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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

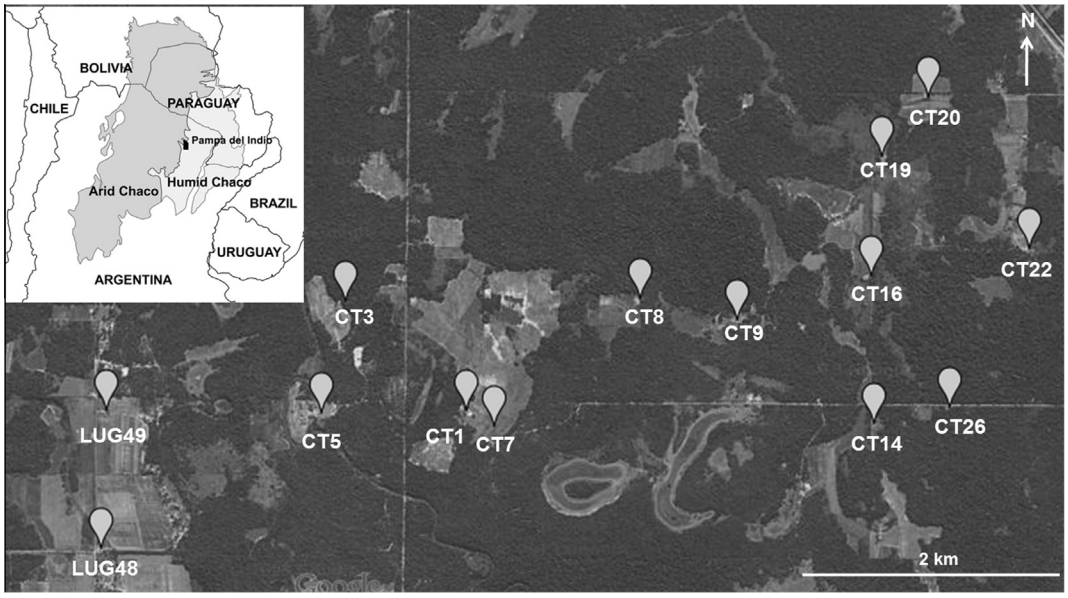

Fig. 1. Map showing the bug collection sites in Campo Los Toros and Santos Lugares villages, Pampa del Indio, Chaco Province, Argentina. Inset: location of the study area in South America highlighting the Arid and Humid Chaco. Map data: Google, Inav/Geosistemas SRL, TerraMetrics.

2.2. DNA extraction and microsatellite genotyping

DNA was successfully extracted from two legs of 228 males, females or third-, fourth- and fifth-instar nymphs following a protocol that included sodium-dodecyl-sulfate (SDS), proteinase K, NaCl and isopropanol (modified from Miller et al., 1988). Ten microsatellite loci, Tims3, Tims5, Tims19, Tims22, Tims23, Tims27, Tims42, Tims56, Tims64 and Tims65, were PCR amplified for multilocus genotyping of domestic and peridomestic bugs as previously described (Marcet et al., 2006, 2008). DNA fragment analysis was performed in an ABI 3130 automated DNA sequencer and allelic sizes and binning were determined with GeneMapper v3.7 (Applied Biosystems) and FlexiBinV2 (Amos et al., 2007).

2.3. Data analysis

2.3.1. Descriptive statistics, linkage disequilibrium, relatedness and null alleles

Mean number of alleles (Na), mean allelic richness based on the smallest sample size (Rs, El Mousadik and Petit, 1996), mean unbiased expected heterozygosity (He, Nei, 1978), mean observed heterozygosity (Ho) and Weir and Cockerham (1984) mulilocus F_{IS} were estimated with FSTAT 2.9.3 (Goudet, 2001) and GENEPOP 4.0 (Rousset, 2008). The presence of null alleles, allelic dropouts and stutter peak scoring was investigated with MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004). Inbreeding within sites was evaluated by computing Queller and Goodnight (1989) relatedness estimator per population (r_{QG}) with Genalex 6.5 (Peakall and Smouse, 2006, 2012).

The differences in the levels of genetic variability according to Na, Rs, He and Ho between domestic and peridomestic sites were evaluated with one-way ANOVAs. Assumptions of normality and homocedasticity were previously assessed with Shapiro–Wilk and Levenés tests respectively. All calculations were made with InfoStat (Di Rienzo et al., 2015).

