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

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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.

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**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 km², 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 km² 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
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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 (Panzerá 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, 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, 1998). Several reports using different nuclear and mitochondrial sequences are not conclusive about resolving the origin and spread of T. infestans (for review see 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 (Panzerá 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 (domestic, peridomestic 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 CITIFA–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 triatamone 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 (Panzerá 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 (Panzerá 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.
### 3.2.2. Non-Andean group

Includes 238 specimens from Argentina, Bolivia (non-Andean region), Brazil, Chile, Paraguay, and Uruguay (Fig. 1, map references 29–52). The number of heterochromatic autosomes varies from 4 to 6 (5.86 ± 0.52) (Table 2, Figs. 2C and D), but most individuals (86.2%) have six C-heterochromatic autosomes. In all individuals, the X chromosomes are euchromatic.

### 3.2.3. Intermediate group

Restricted to Argentina-Bolivia border, including 85 individuals from localities from Salvador Mazza (Argentina) and Tariria (Bolivia) (Fig. 1, map references 17–28). The number of heterochromatic autosomes varies from 7 to 11 (8.20 ± 1.08) (Table 2, Figs. 2E and F). In 72 insects (84.7%), the X chromosomes are euchromatic (similar to non-Andean group), while in the remaining 13 are heterochromatic (similar to Andean group).

### 3.3. Chromosome location of 45S rDNA cluster

_T. infestans_ showed marked variability between populations and between individuals, both in the number of rDNA loci and in the type of chromosomes (autosomes and sex chromosomes) that carried the rDNA genes (Table 2 and Fig. 3). The number of rDNA loci per male haploid genome ranged from 1 to 4 sites. The 45S rDNA cluster is located on one sex chromosome (X chromosome) and even on up to three autosomal pairs. Due to the similar size of the autosomes is very difficult to determine exactly which chromosome pair presents the ribosomal cluster. However, one ribosomal signal is always located in at least one of the four largest autosomal.
pairs. In total, we observed nine ribosomal location patterns: on one autosomal pair (pattern 1A, Fig. 3A); on one autosomal pair plus X chromosome (1A + X, Fig. 3B); on two autosomal pairs (2A, Fig. 3C); on two autosomal pairs plus X (2A + X, Fig. 3D); on two autosomal pairs (with one heterozygote) plus X (1/2 A + 1/2 A + X, Fig. 3E); on one heterozygote autosomal pair plus X (1/2 A + 1/2 A + X, Fig. 3F); on two autosomal pairs (both of them heterozygotes) plus X (1/2 A + 1/2 A + 1/2 A + X, Fig. 3G); on three heterozygote autosomal pairs plus X chromosome and X pattern (1/2 A + 1/2 A + 1/2 A + X, Fig. 3H) and only on the X chromosome (pattern X, Fig. 3I). In all cases, the hybridization signals were located at a terminal or subterminal chromosome position.

Considering the three chromosomal groups previously described, the ribosomal patterns are associated with a particular geographic distribution (Table 2). The Andean group is the most variable, with five patterns exclusively detected in this group.

