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Eco-geographical differentiation among Colombian populations of the Chagas disease vector *Triatoma dimidiata* (Hemiptera: Reduviidae)

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ABSTRACT

Triatoma dimidiata is currently the main vector of Chagas disease in Mexico, most Central American countries and several zones of Ecuador and Colombia. Although this species has been the subject of several recent phylogeographic studies, the relationship among different populations within the species remains unclear. To elucidate the population genetic structure of *T. dimidiata* in Colombia, we analyzed individuals from distinct geographical locations using the cytochrome c oxidase subunit 1 gene and 7 microsatellite loci. A clear genetic differentiation was observed among specimens from three Colombian eco-geographical regions: Inter Andean Valleys, Caribbean Plains and Sierra Nevada de Santa Marta mountain (SNSM). Additionally, evidence of genetic subdivision was found within the Caribbean Plains region as well as moderate gene flow between the populations from the Caribbean Plains and SNSM regions. The genetic differentiation found among Colombian populations correlates, albeit weakly, with an isolation-by-distance model (IBD). The genetic heterogeneity among Colombian populations correlates with the eco-epidemiological and morphological traits observed in this species across regions within the country. Such genetic and epidemiological diversity should be taken into consideration for the development of vector control strategies and entomological surveillance.

1. Introduction

Chagas disease is a parasitic disease in which the pathogenic agent, *Trypanosoma cruzi,* is transmitted by hematophagous insects of the Triatominae subfamily. *Triatoma dimidiata* is the major vector in several Central American countries as well as in regions of Ecuador and Colombia, where it occupies a large diversity of ecotopes and life zones (Dorn et al., 2007). Notably, *T. dimidiata* populations within Colombian seem to differ in several biological, ecological and epidemiological attributes (i.e., life cycle, infection rates) and present marked morphological differences between western and eastern populations (Esteban, 2010).

This species had been found in 47% of Colombia departments, occupying human dwellings, palm trees, bark of dead trees, rocks piles, chicken huts, huts for drying tobacco leaves and wood stoves (Grisales et al., 2010; Guhl et al., 2007). Colombian *T. dimidiata* populations from the Inter Andean Valleys eco-geographical region are frequently found colonizing dwellings and show high rates of *T. cruzi* infection (Guhl et al., 2007; Parra-Henao, personal communication). Hence, these *T. dimidiata* populations are considered more relevant as Chagas vectors than those from Northern Colombia or Caribbean regions, that are mostly sylvatic (Guhl et al., 2007; Ramírez et al., 2005).

An effective surveillance and vector control program must take into account the habitat diversity and the dispersion capacity among populations within and among eco-geographical regions, which is yet to be established for Colombian *T. dimidiata* populations. A recent study based on nucleotide sequence analysis of the mitochondrial NADH dehydrogenase gene subunit 4 (ND4), showed significant genetic differentiation and strong population structure among three localities from the departments of La Guajira, located at Northwestern of the Sierra Nevada de Santa Marta (SNSM) and Cesar, located at Caribbean Plains and Santander, in the Inter Andean Valleys region (Grisales et al., 2010). However, because the sample evaluated was rather small ($n = 40$), representing only a minimal area of the species distribution, a more comprehensive analysis is needed to elucidate the actual genetic structure of *T. dimidiata* populations throughout Colombia and to determine whether the morphological and epidemiological differences observed across regions correlate with genetic differentiation. The present study includes a larger number of individuals representing *T. dimidiata* populations across the complete distribution of the species within the country. Moreover, by analyzing information based on independent and higher resolution genetic markers, we were able to explore the degree of gene flow occurring among the different

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eco-geographical regions. The application of such molecular markers can help improve vector control and surveillance by identifying and characterizing genetically distinct vector populations and developing targeted intervention strategies. Quantitative measures of these aspects can be assessed by using certain parameters such as the best-fitted number of genetic clusters (K) based on allele frequencies distribution, estimation of genetic structure indexes (such as Fst, Rst or Φst) or by statistical procedures that allows the hierarchical partitioning of genetic variation among and within groups (such as AMOVA test). To estimate these parameters independent genetic markers with a significant degree of variability within a species are required.

Mitochondrial DNA (mtDNA) had been extensively used in molecular systematic studies of triatomine species across most countries in Central and South America (Lyman et al., 1999; Mas-Coma and Bargues, 2009). mtDNA loci are considered one of the more sensitive tools to infer population structure at both local and regional levels in several epidemiologically relevant triatomine species such as *T. infestans* (Monteiro et al., 1999; Piccinali et al., 2009, 2011), *Rhodnius prolixus* (Fitzpatrick et al., 2008) and *T. dimidiata* (Blandón-Naranjo et al., 2010; Monteiro et al., 2013), among others. The cytochrome c oxidase subunit 1 (COI) gene has been used at several micro-evolutionary scales ranging from gene flow studies among sylvatic and domestic isolates in *Triatoma infestans* at a local level (Piccinali et al., 2011) to regional studies about phylogenetic relationships and population structure in North American sibling species as *Thala recurva* and *T. rubida* (Pfeiler et al., 2006), and of *Triatoma infestans* populations in South America (Piccinali et al., 2009).