Linkage disequilibrium (LD) exact tests between microsatellite loci pairs for the total sample were calculated using GENEPOP 4.0 (Rousset, 2008). A Bonferroni correction was performed dividing the *p*-value by the total number of comparisons. If significant LD was found between pairs of loci, we applied the criterion proposed by Kaeuffer et al. (2007) to decide whether they should be included or not in the following analyses, particularly in the STRUCTURE and Geneland runs, which rely on the assumption of loci independence. For this purpose, we calculated the correlation coefficient of linkage disequilibrium (rLD) with Linkdos (Garnier-Gere and Dillmann, 1992).

2.3.2. Genetic structure

2.3.2.1. Traditional genetic structure and spatial analyses. Global and pairwise Weir and Cockerham (1984) multilocus F_{ST} statistics between sites were computed with GENEPOP 4.0 (Rousset, 2008) and FSTAT 2.9.3.2 (Goudet, 2001). A Bonferroni correction of the p-value was made as described in Section 2.3.1. Possible isolation-by-distance effects were evaluated with a Mantel test (Mantel, 1967). The correlation between geographic (ln transformed) and genetic (linearized F_{ST}) distance matrices was performed with Genalex 6.5 (Peakall and Smouse, 2006, 2012). Negative F_{ST} values were equaled to zero. The analysis was performed including all the sites and only one site per house, due to the huge difference in the distance range between sites within the same house ($\leqslant$5 m) relative to sites between different houses ($\geqslant$178 m). Sites within houses were chosen at random.

2.3.2.2. Multivariate analysis using discriminant analyses of principal components (DAPC). This exploratory analysis (Jombart et al., 2010) was performed in two different ways: using collection sites as groups, and grouping the individuals with the function find.clusters. In the latter case, the maximal number of clusters was set to 20 and the model with the optimal number of clusters (*K*) was chosen applying the Bayesian Information Criterion, as suggested by Fraley and Raftery (1998). Calculations and graphs were performed with the adegenet package (Jombart, 2008) which runs in the R software environment (R Development Core Team, 2014).

2.3.2.3. Bayesian clustering analyses including spatial information. The Bayesian model-based approach of Pritchard et al. (2000) implemented in STRUCTURE 2.3.4 was applied to data. The number of clusters (*K*) evaluated ranged from 1 to 16. The analysis was performed using 5 replicate runs per *K* value, a burn-in period length of 500,000 and a run length of 1,500,000.

Runs were made with and without prior information on the origin of the individuals to assist the definition of the clusters (LOCPRIOR option). The use of a prior on the origin of the samples has the desirable properties of not finding any structure when none is present, and ignoring the sampling information when the ancestry of individuals is uncorrelated with the sample origin (Hubisz et al., 2009). All the analyses were run under an admixture model with correlated allele frequencies. The selection of the *K* value that better recovered the structure of the data was performed by comparing the rate of change in the log-probability of the data between successive *K* values (Evanno et al., 2005) using STRUCTURE HARVESTER 0.56.4 (Earl and von Holdt, 2012). The results of the 5 different runs for the chosen *K* value were averaged using the fullsearch option implemented in CLUMPP 1.1.2 (Jakobsson and Rosenberg, 2007) and plotted using DISTRUCT 1.1 (Rosenberg, 2004).

A second Bayesian spatial clustering model was run with the Geneland package (Guillot et al., 2005) of the R software environment (R Development Core Team, 2014). This software estimates populations and their spatial boundaries. We applied a spatial model with correlated allele frequencies, null alleles and no uncertainty about the spatial coordinates of the individuals. The number of clusters was set to a maximum of 20, the number of iterations to 2,000,000, the thinning to 1000, the burnin to 25% and the number of different runs to 20. Convergence was checked by comparing the modal number and the composition of the populations across the runs, and verifying that chains did not show transient behaviors or got stuck in one single value as suggested in the software manual (The Geneland Developing Team, 2012).

3. Results

3.1. Variability and Hardy–Weinberg expectations

Levels of variability were rather similar among sites (Table 2). The mean number of alleles ranged between 2.7 and 3.9 whereas mean allelic richness varied between 2.5 and 3.2. Mean expected unbiased heterozygosity was around 0.43 and 0.56 and mean observed heterozygosity between 0.38 and 0.56. The most variable sites were CT5 and CT8.

Table 2
Genetic variability and HWE estimators for 10 microsatellite loci in 16 *T. infestans* sampled sites.