**Table 2**

<table>
<thead>
<tr>
<th>Map reference</th>
<th>No. of C-autosomes</th>
<th>Chromosomal fragments (%)</th>
<th>rDNA pattern locationb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.93 ± 1.16 (15)</td>
<td>0.0</td>
<td>1A (2); 2A (1)</td>
</tr>
<tr>
<td>2</td>
<td>15.55 ± 1.21 (11)</td>
<td>0.0</td>
<td>1A + X (6)</td>
</tr>
<tr>
<td>3–4</td>
<td>14.90 ± 0.88 (10)</td>
<td>0.0</td>
<td>1A + X (11); 2A + X (6); 1A + 1/2 A + X (1)</td>
</tr>
<tr>
<td>5–9</td>
<td>15.62 ± 1.37 (34)</td>
<td>0.0</td>
<td>1A (2); 1A + X (2); 1A + 1/2 A + X (2)</td>
</tr>
<tr>
<td>10–11</td>
<td>16.29 ± 1.65 (17)</td>
<td>0.0</td>
<td>1A (3); 2A (2); 1A + X (1); 1A + 1/2 A + X (1)</td>
</tr>
<tr>
<td>12–16</td>
<td>15.20 ± 1.21 (15)</td>
<td>0.0</td>
<td>1A (2); 1A + X (3)</td>
</tr>
<tr>
<td>Andean Group</td>
<td>15.64 ± 1.35 (102)</td>
<td>0.0</td>
<td>55 individuals: 1A (26); 1A + X (22); 2A (3); 2A + X (2); 1A + 1/2 A + X (2)</td>
</tr>
<tr>
<td>17–25</td>
<td>8.16 ± 1.19 (32)</td>
<td>18.8</td>
<td>X (1); 1/2 A + X (6); 1/2 A + 1/2 A + X (1); 1/2 A + 1/2 A + 1/2 A + X (1)</td>
</tr>
<tr>
<td>26–28</td>
<td>8.23 ± 1.01 (53)</td>
<td>83.0</td>
<td>X (9); 1/2 A + X (4)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>8.20 ± 1.08 (85)</td>
<td>58.8</td>
<td>22 individuals: X (10); 1/2 A + X (10); 1/2 A + 1/2 A + X (1); 1/2 A + 1/2 A + 1/2 A + X (1)</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29–30</td>
<td>6.15 ± 0.38 (13)</td>
<td>0.0</td>
<td>X (5)</td>
</tr>
<tr>
<td>31–32</td>
<td>6.17 ± 0.41 (6)</td>
<td>16.7</td>
<td>X (2)</td>
</tr>
<tr>
<td>33–34</td>
<td>5.58 ± 0.56 (33)</td>
<td>24.2</td>
<td>X (3)</td>
</tr>
<tr>
<td>35–36</td>
<td>5.27 ± 0.77 (22)</td>
<td>0.0</td>
<td>X (3)</td>
</tr>
<tr>
<td>37–39</td>
<td>6.20 ± 0.56 (15)</td>
<td>0.0</td>
<td>X (4)</td>
</tr>
<tr>
<td>40</td>
<td>6.05 ± 0.23 (19)</td>
<td>15.8</td>
<td>X (2)</td>
</tr>
<tr>
<td>41</td>
<td>6.00 ± 0.00 (5)</td>
<td>0.0</td>
<td>ND</td>
</tr>
<tr>
<td>42</td>
<td>5.17 ± 0.58 (12)</td>
<td>0.0</td>
<td>X (2)</td>
</tr>
<tr>
<td>43–46</td>
<td>6.04 ± 0.52 (27)</td>
<td>0.0</td>
<td>X (3)</td>
</tr>
<tr>
<td>47–48</td>
<td>6.00 ± 0.00 (12)</td>
<td>0.0</td>
<td>X (1)</td>
</tr>
<tr>
<td>49–50</td>
<td>5.99 ± 0.12 (70)</td>
<td>0.0</td>
<td>X (2)</td>
</tr>
<tr>
<td>51</td>
<td>6.00 ± 0.00 (2)</td>
<td>0.0</td>
<td>ND</td>
</tr>
<tr>
<td>52</td>
<td>6.00 ± 0.00 (2)</td>
<td>0.0</td>
<td>X (1)</td>
</tr>
<tr>
<td>Non-Andean</td>
<td>5.86 ± 0.52 (238)</td>
<td>4.4 %</td>
<td>28 individuals: all X pattern</td>
</tr>
</tbody>
</table>

- Partial C-banding data from Panzera et al. (2004).
- Partial FISH data from Panzera et al. (2012).
Two of them (patterns 1A and 1A + X) involved 90% of the individuals analyzed (48/55). Most of the collection sites are polymorphic, being the populations from Cochabamba and Chuquisaca the most variable (Table 2) and the populations from La Paz the most homogeneous.

The non-Andean group showed only one ribosomal pattern: rDNA loci located on the X chromosome (Fig. 3I and Table 2), involving 28 individuals from six countries, including sylvatic and peridomestic specimens from the Bolivian Chaco region (Fig. 1, map references 29–30).

