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Evolutionary and dispersal history of *Triatoma infestans*, main vector of Chagas disease, by chromosomal markers

Francisco Panzera [a,*], María J. Ferreiro [a], Sebastián Pita [a], Lucía Calleros [a], Ruben Pérez [a], Yester Basmadjián [b], Yenny Guevara [c], Simone Frédérique Brenière [d], Yanina Panzera [a]

[a] Sección Genética Evolutiva, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay
[b] Departamento de Parasitología y Medicina, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
[c] Laboratorio de Citogenética Alberto Tellería Cáceres, Universidad Nacional Mayor de San Marcos, Lima, Peru
[d] INTERTRYP (Interactions hôtes-vecteurs-parasites dans les infections par trypanosomatidae), Institut de Recherche pour le Développement (IRD), Montpellier, France

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ABSTRACT

Chagas disease, one of the most important vector-borne diseases in the Americas, is caused by *Trypanosoma cruzi* and transmitted to humans by insects of the subfamily Triatominae. An effective control of this disease depends on elimination of vectors through spraying with insecticides. Genetic research can help insect control programs by identifying and characterizing vector populations. In southern Latin America, *Triatoma infestans* is the main vector and presents two distinct lineages, known as Andean and non-Andean chromosomal groups, that are highly differentiated by the amount of heterochromatin and genome size. Analyses with nuclear and mitochondrial sequences are not conclusive about resolving the origin and spread of *T. infestans*.

The present paper includes the analyses of karyotypes, heterochromatin distribution and chromosomal mapping of the major ribosomal cluster (45S rDNA) to specimens throughout the distribution range of this species, including pyrethroid-resistant populations. A total of 417 specimens from seven different countries were analyzed.

We show an unusual wide rDNA variability related to number and chromosomal position of the ribosomal genes, never before reported in species with holocentric chromosomes. Considering the chromosomal groups previously described, the ribosomal patterns are associated with a particular geographic distribution. Our results reveal that the differentiation process between both *T. infestans* chromosomal groups has involved significant genomic reorganization of essential coding sequences, besides the changes in heterochromatin and genomic size previously reported. The chromosomal markers also allowed us to detect the existence of a hybrid zone occupied by individuals derived from crosses between both chromosomal groups. Our genetic studies support the hypothesis of an Andean origin for *T. infestans*, and suggest that pyrethroid-resistant populations from the Argentinean-Bolivian border are most likely the result of recent secondary contact between both lineages. We suggest that vector control programs should make a greater effort in the entomological surveillance of those regions with both chromosomal groups to avoid rapid emergence of resistant individuals.

1. Introduction

Triatoma infestans (Hemiptera, Reduviidae, Triatominae) is an important vector of the flagellate protozoan *Trypanosoma cruzi*, causative agent of Chagas disease or American trypanosomiasis. In the early 1990s, *T. infestans* had a wide geographical distribution extending over more than 6 million km^2, including parts of seven South American countries, and was responsible for well over half of the 18 million people affected by Chagas disease (WHO, 1991). Since then, control interventions in the framework of the "Southern Cone Initiative" have substantially reduced the distribution of *T. infestans* to less than 1 million km^2 and 9.8 million people infected (Schofield et al., 2006). At present, domestic *T. infestans* mainly persists in the Andean valleys of Bolivia, southern Peru, and parts of the Gran Chaco region of Argentina, Bolivia and Paraguay. One of the challenges facing the control of *T. infestans* in these regions is the recent detection of pyrethroid resistance

* Corresponding author. Address: Sección Genética Evolutiva, Facultad de Ciencias, Universidad de la República, Calle Iguá 4225, 11400 Montevideo, Uruguay. Tel.: +598 25258619; fax: +598 25258617.
E-mail addresses: fcopanzera@gmail.com, panzera@fcien.edu.uy (F. Panzera).

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(Depickère et al., 2012; Lardeux et al., 2010; Picollo et al., 2005). *T. infestans* is known to comprise two main evolutionary lineages recognized by molecular markers (nuclear and mitochondrial sequences) (Bargues et al., 2006; Giordano et al., 2005; Monteiro et al., 1999; Piccinali et al., 2009, 2011; Quisberth et al., 2011; Torres-Pérez et al., 2011) and phenetic characteristics (Calderón-Fernández et al., 2012; Hernández et al., 2008). These lineages, known as the Andean and non-Andean groups, are defined by substantial differences (from 30% to 50%) in the amount of nuclear DNA due to different quantities of highly repeated DNA revealed as heterochromatin by C-banding (Panzera et al., 2004). Based on the existence of sylvatic populations, two main hypotheses have been proposed to clarify the origin and spread of *T. infestans* throughout South America. The "Andean" hypothesis suppose that the high-altitude valleys of Bolivia is the center of origin and dispersal (Schofield et al., 1988; Usinger et al., 1966), while the "Chaco" hypothesis assumes that the origin is the subtropical lowlands regions of southern Bolivia, north-western Paraguay and north of Argentina (Carcavallo et al., 1998). Several reports using different nuclear and mitochondrial sequences are not conclusive about resolving the origin and spread of *T. infestans* (for review sees Torres-Pérez et al., 2011). To better understand their chromosomal organization and population differentiation, we analyzed the karyotypes, heterochromatin distribution and chromosomal location of the major ribosomal cluster (45S rDNA) in specimens from all countries of its original distribution, with emphasis on sylvatic and pyrethroid-resistant populations not previously studied. In Triatominae, these chromosomal markers are the main source of karyological variation in population differentiation and speciation processes (Panzera et al., 2010, 2012; Pita et al., 2013). We try to respond to the following questions: (i) what are the main karyological changes that occurred during the dispersal of *T. infestans*? and (ii) do the pyrethroid-resistant individuals detected along the Argentina-Bolivia border represent ancient or recent populations? Our findings provide further information about the origin and spread of *T. infestans*, including the origin of the pyrethroid resistance populations in non-Andean regions.