Sample	Habitat	Na	Rs	Ho	He	F_{IS}
CT1D	D	3.7	3.03	0.475	0.518	0.087*
CT14D	D	2.7	2.56	0.425	0.441	0.038
CT16	D	3.20	2.96	0.500	0.509	0.019
CT26	D	3.00	2.63	0.440	0.488	0.100
CT5	D	3.70	3.06	0.563	0.550	−0.024
CT8	D	3.40	3.24	0.486	0.564	0.148
CT1P	P	2.9	2.81	0.471	0.492	0.046
CT14D	P	3.5	3.00	0.511	0.531	0.036
CT19	P	3.60	2.81	0.518	0.482	−0.077
CT20	P	2.70	2.49	0.404	0.438	0.080
CT22	P	2.70	2.46	0.450	0.450	0.000
CT7	P	3.60	2.93	0.506	0.536	0.061
CT9	P	3.90	2.68	0.384	0.433	0.113*
LUG48	P	3.50	2.86	0.454	0.471	0.037
LUG49	P	3.10	2.69	0.483	0.506	0.043*
CT3	NA	2.80	2.80	0.500	0.462	−0.091
Average	D	3.38	2.96	0.493	0.520	–
Average	P	3.21	2.71	0.457	0.476	–

D: domestic site; P: peridomestic site; Na: mean number of alleles; Rs: mean allelic richness based on the smallest sample size; Ho: mean observed heterozygosity; He: mean unbiased expected heterozygosity; F_{IS} = Weir and Cockerham mulilocus F_{IS}; NA: not available.
* $p < 0.05$.

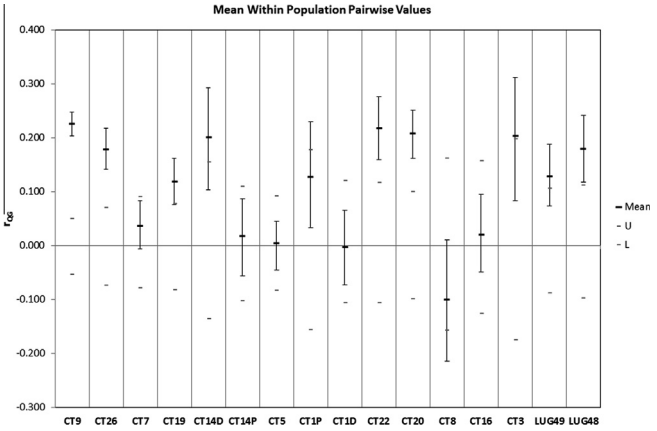

Fig. 2. Values of mean pairwise relatedness within collection sites according to Queller and Goodnight estimator (r_{QG}). Grey lines are 95% confidence intervals obtained by permuting values around the null hypothesis of no differentiation from a relatedness of 0. Bars are the 95% confidence intervals around population means obtained by bootstrap re-sampling.

When sites were grouped as domestic or peridomestic, mean values of variability were higher for the domestic group (Table 2) and the ANOVAs revealed a significant group effect over genetic variability. Levels of variation were significantly higher in domestic than in peridomestic sites according to Rs ($F_{(1,13)} = 5.81$, $P = 0.031$) and He ($F_{(1,13)} = 4.83$, $P = 0.047$) but not to Ho or Na (Table S1).

Departures from Hardy Weinberg expectations (HWE) were found in CT1D, CT9 and LUG49, indicating an excess of homozygotes (Table 2). This result could be due to substructuring, null alleles and/or inbreeding. The presence of substructuring within sites was further analyzed in Section 3.3.4. According to MICROCHECKER, there was an excess of homozygotes for most allelic size classes in some loci in these three populations, which is compatible with the presence of null alleles. These loci were Tims19 in CT9 (frequency of null alleles = 0.16), Tims22 and Tims65 in CT1D (0.15 and 0.23), and Tims19, Tims27 and Tims56 in LUG49 (0.17, 0.19 and 0.16, respectively). When we revised the total sample, we found that only 9 PCR reactions failed after three different attempts. Five of them corresponded to the locus Tims65 (56%), two to the locus Tims65 (22%), one to Tims5 (11%) and one to Tims65 (11%).

To investigate the possibility of higher inbreeding on these three sites, we estimated the coefficient of relatedness among individuals within all sampled sites in order to see if r_{QG} was higher in CT1D, CT9 and LUG49 in comparison with other sites. Relatedness was significantly higher than zero only for the individuals within CT9, CT26, CT22 and CT20 (Fig. 2). However, the mean r_{QG} value for CT9 was not significantly higher than for the other three sites (Fig. 2).

3.2. Linkage disequilibria

We found significant LD between the alleles of loci Tims3 and Tims64 after Bonferroni correction ($p = 0.0006$), an association not found in two previous studies using the same markers (Marcet et al., 2008; Piccinali et al., 2011). The value of rLD was 0.084, far from the rLD $\geqslant$ 0.50 value observed for strongly linked loci, which are capable of producing spurious clustering (Kaeuffer et al., 2007). For this reason we decided to include all the loci in the following analyses.