The Intermediate group shows four ribosomal patterns in the 22 individuals analyzed (Table 2). One of them is similar to that observed in the non-Andean group: on the X chromosome (10 individuals)(Fig. 3I). The other three patterns are exclusively found in this group: 1/2 A + X pattern in 10 individuals (Fig. 3F), and two patterns showing 2 and 3 heterozygote autosomal pairs plus X chromosome found in one individual for each one (Fig. 3G and H).

4. Discussion

4.1. Chromosome number

All individuals have 20 autosomes plus two sex chromosomes (2n = 22). However, some individuals have chromosomal fragments or extra-chromosomes (Table 2 and Fig. 2E). One distinctive cytogenetic feature of heteropteran insects, including Triatominae, is the holocentric or holokinetic nature of their chromosomes (Hughes-Schrader and Schrader, 1961). Unlike what is observed in monocentric chromosomes, chromosomal fragments in holocentric systems attach to spindle fibres and migrate normally during cell divisions, becoming what are known as B chromosomes (Mola and Papeschi, 2006). In true bugs however, B chromosomes seem very uncommon and have been detected in only 12 of the 465 species so far studied (Kuznetsova et al., 2011). In Triatominae, chromosomal fragments usually occur as byproducts of a translocation among non-homologous autosomes, as has been observed in Mepraia gajardoi and T. infestans (Pérez et al., 2004; Poggio et al., 2013). In these mutant individuals, an autosomal trivalent and chromosomal fragments were observed both in mitosis and meiosis. However, the individuals of T. infestans with chromosomal fragments here analyzed are quite different, as they show normal meiotic segregation without autosomal associations. These chromosomal fragments may reflect the occurrence of small fissions or the presence of unstable chromosome regions (fragile sites) that are prone to breakage in mitotic metaphases. The frequency of individuals with chromosomal fragments varies greatly according to the populations studied (Table 2), and its appearance probably is associated with populations resistant to pyrethroids (see below Intermediate group and pyrethroid resistance).

4.2. Chromosome location of 45S rDNA genes

The striking intraspecific rDNA variability detected in T. infestans is unusual in insects, and exceptional in holocentric chromosomes.
Considering almost 100 heteropteran species analyzed to date, the chromosome position of the ribosomal genes appears to be a species-specific character. The number of rDNA loci described in *T. infestans* (from 1 to 4 sites) is very high in comparison with the 1 or 2 loci found in most heteropteran species analyzed to date (Bardella et al., 2013; Panzera et al., 2012).

The existence of individuals with ribosomal genes located on four different chromosomes (X chromosome and up to three autosomal pairs) allows clear recognition of hybrid individuals revealed by the occurrence of heterozygous patterns for a particular locus (Fig. 3E–H). The high rDNA variation in *T. infestans* also suggests that some structural characteristic of their chromosomes promotes changes in the number and position of ribosomal genes. The heterologous chromosome associations between several autosomes and sex chromosomes during cell divisions in *T. infestans* could favor the high mobility of rDNA loci through of transposition and/or ectopic recombination, which are the principal mechanisms suggested for rDNA changes in other organisms (Schubert and Wobus, 1985).

### 4.3. Geographic distribution of Andean and non-Andean groups

The Andean group of *T. infestans* is distributed in high-altitude regions (above 1700 masl) of the Andean valleys of Bolivia and Peru, and also in lower altitudes such as the city of Nazca (588 masl). The non-Andean group is found in lowland regions (0 to 1400 masl) in various countries including Bolivia (Chaco region) but also at higher altitudes in some localities of Argentina such as Palo Blanco (1800 masl) or San Carlos (2088 masl) (Fig. 1). These data confirm that Andean insects are able to colonize lowlands, and that non-Andean insects would be able to colonize highlands, as suggested previously (Panzera et al., 2012). Thus, the occurrence of a chromosomal group in a particular region may be associated with selection mechanisms linked to altitude as well as with the historical dispersal routes of each chromosomal group. Our chromosomal data from Peru and Chile are consistent with the presence of the two lineages in Chile, as suggested by mitochondrial sequences (Torres-Pérez et al., 2011). The colonization of northern Chile by the Andean group has probably also occurred from the coastal regions of Peru, and not only through the Andes. By contrast, we have not detected both lineages in Argentina, as has been suggested by microsatellite markers (Pérez de Rosas et al., 2011).

