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# Fine-scale genetic structure of *Triatoma infestans* in the Argentine Chaco

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ABSTRACT

The patterns of genetic structure in natural populations provide essential information for the improvement of pest management strategies including those targeting arthropod vectors of human diseases. We analyzed the patterns of fine-scale genetic structure in *Triatoma infestans* in a well-defined rural area close to Pampa del Indio, in the Argentine Arid-Humid Chaco transition, where a longitudinal study on house infestation and wing geometric morphometry is being conducted since 2007. A total of 228 insects collected in 16 domestic and peridomestic sites from two rural communities was genotyped for 10 microsatellite loci and analyzed. We did not find departures from Hardy–Weinberg expectations in collection sites, with three exceptions probably due to null alleles and substructuring. Domestic sites were more variable than peridomestic sites suggesting the presence of older bug populations in domestic sites or higher effective population sizes. Significant genetic structure was detected using F-statistics, a discriminant analysis of principal components and Bayesian clustering algorithms in an area of only 6.32 km$^2$. Microsatellite markers detected population structuring at a finer geographic scale (180–6300 m) than a previous study based on wing geometric morphometry (>4000 m). The spatial distribution of genetic variability was more properly explained by a hierarchical island than by an isolation-by-distance model. This study illustrates that, despite more than a decade without vector control interventions enhancing differentiation, genetic structure can be detected in *T. infestans* populations, particularly applying spatial information. This supports the potential of genetic studies to provide key information for hypothesis testing of the origins of house reinfestation.

## 1. Introduction

The patterns of genetic structure in natural populations offer insight on the evolutionary processes and complexities of the dynamics of natural populations, and relevant information that can be utilized for wildlife conservation and pest management, including arthropod vectors of human diseases (DeSalle and Amato, 2004; McCoy, 2008; Monteiro et al., 2001). These studies often require sizable sampling efforts and analysis at a small spatial scale, a scale that includes, for example, the range of dispersal of the species of interest and the movements of humans in their daily activities. This is particularly important for species with limited vagility or which occupy patchy habitats (Lowe et al., 2004; Smouse et al., 2008).

A good description of this fine-scale population structure poses a real challenge to population genetic tools because when populations are close to each other and migration rates are not very low, structuring tends to be weak and population genetic methods may fail to detect it (Faubet et al., 2007; Putman and Carbone, 2014). However, recent developments in Bayesian model-based analyses (Beaumont and Rannala, 2004) and other techniques such as discriminant analysis of principal components of genetic data (Jombart et al., 2010), combined with spatial information (François and Durand, 2010; Guillot et al., 2009), proved to be very powerful to address difficult questions in ecology, evolution and conservation biology related to the genetic structure of the species.

*Triatoma infestans* is an hemipteran bug and the main vector of *Trypanosoma cruzi*, the etiological agent of Chagas disease, in South America. This species lives mainly in warm and dry rural areas and in close association with human dwellings, including domiciles and peridomestic structures. National vector control programs supported and coordinated by an international governmental initiative to interrupt the transmission of *T. cruzi* drastically reduced the area of distribution of *T. infestans*. However, in some regions of Argentina, Bolivia and Paraguay, the success of these actions was limited and infestation usually persisted after insecticide spraying campaigns. Most of these areas belong to the Gran Chaco ecoregion where the estimated prevalence of vector-borne transmission of *T. cruzi* is the highest across Latin America (World Health

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Organization, 2015). The reasons for the lower effectiveness of traditional vector control strategies in this area are still poorly understood and subject of research (Gürtler, 2009; Gürtler et al., 2007).

Several studies analyzed the genetic, kariotypic and phenoptypic structure of *T. infestans* populations at a country (Catalá and Dujardin, 2001; Dujardin et al., 1998; Pérez de Rosas et al., 2007; Waleckx et al., 2011) or regional scale (Bargues et al., 2006; Gumiel et al., 2003; Panzera et al., 2004; Piccinali et al., 2009; Torres-Pérez et al., 2011). However, research efforts on this topic at finer spatial scales (from meters up to 10–15 km) are much more limited. A study made in the Bolivian Andean Valleys of La Paz and Cochabamba using enzyme electrophoresis showed a population structure pattern compatible with an isolation-by-distance (IBD) model and that the panmictic unit of *T. infestans* could be as small as a single domestic or peridomestic structure (Brenière et al., 1998). Later studies based on microsatellites in the same region and in the Bolivian highlands of Chuquisaca detected genetic differentiation at different hierarchical geographic levels, including adjacent households within a single locality, but not an IBD pattern (Brenière et al., 2013; Pizarro et al., 2008). A similar geographic pattern of variability found in the urban–rural interface in Arequipa, Peru, was attributed to passive bug transportation by humans (Foley et al., 2013).

This high structuring of *T. infestans* populations also was found in the western Gran Chaco (i.e., the Arid Chaco). In Santiago del Estero, Argentina, wing geometric morphometry (Schachter-Broide et al., 2004) and microsatellites (Marcet et al., 2008) showed extensive differentiation among insect capture sites and house compounds in an area under long-term vector control interventions. In Catamarca, Argentina, in an area subject to recent insecticide spraying, Pérez de Rosas et al. (2008, 2013) also reported important substructuring within domestic and peridomestic sites and a positive spatial correlation of genetic variability up to 400 m. Most of these studies were performed in areas under regular vector vigilance or which had been sprayed with insecticides 3–5 years before. The latter has been shown to affect the genetic structure of *T. infestans* populations (Pérez de Rosas et al., 2007).