3.3. Population structure

3.3.1. F_{ST} and Mantel test

The estimation of global differentiation among sites was highly significant and close to 8% ($F_{ST} = 0.081$, $P < 0.001$). When analyzed

separately for each life stage, F_{ST} was higher for nymphs than for adults and for males than for females ($F_{ST\ nymphs} = 0.126$, $P < 0.001$; $F_{ST\ males} = 0.097$, $P < 0.001$; $F_{ST\ females} = 0.090$, $P < 0.001$), although the differences among stages were not statistically significant.

Pairwise F_{ST} values were significant for 58 (48%) from 120 comparisons, with values varying between 0.20 and 0.05 (Table 3). Population CT9 was different from any other collection site, and CT26, CT7, CT22, LUG48 and LUG49 differed from most of all the other sites, whereas CT3, CT8 and CT16 were not differentiated from almost any population. No significant differentiation was found between sites from the same house.

The correlation between geographic and genetic distances was not significant when all the sites were included ($r = 0.148$, $P \geqslant 0.065$, Fig. S2). However, a significant but low correlation was found when only one site per house was included ($r = 0.204$, $P \leqslant 0.041$, Fig. S2).

3.3.2. Discriminant analyses of principal components

We retained 24 PCs (95% of total variance) and 4 linear discriminant functions (71% of total variation, Fig. S3) for the DAPC using collection sites. Five sites were visualized as separate groups with partial overlap in the scatterplots (Fig. 3). The scatterplot with the first two discriminant functions showed a clear separation of CT9, CT26 and LUG49 from the other sites, followed in less degree by CT7. The scatterplot with the third and fourth discriminant functions allowed the differentiation of CT22. These five sites exhibited a high consistency between prior and posterior assignment of bugs: 85% for CT9 and CT26, 80% for LUG49, 78% for CT7, and 75% for CT22.

The total number of PCs (53) was retained in order to find the number of clusters using the find.clusters option. The value of K that better described the data according to BIC was between 5 and 8. We conducted the analysis with both extreme values. For $K = 5$, we retained 24 PCs (95% of total variance) and 3 linear discriminant functions for the dapc function. The scatterplot showed five well-differentiated groups but no one was integrated by individuals from a single site (Fig. S4). This result probably reflects a high degree of admixture between sites. However, individuals from CT26 were the majority in cluster 1 (15/39, 38%), and CT9 in clusters 4 and 5 (11/42, 26%, and 17/51, 33%, respectively). A similar result was found with 8 clusters, which were all composed by individuals from different sites. Three of them had a majority of CT9 individuals (46%, 43% and 29%), one of CT26 individuals (36%), one of LUG48 (28%), one of CT19 and LUG49 (43%), and the remaining two were highly mixed.

3.3.3. Structure

The optimal value of K for the runs without individual origin prior was 1, indicating that only one genetic cluster was detected. However, when prior information was used, the optimal value of K to describe the data was 3 (Fig. S5). Most individuals exhibited variable degrees of admixture, but individuals from the same collection site were more similar to each other than from different sites (Fig. 4). The genome of individuals from CT9 mostly belonged to one cluster, from CT26 to another, and from CT22, LUG48 and LUG49 to a third cluster. Individuals from CT7 had similar proportions of each cluster, a feature unique in the data, and the remaining sites had variable proportions of admixture, with preponderance of the third cluster genome.

In order to detect possible substructuring within CT1D, CT9 and LUG49, we ran STRUCTURE separately for these three sites. For CT1D and LUG49 the optimal number of clusters was 1, indicating no substructure within these sites. However, for CT9 the optimal number of clusters was 3. Most insects collected in this place had more than 70% of the genome of one of the three groups, but

Table 3
Genetic and geographic distances between pairs of study sites. Below the diagonal: pairwise F_{ST}s. Above the diagonal: geographic distances in meters. Bold: significant values after Bonferroni correction.