### 4.4. Origin and dispersal of *T. infestans* in the light of chromosomal markers

Two main hypotheses have been proposed to explain the origin and spread of *T. infestans* throughout South America. The “Andean” hypothesis assumed that the Andean valleys of Bolivia represent the geographic area of the most ancient populations (Schofield, 1988; Usinger et al., 1966), where sylvatic populations are found amongst rock piles associated with wild rodents (Dujardin et al.,...
This hypothesis also suggests that the domiciliation of T. infestans began in this region, associated with the domestication of wild guinea-pigs by pre-Columbian Andean cultures and was then spread in association with human migrations along two main routes: a north-western one towards Peru and then to north of Chile, and a southern route to Argentina, Chile, Paraguay, Brazil, and Uruguay (Schofield, 1988). The second theory, called “Chaco” hypothesis, assumed that T. infestans originated in the dry subtropical woodlands of southern Bolivia, Paraguay, and north Argentina (Carcavallo, 1998). Several studies have confirmed the presence of sylvatic populations in this region, usually occupying hollow trees, probably associated with birds, and presenting low or no infection with T. cruzi (Ceballos et al., 2009; Noireau et al., 2000; Rolón et al., 2011; Waleckx et al., 2011). Analyses with nuclear and mitochondrial sequences show that the sylvatic and domestic populations from Andean-Bolivia and Chaco regions show similar values of haplotype diversity (Bargues et al., 2006; García et al., 2013; Piccinalli et al., 2009, 2011; Quisberth et al., 2011; Waleckx et al., 2011; 2012). Furthermore, molecular phylogeographic including coalescent analyses are not conclusive about resolving the Andean or Chaco origin of T. infestans (Torres-Pérez et al., 2011). However in this paper, the high rDNA diversity observed in the Andean group compared to the monomorphic non-Andean group strongly suggests that the Andean group is the ancestral in T. infestans, with the non-Andean group being a derivative. Considering the two most common patterns of the Andean group (1A and 1A+X patterns in 90% of the individuals analyzed), a single transposition event (from an autosomal to X chromosome) or autosomal loss of rDNA loci (in 1A+X pattern) could explain the X pattern in the non-Andean group. An ancestral origin from non-Andean group (Chaco hypothesis) would have to imply several events of transposition and duplication of ribosomal genes occurring simultaneously, which is highly unlikely.

In light of our chromosomal data, we support the hypothesis that T. infestans originated in the Andean valleys of Bolivia, as a sylvatic species with polymorphic populations in terms of high amounts of heterochromatic autosomes (14–20) and variable ribosomal cluster positions. The most variable populations were those of Cochabamba and Chuquisaca, suggesting these as the original regions. Interestingly, the populations from La Paz are the most homogeneous in their ribosomal patterns (Table 2), similar as observed with cytobence b sequences, suggesting ancient isolation and bottleneck in this area (Waleckx et al., 2011). The emergence of the non-Andean group involved a deep genomic rearrangement reflected in a reduction of heterochromatin to 3 autosomal pairs (and consequently in the overall genome size), lost the C-heterochromatin in the X chromosome and a ribosomal cluster situated only on the X chromosome. Molecular data based on nuclear and mitochondrial sequences suggests that this divergence occurred between 59,000 and 588,000 years ago (Bargues et al., 2006; Torres-Pérez et al., 2011) although this would be long before human presence in these regions. Perhaps each chromosomal group would have independently undergone a process of diversification as sylvatic populations associated with different habitats and hosts – the Andean group with rock piles and rodents, and the non-Andean group associated with trees and birds. Subsequent human influence could have led to multiple and independent domestication processes in both groups, as suggested by molecular markers (Waleckx et al., 2011). A ribosomal divergence based on ecological preference can be tested because it predicts that sylvatic populations in non-Andean regions would have ribosomal genes only on the X chromosome, as observed in sylvatic specimens from Bolivian Chaco (Table 2). FISH analyses of sylvatic specimens from Argentinean Chaco with putative ancestral mitochondrial haplotypes (Piccinalli et al., 2011) may help to clarify this issue. A hypothesis of ancestral diversification of sylvatic T. infestans associated with different habitats and hosts is in agreement of differentiation of sensilla patterns observed between both chromosomal groups (Hernández et al., 2008).

