologically very similar, and until 1964 were considered the same species (Corrêa and Espínola, 1964). However, their status as separate species has been confirmed by experimental hybrid crosses (Belisário et al., 2007), and they have been differentiated by cytogenetic analysis (Dos Santos et al., 2007).

T. pseudomaculata is the second most epidemiologically important species in the northeast of Brazil after *T. brasiliensis* (Carbajal de la Fuente et al., 2009). This species typically exhibits wild and peridomiciliar behavior; however, it can occasionally occur in human habitations (Silveira and Vinhaes, 1998). It is considered endemic to the Caatinga and Cerrado Provinces (Morrone, 2006), but has been reported in the States of Alagoas, Bahia, Ceará, Distrito Federal, Goiás, Maranhão, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, Sergipe, and Tocantins (Gurgel-Gonçalves et al., 2012). The objective of the present study was to cytogenetically analyze different populations of *T. pseudomaculata* from different Brazilian states, in order to investigate possible intraspecific chromosomal variation between them.

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MATERIAL AND METHODS

Three adult *T. pseudomaculata* males from each population (Pernambuco, Ceará, Paraíba, Bahia, Rio Grande do Norte, and Piauí) were analyzed. The insects were donated by "Insetário de Triatominae" of the Biological Sciences Department of the Faculty of Pharmaceutical Sciences, State University of São Paulo, Campus Araraquara, São Paulo, Brazil. Microscope slides with the biological material (seminiferous tubules) were prepared by the crushing technique, and stained with lacto-acetic orcein (De Vaio et al., 1985) with modifications by Alevi et al. (2012) and C-banding (Sumner, 1972). The samples were observed using a Jenaval light microscope (Zeiss) coupled to a digital camera and an AxioVision LE 4.8 image analyzer (Zeiss). The images were magnified by 1000X.

RESULTS

All of the specimens exhibited the same cytogenetic characteristics, i.e., 22 chromosomes (20 autosomes and XY), a chromocenter formed by the X and Y sex chromosomes and one pair of autosomes, and heterochromatin blocks in three or four pairs of autosomes (Figure 1 and Table 1).

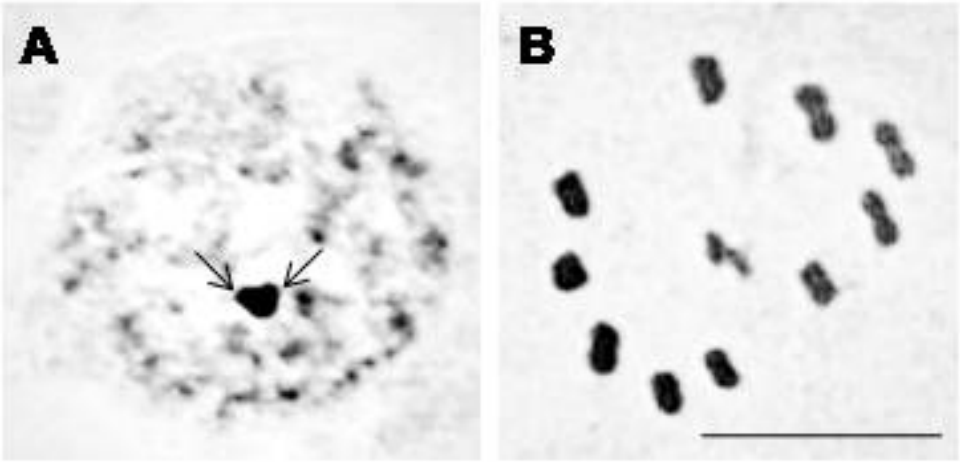

Figure 1. Cytogenetic characteristics of *Triatoma pseudomaculata* from Paraíba, Brazil. **A.** Chromocenter formed by X and Y sex chromosomes plus one pair of autosomes (arrows) during initial prophases. **B.** Karyotype with 22 chromosomes and heterochromatin in three or four autosomes during metaphase I. Bar: 10 µm.

Table 1. Cytogenetic characteristics of different populations of *Triatoma pseudomaculata*.

State	Karyotype	Chromocenter	Heterochromatin
Pernambuco[1]	22 (20A + XY)	X, Y, and one pair of autosomes	Some autosomes + Y
Ceará[1]	22 (20A + XY)	X, Y, and one pair of autosomes	Some autosomes + Y
Paraíba	22 (20A + XY)	X, Y, and one pair of autosomes	Some autosomes + Y
Bahia[1]	22 (20A + XY)	X, Y, and one pair of autosomes	Some autosomes + Y
Rio Grande do Norte	22 (20A + XY)	X, Y, and one pair of autosomes	Some autosomes + Y
Piauí[1]	22 (20A + XY)	X, Y, and one pair of autosomes	Some autosomes + Y

[1]Dos Santos et al. (2007).

DISCUSSION

The Triatominae subfamily exhibits very little variation in chromosomal number, because all of the species have between 21 and 25 chromosomes (Panzera et al., 1998; Alevi et al., 2013). Variation usually occurs by the fragmentation of the X sexual chromosome, resulting in different mechanisms of sexual determination (XY, X_1X_2Y, or $X_1X_2X_3Y$) (Ueshima, 1966). However, although the number of chromosomes is fairly conserved, triatomines exhibit a large variety of C-banding patterns and interspecific and intraspecific chromosome behaviors during male meiosis (Panzera et al., 1997, 1998, 2004; Alevi et al., 2015b).

Intraspecific chromosome variation in the Triatominae has been analyzed in 10 species (Table 2). Six species exhibit chromosomal variation, which resulted in important publications, as the division of different *T. infestans* into Andean and non-Andean populations (Panzera et al., 2004), and indicates that cryptic speciation has occurred in *T. sordida* (Panzera et al., 1997) and *Panstrongylus geniculatus* (Crossa et al., 2002).

Table 2. Intraspecific variation in triatomine species.

Species	Intraspecific chromosome variation	Reference
R. ecudoriensis	Present	Pita et al. (2013)
R. pallescens	Present	Gómez-Palacio et al. (2008)
P. geniculatus	Present	Crossa et al. (2002)
T. dimidiata	Present	Panzera et al. (2006)
T. infestans	Present	Panzera et al. (2004)
T. sordida	Present	Panzera et al. (1997)
R. neglectus	Absent	Alevi et al. (2015c)
P. megistus	Absent	Alevi et al. (2015d)
T. brasiliensis	Absent	Panzera et al. (2000)
T. pseudomaculata	Absent	This study

Cytogenetic analysis of *T. pseudomaculata* revealed that this species, in addition to the three other species already analyzed, exhibits no intraspecific variation (Table 2). Our results for the States of Pernambuco, Ceará, Bahia, and Piauí were the same as those obtained by Dos Santos et al. (2007). These data confirm that all of the populations analyzed were *T. pseudomaculata*, and although they may be subject to different selection pressures they have maintained the genetic integrity that characterizes the species.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; Process #2013/19764-0), Fundação de Apoio à Pesquisa e Extensão de São José do Rio Preto (Process #94/2015) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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