	CT9	CT26	CT7	CT19	CT14D	CT14P	CT5	CT1P	CT1D	CT22	CT20	CT8	C16	CT3	LUG49	LUG48
CT9		1507	1699	1406	1135	1136	2757	1811	1809	1943	1897	643	908	2549	4134	4386
CT26	**0.199**		2952	1639	494	493	4084	3105	3105	1140	2009	2144	998	4000	5488	5600
CT7	**0.090**	**0.086**		3047	2467	2468	1136	178	180	3628	3509	1237	2603	1274	2540	2695
CT19	**0.065**	**0.114**	**0.077**		1690	1690	4007	3131	3128	1071	498	1828	747	3633	5313	5679
CT14D	**0.087**	**0.136**	**0.072**	0.061		1	3601	2623	2623	1495	2134	1741	952	3548	5006	5107
CT14P	**0.105**	0.056	0.053	0.033	0.069		3602	2624	2624	1494	2134	1742	952	3549	5007	5108
CT5	**0.134**	**0.071**	**0.088**	0.054	0.050	0.014		979	979	4700	4431	2191	3662	740	1405	1680
CT1P	**0.157**	**0.035**	**0.078**	0.086	0.092	0.014	0.037		5	3750	3584	1306	2719	1099	2383	2575
CT1D	**0.060**	**0.095**	**0.046**	−0.010	0.027	−0.001	0.021	0.025		3748	3581	1303	2717	1096	2384	2577
CT22	**0.106**	**0.151**	**0.101**	**0.061**	**0.074**	**0.074**	**0.083**	**0.114**	0.031		1171	2538	1040	4448	6072	6322
CT20	**0.053**	**0.135**	**0.077**	0.028	0.049	0.083	0.098	0.095	0.012	0.038		2278	1218	4012	5705	6109
CT8	**0.101**	**0.051**	0.024	0.019	0.023	−0.024	−0.022	−0.011	−0.032	0.051	0.048		1500	1917	3538	3854
CT16	**0.051**	**0.107**	0.047	0.001	0.022	0.031	0.039	0.053	−0.030	−0.002	−0.014	−0.013		3415	5032	5293
CT3	**0.113**	**0.120**	0.073	0.079	0.030	0.039	0.008	0.057	0.018	**0.062**	0.088	−0.005	0.025		1698	2243
LUG49	**0.132**	**0.177**	**0.111**	0.056	**0.108**	**0.075**	**0.058**	**0.124**	0.048	**0.109**	**0.097**	0.032	0.057	0.102		887
LUG48	**0.147**	**0.178**	**0.078**	**0.079**	**0.079**	**0.088**	0.076	0.169	0.045	**0.057**	**0.091**	0.039	0.025	0.063	**0.083**	

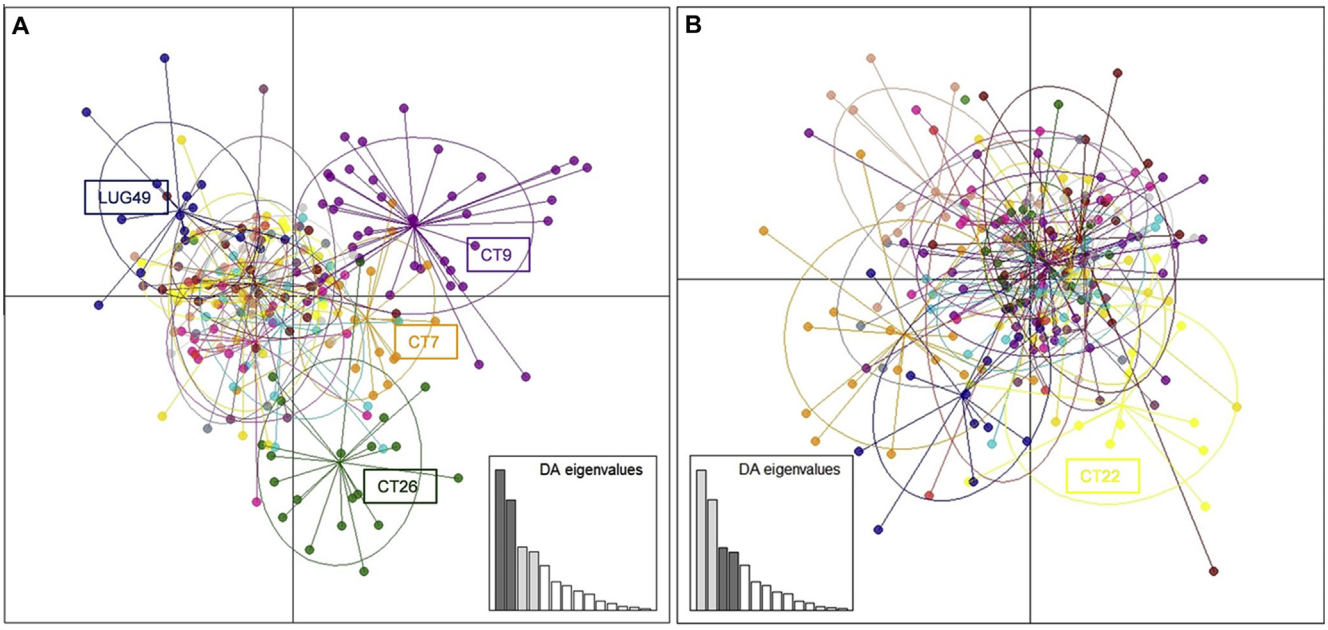

Fig. 3. Scatterplots of DAPC based on collection sites. These scatterplots show (A) the first two principal components of the DAPC, (B) the third and fourth principal components of the DAPC. Sites are shown by different colors and inertia ellipses, whereas dots represent individuals. Sites that were differentiated from the rest are shown with a legend.