4.5. The Intermediate chromosomal group

One of the most significant results of this study was the detection of a third chromosome group (Intermediate), restricted to a small lowland region about 70 km wide along the Argentina-Bolivia border (Fig. 1, map references 17–28). This Intermediate chromosomal group has three chromosome characteristics. First, the number of heterochromatic autosomes (7–11) is lower than Andean group (14–20) but higher than non-Andean group (4–6). Second, the Intermediate group includes individuals both with and without heterochromatic X chromosome, as observed in Andean and non-Andean individuals. Third, about 50% of individuals have an exclusive rDNA pattern (1/2 A + X). Considering these three chromosomal characteristics and Mendelian inheritance of C-heterochromatin as both of the ribosomal genes, two opposing hypotheses can be suggested for the origin of the Intermediate group.

The first hypothesis, that we called “ancestral origin”, assumes that the Intermediate group represents a middle step of gradual heterochromatin variance that occurred during the early divergence between Andean and non-Andean groups. The three chromosomal groups of T. infestans might reflect the ancestral processes of chromosomal differentiation, forming a north–south cline or gradient of heterochromatin. The current geographic distribution of the Intermediate group along the Argentina-Bolivia border would thus represent the original area where the Andean and non-Andean groups diverged.

The alternative hypothesis, called “secondary contact”, assumes that this group originated recently from crosses between Andean and non-Andean individuals, which were previously isolated. Following divergence between the Andean and non-Andean groups, the two forms would have continued to differentiate in isolation – for example through adaptation to different environments. However, when the two groups were subsequently brought back together, for example through anthropogenic activities such as human migration and domestic vector control, interbreeding between them would have given rise to the Intermediate group. This second hypothesis assumes that the Intermediate group is very recent formation and not a relic of the ancient process of divergence between Andean and non-Andean groups.

The C-heterochromatin traits observed in the Intermediate group, namely the number of heterochromatic autosomes and the X chromosomes with and without C-bands, are consistent with either hypothesis. The same statement was achieved by analysis with antennal phenotypes (Hernández et al., 2008). However, the high frequency of the heterozygous rDNA pattern (1/2 A + X, Fig. 3F) exclusively detected in the Intermediate group can only be explained assuming the hypothesis of a secondary contact. This pattern would be easily derived by crosses between non-Andean individuals (X pattern) with Andean individuals having the 1A+X and 1A patterns (the two most frequently observed in Andean individuals). All F1 progeny would have the heterozygous pattern, and crossing between them would give offspring showing heterozygous and parental patterns. By contrast, it is very unlikely that a population that includes a high frequency of heterozygotes (about 50%) could be maintained for long time, as the ancestral hypothesis presupposes. This high frequency strongly indicates these individuals are of recent origin (hypothesis of secondary contact) and suggests that Argentina-Bolivia border represents a very recent hybrid zone formed by crosses of two parental populations (Andean and non-Andean groups) generating intermediate or “mixed” genotypes (with chromosomal characteristics of the two
main chromosome groups). Experimental crosses between these groups showed that the F1 progeny have intermediate amounts of heterochromatin (Panzer et al., 2004). Epidemiological data from vector control activities also support a recent origin of the Intermediate group (see below). In conclusion, our FISH data strongly suggests that the Intermediate chromosomal group detected along the Argentina-Bolivia border is due to a recent secondary contact between Andean and non-Andean groups, rather than an ancient variation resulting from north–south cline of heterochromatin.

4.6. Intermediate group and pyrethroid resistance

Considering that the Intermediate group is situated at relatively low altitudes (387–958 masl), it seems most likely to have been occupied by non-Andean individuals prior to secondary contact with Andean populations. This assumption is supported by the high frequency of individuals with ribosomal X pattern (similar to non-Andean specimens). It thus seems likely that insects from Andean regions moved to southern Bolivia, reaching the Argentina-Bolivia border, perhaps by passive human transport. When these Andean individuals cross with non-Andean, producing intermediate individuals resistant to pyrethroids, which was not previously present in this region. Epidemiological information supports this scenario. Data provided by the Vector Control Program of Salta Province point out that, in the localities of Salvador Mazza, the spraying of houses with deltamethrin between 1995 and 1998 led to very low house infestation rates (ranging between 0.5 and 0.8%). This indicates that this region was then occupied by populations susceptible to deltamethrin. Since 1998 however, and despite continued vector control activities, there has been a gradual increase of insects in houses, reaching house infestation levels of 50 to 80% in 2004. Similar control failures were reported at the same time in the Bolivian city of Yacuiba, close to Salvador Mazza, across the Argentina-Bolivia border (Cardozo et al., 2010).