By contrast, almost no information on the genetic structure of *T. infestans* is available for the Humid Chaco, which owes its name to its higher average annual precipitation (750–2,200 mm) in comparison with the Arid Chaco (300–700 mm); both subregions also differ in biophysical and social aspects (Guinzburg and Adámoli, 2005; Torrela and Adámoli, 2005). A recent study, which is part of a research project on the eco-epidemiology and control of Chagas disease in the transition between the Humid and Arid Argentine Chaco, analyzed the occurrence of spatial structuring in *T. infestans* populations at a micro- and meso-scale using wing geometric morphometry (Gaspe et al., 2012). *T. infestans* populations were significantly structured only at a scale of more than 4 km of distance in a well-defined endemic area where no systematic vector control actions had been conducted during the previous 12 yr (Gaspe et al., 2012). This result differed from those reported previously for the Arid Chaco and for Bolivia, and could be due to the distinct local history of vector control, the eco-bio-social characteristics of the area, and/or the lack of resolution of the chosen marker.

As part of the same longitudinal research program, in the current study we investigate whether a lower scale structuring pattern can be detected in the same area using microsatellites as genotypic markers and several population genetic approaches. This data will provide the baseline information for ongoing studies that focus on the sources of house reinfestation after insecticide spraying.

The main goal of the present work is to describe the fine-scale genetic structure of *T. infestans* populations in an area of the Argentine Chaco without recent vector control interventions.

## 2. Materials and methods

### 2.1. Study area and bug samples

Fieldwork was conducted in a rural section of the municipality of Pampa del Indio (25°55′S 56°58′W), Province of Chaco, Argentina. This area belongs to the transition between the Humid and Arid Chaco ecoregions. Historically, vector control activities in the municipality had been sporadic, with a community-wide insecticide spraying campaign conducted in 1995 and a few houses treated by the villagers or the staff from the local hospital in 2006.

A community-wide intervention comprising a well-defined rural section with 12 villages was launched in October 2007. A total of 327 houses was georeferenced and evaluated for the presence of *T. infestans* in all of its sites, including domestic and peridomestic structures (Gurevitz et al., 2011). Domiciles were the places where people used to sleep, whereas peridomestic sites included storerooms, latrines, kitchens, corrals, chicken coops and chicken nests, among others. Timed manual searches of triatomines were performed by skilled personal of the Provincial Vector Control Program using a dislodging spray (0.2% tetramethrin). Domiciles were inspected by one person for 20 min and peridomestic sites by one person for 15 min. Collected bugs were put in labeled plastic bags and transported to the laboratory for further processing. Immediately after bug searches, all the sites within each house were sprayed with deltamethrin (K-Othrin, Bayer, Argentina) at a standard dose (25 mg/m$^2$).

Bug colonies were found in 30.8% of all houses in the surveyed area (Gurevitz et al., 2011). We selected the village of Campo Los Toros for the present study because: (a) it was one of the most infested villages in the area, with a prevalence of *T. infestans* infestation greater than 60% in both domiciles and peridomiciles (Gurevitz et al., 2011); (b) it was one of the villages with more insects collected during post-spray vector surveys (Gurevitz et al., 2013). The two houses of Santos Lugares were included due to its close proximity to Campo Los Toros and high bug abundance. Only sites with at least 6 bugs available for DNA extraction were selected for microsatellite genotyping. We found 16 sites from 14 houses that fulfilled this requisite (Table 1, Fig. 1). Distances between houses ranged from 180 m to 6300 m, with a mean value of 2700 m, and distances between sites within the same house were $\leqslant$5 m. The total surface covered was of 6.32 km$^2$.

**Table 1**
Description of the collection sites and sample sizes of *T. infestans*. Bug abundance is the total number of bugs collected in the site by one operator during 20 min in domiciles or 15 min in other peridomestic sites.

| House | Sample name | Site description | Bug abundance | Number of genotyped bugs |
|---|---|---|---|---|
| CT1 | CT1P | Chicken coop | 9 | 7 |
| | CT1D | Domicile | 13 | 12 |
| CT5 | CT5 | Domicile | 30 | 16 |
| CT7 | CT7 | Domicile | 26 | 18 |
| CT8 | CT8 | Domicile | 10 | 7 |
| CT9 | CT9 | Chicken coop | 155 | 40 |
| CT14 | CT14D | Domicile | 36 | 12 |
| | CT14P | Kitchen | 18 | 8 |
| CT16 | CT16 | Domicile | 17 | 9 |
| CT19 | CT19 | Kitchen | 53 | 19 |
| CT20 | CT20 | Chicken nest | 14 | 14 |
| CT22 | CT22 | Chicken nest | 12 | 12 |
| CT26 | CT26 | Kitchen /Storeroom | 91 | 20 |
| CT3 | CT3 | Unknown | 6 | 6 |
| LUG48 | LUG48 | Storeroom | 15 | 13 |
| LUG49 | LUG49 | Chicken coop | 17 | 15 |
| Total | | | | 228 |

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