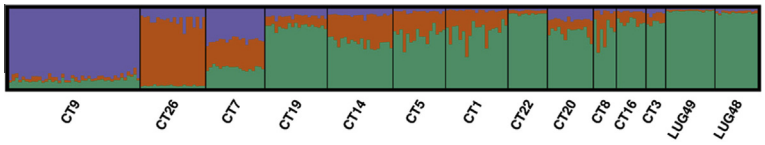

Fig. 4. Population structure inferred with STRUCTURE. Each bar represents an individual and each color the proportion of its genome assigned to each cluster. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Frequencies of Geneland clusters across 20 independent runs.

Clusters	Frequency alone (%)	Grouped with others (%)	Site(s)
CT9	100	0	
CT22	100	0	
CT26	90	10	CT14P, CT14D
CT3, CT5	80	20	CT14P, CT14D
CT1D, CT1P, CT7, CT8	75	25	CT14P, CT14D
CT16, CT19	75	20	CT20
		5	CT14P, CT14D
CT20	75	20	CT16, CT19
		5	CT14P, CT14D
LUG48	35	65	LUG49
LUG49	35	65	LUG48
CT14P, CT14D	40	10	CT26
		20	CT3, 5
		25	CT1D, CT1P, CT7, CT8
		5	CT16, 19

there were also some individuals with high degrees of admixture (Fig. S6).

3.3.4. Geneland

The modal number of populations was consistent across different runs and equal to 9, but there was some heterogeneity in the clustering of the sites. Sites CT9 and CT22 were always recovered as separated populations and site CT26 in all but one run (Table 4). Sites CT1D, CT1P, CT7 and CT8, CT3 and CT5, and CT16 and CT19 were always clustered together, mostly alone and sometimes with CT14D and P or CT20. LUG48 and LUG49 were clustered together or in independent groups (Table 4).

4. Discussion

4.1. Variability and HWE

This study, unlike previous ones, analyzes the patterns of fine-scale genetic structure of *T. infestans* in an area of the Gran Chaco with more than 10 years since the last vector control intervention. General levels of variability were lower for this area than in a previous study using the same markers in the Arid Chaco of Santiago del Estero (Marcet et al., 2008). This result agrees with surveys based on mitochondrial DNA (Ceballos et al., 2011; Piccinali et al., 2009), and suggests that populations of *T. infestans* from northern Argentina (with the exception of sylvatic dark morphs) harbor lower variability than in other areas.

Our results indicate that in this study area, sites generally behave as demes, in agreement with the proposal of Brenière et al. (2013) of considering a group of insects collected in the same site or in very closely located sites (a few meters) at the same time as a population, both for *T. infestans* and other triatomines. However, in some cases, panmictic units were higher than one site (see below) suggesting that the concept of "one site, one population" could lead to an overestimation of the number of *T. infestans* populations. This pattern is different from those reported in the Arid Chaco where 43% of the surveyed sites in Santiago del Estero (Marcet et al., 2008) and 100% of the surveyed sites in

Catamarca (Pérez de Rosas et al., 2013) were not found in HWE mainly due to the presence of substructuring within sites.

Excesses of homozygotes in neutral markers as microsatellites could be the result of different factors including null alleles, inbreeding and substructuring. We tried to distinguish among these three possibilities to explain the departures in HWE found at CT1 D, CT9 and LUG49. When pedigrees from individuals are not available, inbreeding is often estimated using relatedness measures (Liautard and Sundström, 2005). The relatedness between two individuals can be interpreted as the expected fraction of alleles that are shared identical by descent (Blouin, 2003). For example, for monozygotic twins, r or the coefficient of relatedness is expected to be one and for unrelated individuals zero. We found that most sites had average values of r between individuals not different from zero. CT9 was one exception with an r equal to 0.23 (close to 0.25 as for half-sibs, for example). However, other sites in HWE had similar r values. An extra argument supporting this point is that inbreeding produces excess of homozygotes in all loci and not in a few in particular as in this case (Hedrick, 2011).