The elimination of T. infestans by pyrethroid insecticides in Brazil, Uruguay, Chile, and its drastic reduction in large parts of Paraguay and Argentina, clearly indicates that pyrethroid resistance was very uncommon in non-Andean regions. By contrast, in the Bolivian Andes, pyrethroid resistant populations appear to be much more frequent, being detected in several localities from the departments of Cochabamba, Chuquisaca, Sucre, and Potosí (Depickère et al., 2012; Lardeux et al., 2010; Roca Acevedo et al., 2011). This suggests that Andean populations have particular genetic backgrounds that enable the development of resistance to pyrethroid insecticides more rapidly than non-Andean populations. Although, there are probably different mechanisms responsible for resistance to pyrethroids in T. infestans (Germán et al., 2010), genetic analyses of experimental crosses between resistant and susceptible individuals showed that the inheritance of deltamethrin resistance is autosomal and with incomplete dominance, involving at least three genes (Cardozo et al., 2010). This inheritance fashion indicates that, under pyrethroid selective pressure, the resistant character may spread easily to susceptible insects.

A striking observation is the high frequency of chromosome fragments in the pyrethroid resistant populations from Salvador Mazza (Table 2). In other insects, chromosome rearrangements have been suggested to be involved in insecticide resistance. In Myzus persicae, an aphid with holocentric chromosomes, an autosomal translocation is responsible for organophosphate and carbamate resistance by the amplification of esterase gene E4 due to a position effect caused by the repositioning of heterochromatin (Blackman et al., 1978). A similar phenomenon may have occurred in T. infestans, where changes in position and amount of heterochromatin are very frequent. It is likely that homologous recombination between individuals with different amounts of heterochromatin (Andean and non-Andean groups) may modify the gene expression of euchromatic regions adjacent to the heterochromatin (position-effect variegation).

The emergence of resistant populations along the Argentina-Bolivia border supports a close relationship between the Intermediate genomes and pyrethroid resistance. According to this idea, we can predict that nearby regions occupied by Andean and non-Andean groups, or non-Andean regions experiencing human migration from Andean regions (such as Gran Chaco region), would have a greater likelihood of developing pyrethroid resistance. Recently, several domestic populations of T. infestans from the Bolivian Chaco (Izozog, Santa Cruz Department) appear to be the result of a mixture of Andean and non-Andean groups probably generated by the passive transport of triatomines from the Andean to the Gran Chaco region (Quisberth et al., 2011). Although T. infestans populations from Santa Cruz Department are currently considered amongst the most susceptible to deltamethrin (Depickère et al., 2012), these ones could have a high probability of developing pyrethroid resistant in the near future.

5. Conclusions

This paper reveals that the differentiation process between the two T. infestans chromosomal groups has involved significant genomic reorganization of heterochromatin and essential coding sequences. Furthermore, the detection of pyrethroid-resistant insects in a hybrid zone between both chromosomal groups suggests a correlation between genomic variability and insecticide-resistant populations. Vector control programs should make a greater effort in hybrid-zones surveillance to avoid rapid emergence of resistant individuals. As an effective control of Chagas disease depends on vector elimination, our study underscores the importance of chromosomal and genomic studies to provide a better understanding of the dynamics of domestic insect population.

Authors’ contributions

F.P., R.P., Y.P.: Design, coordination and wrote the different versions of the manuscript. F.P., M.J.F., S.P., I.F., L.C., Y.B., Y.G., S.F.B., Y.P.: performed data and their analyses, helped to draft the manuscript. F.P., Y.B., Y.G., S.F.B.: contributed to the triatomine collection. All authors read and approved the final version of the manuscript.

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