Multilocus microsatellite analysis (MMA) have been developed and applied for population studies of several triatomine species over a wide range of geographic and evolutionary scales: *R. prolixus* (Fitzpatrick et al., 2009), *Rhodnius pallescens* (Gómez-Sucerquia et al., 2009), *Triatoma pseudomaculata* (Harry et al., 2008a) and *T. infestans* (Marcet et al., 2008; Pizarro et al., 2008; Pérez de Rosas et al., 2008, 2011; Richer et al., 2007). For *T. dimidiata* however, only a limited number of studies have applied MMA for genetic population studies. Low genetic differentiation and variability of *T. dimidiata* populations from several villages in the Yucatan Peninsula of Mexico (Dumonteil et al., 2007) was detected using eight previously reported microsatellite loci (Anderson et al., 2002), of which only four loci had actually provided reliable genotypic information (Dumonteil et al., 2007). Those results revealed the need to identify new microsatellite loci for *T. dimidiata* species and/or to optimize the performance of those already published. Therefore the objectives of this work were (i) to evaluate and optimize the performance of previously published microsatellite loci from different triatomine species in *T. dimidiata* individuals from different areas across the species geographical distribution, and (ii) to study the genetic structure of Colombian *T. dimidiata* populations by using both COI nucleotide sequences and microsatellites markers.

2. Materials and methods

2.1. Sample origin

The capture origin of each *T. dimidiata* from Colombia included in the analyses is detailed in Fig. 1 and Table 1. Samples were collected in 12 communities groups from seven departments that belong to three different eco-geographical regions, considered relevant for the presence of triatomine vectors in Colombia: Inter Andean Valleys, Caribbean Plains and SNSM (Fig. 1, Table 1). The sampling includes both rural and urban capture sites, encompassing a variety of eco-epidemiological attributes, which aimed to represent the ecological diversity of *T. dimidiata* populations throughout the geographical distribution of the species in Colombia.

Bug captures were carried out during 2003–2009 in collaboration with local personnel from the Ministry of Health. Sylvatic collections were performed with live-baited traps (Noireau et al., 1999). Domiciliary and peridomiciliary collections were made by the traditional time manual collection method using a dislodging spray (Gürtler et al., 1999) and by homeowners. A maximum of three insects per house were included in the sample. Captures from palm trees were obtained through palm dissection (Fitzpatrick et al., 2008), having previously obtained consent from the landowners. All specimens were identified as *T. dimidiata* according to morphological characters (Lent and Wygodzinsky (1979) and kept in 70% ethanol until processed for DNA extraction.

Genomic DNA was obtained from four legs of each insect or from thorax muscle, following an insect DNA extraction protocol (Collins et al., 1987). Additionally, in order to optimize the microsatellite PCR conditions, high quality DNA from fresh specimens was purified using the Wizard Genomic Purification Kit (Promega®) following the manufacturer recommendations.

2.2. PCR and sequencing

A 402-bp fragment of the COI gene was PCR-amplified for each specimen, using the primers LCO1490f (5′-GGTCWMCAAATCAT AAAGATATTGG-3′) and HCO2198r (5′-TAWACTTCAGGGTGWCCAA ARAATCA-3′), slightly modified from the original publication (Folmer et al., 1994) to improve their performance in this species. PCR reactions were conducted in a final volume of 35 μl using 3 μl of 10 ng/μl DNA templates, 3.5 μl of 1× PCR buffer, 4.4 μl of 2 mM dNTP, 1.4 μl of each 0.4 μM primer, 3.5 μl of 50 mM $MgCl_2$ and 1 U/μl of Taq DNA polymerase (Promega®). Amplification conditions were: 95 °C for 5 min; 35 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 60 s; 72 °C for 10 min. PCR products were sent to Macrogen Inc., Korea for purification and sequencing.

Both strands of the COI fragment were sequenced for all samples ($n = 82$). A multiple sequence alignment was obtained with the CLUSTAL W algorithm (Thompson et al., 1997) implemented in BioEdit v. 7.0.5 (Hall, 1999). The number of segregating sites (S), nucleotide diversity (π), number of haplotypes (h) and haplotype diversity (Hd) were estimated with DnaSP v.5.10 (Librado and Rozas, 2009). Population differentiation based on nucleotide and haplotype diversity was estimated by Kst and Hst (Hudson et al., 1992), with a permutation test of 1000 replicates and significance level of $p < 0.001$, using DnaSP v.5.10 (Librado and Rozas, 2009). A median joining (MJ) haplotype network was obtained using Network v.4.6.1.1 (http://www.fluxus-engineering.com), using default parameters (equal character weight = 10; epsilon value = 10; transversions/transitions weight = 1:1 and connection cost as a criterion).

2.3. Multilocus microsatellite analysis (MMA)

The performance of microsatellite loci previously published for triatomine species was assessed for *T. dimidiata*. Twenty seven markers were evaluated: eight from *T. dimidiata* (Anderson et al., 2002), ten from *T. infestans* (Marcet et al., 2006), six from *T. pseudomaculata* (Harry et al., 2008a) and three from *R. prolixus* (Harry et al., 2008b). Each primer set was first evaluated in 10 *T. dimidiata* insects from different locations (Belize, Costa Rica, Honduras, Nicaragua and Colombia), aiming to account for most of the genetic diversity observed within the species across its geographic distribution. Amplification conditions for each locus optimized for *T. dimidiata* are presented in Table S1. DNA fragment detection was performed using an automated DNA sequencer ABI 3130 (Applied Biosystems®). Fragment size determination with one bp resolution and allele classification was performed with GeneMapper v.3.7 (Applied Biosystems®) software.