The presence of null alleles can lead to an underestimation of heterozygosity because heterozygous individuals for a null allele are wrongly computed as homozygous. The analysis performed with MICROCHECKER suggested that null alleles in different loci could be present in sites in non HWE. We did not detect individuals with negative PCR amplifications for loci Tims22, Tims27 and Tims56. However, 77% of the 9 PCR failures were found at loci Tims19 and Tims65. These two loci also showed departures from HWE in several populations studied in Santiago del Estero (Marcet et al., 2008) and in sylvatic and peridomestic *T. infestans* from Chaco (Piccinali et al., 2011). The evidence thus agrees with the presence of null alleles for Tims19 causing departures from HWE in CT9 and LUG49 and in Tims65 in CT1 D, respectively.

Substructuring can produce an apparent excess of homozygotes when more than one differenced population is pooled, a pattern known as Wahlund effect. The STRUCTURE analyses did not support this hypothesis in the case of CT1 D and LUG48 because only one genetic cluster was present in these sites. However, three different genetic groups were found for CT9. This pattern was also recovered in the find.cluster function-based DAPC, where individuals from CT9 were assigned to different groups. Interestingly, this site also showed some heterogeneity for adult wing shape in insects collected after insecticide spraying (Gaspe et al., 2013). This pattern is surprising because CT9 was a rather isolated site surrounded by patches of forest, and was not connected to any other house by dirt roads. Nevertheless, its genetic heterogeneity suggests the immigration of insects with three different genome compositions that were not found in other neighboring sites. In summary, null alelles in CT1 D and LUG48 and null alleles combined with substructuring in CT9 apparently underlie the departures from HWE in these sites.

4.2. Domestic versus peridomestic variability

Domiciles were genetically more variable than peridomestic sites including kitchens, storerooms and chicken coops. Possible explanations for such pattern are the presence of older bug populations in domestic sites, or higher effective population sizes. This outcome seems to contradict the results of Gurevitz et al. (2011) who reported higher bug abundance in chicken nests (“nideros”), storerooms and kitchens in the same study area. However, because some peridomestic sites are easier to inspect than domiciles (because they are smaller and less labor-intensive), domestic bug abundance could be underestimated. Another possibility is that peridomestic populations, although reaching larger populations sizes, were descendents from fewer founder individuals or were more exposed to population bottlenecks due to a less stable environment than domestic habitats. In addition, domiciles could be more prone to receive bugs from different sources (Gürtler et al., 2014) attracted by artificial lights (Minoli and Lazzari, 2006), which could increase the levels of genetic variability.

4.3. Population genetic structure

We were able to detect significant genetic structure in a rural area of only 6.32 km^2 in the Argentine Chaco undisturbed by massive insecticide spraying for the last 12 years. We found genetic differentiation between sites separated by 180–6000 m, at a lower geographic scale than in a previous study (>4000 m) based on wing geometric morphometry (Gaspe et al., 2012). All the analyses performed were highly consistent and agreed in the presence of several different genetic groups. Analyses based on the hypothesis that each site represented a deme, like the DAPC applied to sites and the pairwise F_{ST}s, showed that at least five sites could be considered as different populations: CT9, CT7, CT22, CT26 and LUG49 (Table 3 and Fig. 3). However, individual-based analyses performed with STRUCTURE found only three genetic clusters, one predominant in CT9 individuals, other in CT26 individuals and the third in the rest of the individuals. One exception to this pattern was the bugs from CT7, which showed similar proportions of each genetic cluster. One possible explanation for such a discrepancy is that, under different models of migration, the algorithm underlying STRUCTURE allows the detection of the uppermost hierarchical level of structuring (Evanno et al., 2005).

When spatial coordinates were included, a higher resolution of the genetic structure was achieved. Sites non differentiated in previous analyses were recovered in different populations, like CT3 and CT5 and CT16 and CT19 (Table 4). Others were grouped with other sites like CT8 and CT1D and P with CT7. Interestingly, all the spatial genetic groups included sites separated by no more than 1300 m, a value within the 1500–2000 m limit for active flight dispersal estimated for *T. infestans* (Schweigmann et al., 1988).

Spatial information appeared to be quite relevant to detect population structure, using sites as “a priori” populations or through spatially explicit models. When such information was not used, patterns of structuring were hard to interpret (DAPC using clusters) or absent (STRUCTURE without collection sites as prior). This finding shows one important aspect (and a possible limitation) of genetic data to detect fine-scale population patterns. When the degree of differentiation between houses is low, genetic data cannot “speak” by themselves, at least when a limited number of hypervariable nuclear markers as those currently available for *T. infestans* are used. The origin of the bugs (collection sites) and distances among houses are key pieces of information that need to be collected. This is particularly important for areas under no regular vector control and/or recent insecticide spraying campaigns and where insecticides do not increase the degree of differentiation between houses. Pérez de Rosas et al. (2007) found that the genetic structure of *T. infestans* populations was profoundly affected by the application of insecticide in the area and by the time elapsed since the treatment. Localities which had been sprayed with insecticides 1–5 years ago had F_{ST} values 3–17 times higher than localities treated 9–20 years ago or never, making more easy the detection of genetic structure by most population genetic methods.