2. Methods

2.1. Material analyzed

All specimens of *T. infestans* came from natural populations and most of them were collected within the framework of the project SSA/ATU INCOCT 2004 515942 (Catalá et al., 2007). These specimens were also analyzed by cuticular hydrocarbon and antennal phenotypes (Calderón-Fernández et al., 2012; Hernández et al., 2008, respectively). The geographic origin of each population, year of collection and habitat (domiciliary, peridomiciliary and sylvatic) are given in Table 1 and Fig. 1. It is noteworthy that in several collection sites here analyzed (Uruguay, Brazil, Chile and some from Argentina and Paraguay), currently there are no specimens of this species, being eliminated by vector control activities. Andean sylvatic specimens were mainly collected amongst rock piles, while the "dark morphs" from the Bolivian Chaco (non-Andean region) were caught in hollow trees (Fig. 1, map reference 29) (Noireau et al., 2000).

Since 2002, control services for Chagas disease from Argentina and Bolivia reported low efficiency of deltamethrin and other pyrethroids for the treatment of rural sites close to Salvador Mazza (Salta Province, Argentina) and Yacuiba cities (Tarija Department, Bolivia) (Fig. 1, map references 17–28). Pyrethroid resistance of insects collected from Yacuiba (Tarija, Bolivia) and Salvador Mazza (Salta, Argentina) were determined with topical application bioassays (WHO, 1994), by María Inés Picollo (Centro de Investigaciones de Plagas e Insecticidas CITEFA-CONICET, Argentina), within the framework of the project SSA/ATU. Pyrethroid resistance in individuals here analyzed from Salvador Mazza has also been experimentally confirmed by Cardozo et al. (2010). Furthermore several reports using triatomine samples collected in this area since 2002 showed high resistance levels, with resistance ratios (RRs) ranging from 50.5 to 183 (Lardeux et al., 2010; Picollo et al., 2005; Toloza et al., 2008).

2.2. Chromosome preparations and banding procedures

Gonads from adult insects were removed and fixed in ethanol–acetic acid (3:1). Chromosome squashes were prepared in 45% acetic acid. C-banding was used to establish the diploid chromosome number (2n) and the number of C-heterochromatic chromosomes (Panzera et al., 2004). We applied the fluorescence *in situ* hybridization (FISH) to determine the chromosome location of the 45S ribosomal clusters (Pita et al., 2013).

For each specimen, at least 20 cells were analyzed to determine chromosome traits. Slides were examined under a Nikon Eclipse 80i microscope and the images were obtained with a DS-5Mc-U2 digital camera. In males, mitotic (prometaphase) and meiotic (metaphase I or II) plates were observed. In total, 425 *T. infestans* specimens were examined by C-banding and 105 by FISH techniques (Table 2, Figs. 2 and 3). Of the latter, 70% of them were analyzed by both techniques (data not shown).

3. Results

3.1. Chromosome number

All specimens showed the same normal diploid number (2n = 22) constituted by 20 autosomes and two sex chromosomes (XY in males, XX in females) (Fig. 2). However, in some individuals we observed the occurrence of chromosomal fragments or extra-chromosomes (Fig. 2E). These are the smallest of the chromosome complement, both euchromatic and heterochromatic, and their frequency varied from 1 to 3 amongst individuals. These chromosomal fragments appear in gonial mitotic prometaphases, both in males and females, but we did not detect any alteration in the meiotic segregation of carrier individuals. The frequencies of individuals with chromosomal fragments were very variable, but the populations from Salvador Mazza showed the highest frequency (more than 80% of individuals) (Table 2).

3.2. Distribution of C-heterochromatin

Each specimen exhibited a specific C-banding pattern. All populations were polymorphic, with variations in the number of chromosomes with C-heterochromatin and in the chromosome position of C-blocks (one or both chromosomal ends). All individuals show a C-heterochromatic Y chromosome while the X chromosome is euchromatic or heterochromatic. The number of autosomes with C-heterochromatin differentiates three distinct groups, two of them previously described (Panzera et al., 2004) and a third group here named as "Intermediate group" (Table 2 and Fig. 2):

3.2.1. Andean group

Includes 102 insects from Bolivia and Peru (Fig. 1, map references 1–16). The number of heterochromatic autosomes with C-blocks varies from 14 to 20, with a mean of 15.64 and a standard deviation (SD) of 1.35 (Table 2, Fig. 2A and B). All individuals have X chromosome with C-heterochromatin.

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