Although we found a correlation between geographic and genetic distances, the percentage of variation explained by the model is too low to consider the IBD as a proper model to describe the spatial structuring of *T. infestans* populations in this area, in contraposition with a previous fine-scale study in the southern Arid Chaco (Pérez de Rosas et al., 2013). Taking all our results together, a hierarchical island model with stratified migration seems to better describe the patterns of genetic variability found in this area, with the groups detected by STRUCTURE as the three

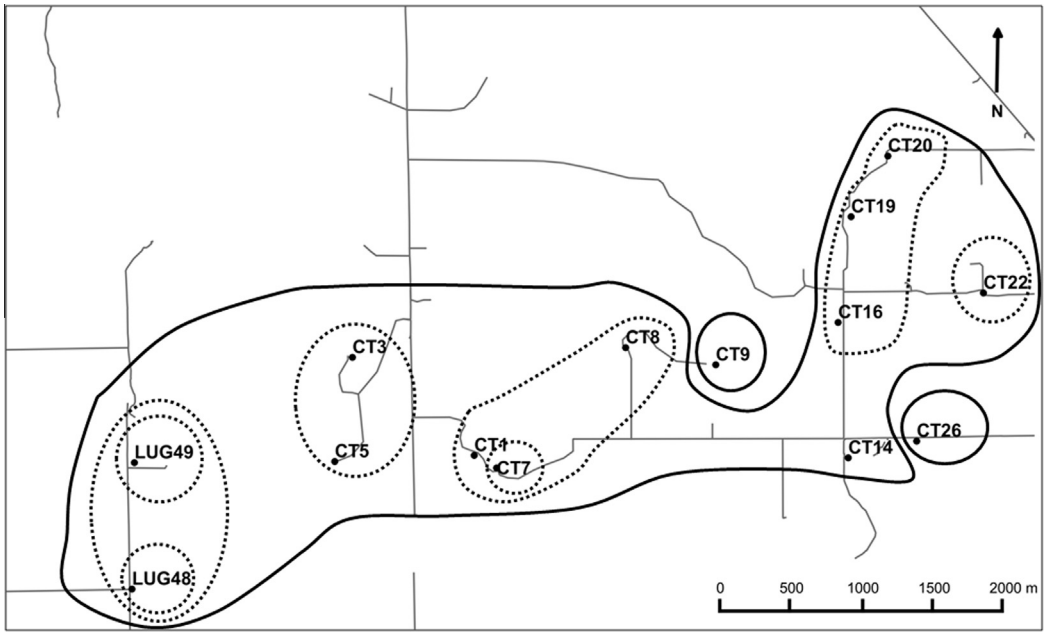

Fig. 5. Map showing a consensus of the genetic structure recovered by all methods. Solid lines: main groups recovered by STRUCTURE. Dotted lines: groups recovered by DAPC, Geneland and F_{ST}s.

main populations and the groups detected by other methods like Geneland and DAPC as the subpopulations. A consensus of the results of all the methods is shown in Fig. 5.

Regarding the utility of this genetic structure to determine the origin of the insects collected after insecticide applications, our findings showed that the presence of residual foci in the area (Gaspe et al., 2013; Gurevitz et al., 2012, 2013) probably can be tested in those houses where bugs were considerably differentiated from the remaining ones (e.g., CT9, CT26 and CT7). Migration events can also be more easily detected between close and highly differentiated sites such as between CT8 and CT9 and between CT1 and CT7.

Our study has some limitations. The low number of hypervariable nuclear markers isolated for *T. infestans* as well as the low number of individuals available for genotyping in some sites may yield inaccurate estimates of genetic variability and low power to detect departures from HWE in those sites. In addition, the degree of genetic structure in the area could be underestimated.

5. Conclusion

Our work illustrates that fine-scale genetic structure patterns of *T. infestans* can be detected even in villages under no systematic vector surveillance and without recent insecticide spraying and that microsatellites can perform as very sensitive markers at fine spatial scales. These data provide the cornerstone for future hypothesis testing of the origin of domestic and peridomestic bugs collected after insecticide spraying in the same area, and will offer valuable information on the process of reinfestation in the transition between the Arid and Humid Argentine Chaco.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2015.05.030.

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