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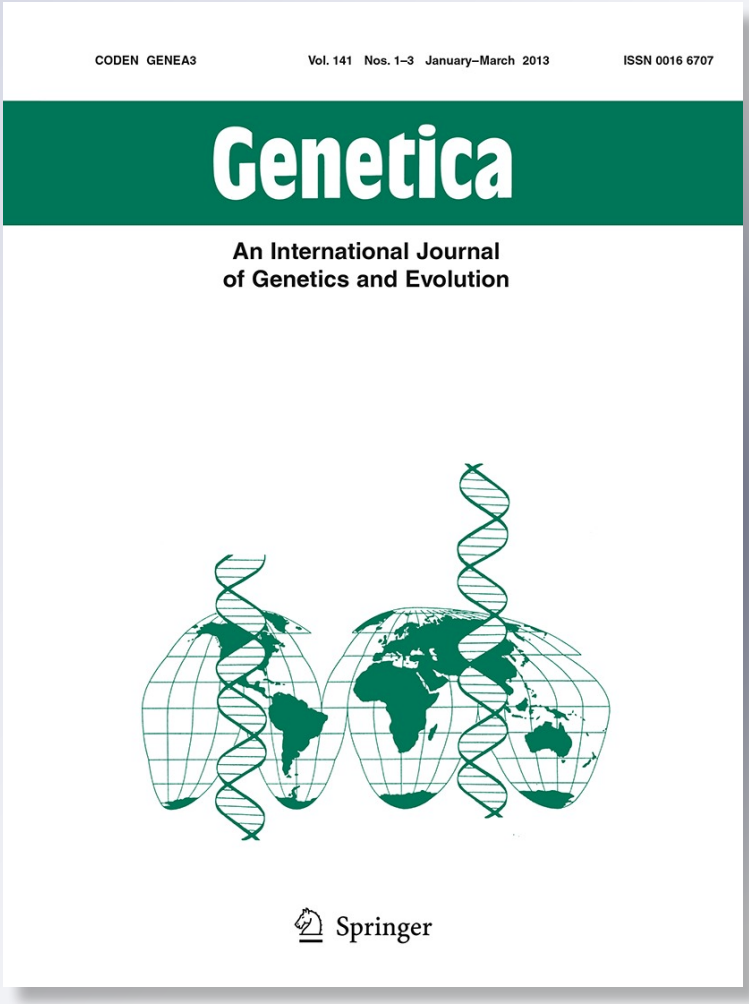

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Fine-scale genetic structure in populations of the Chagas' disease vector *Triatoma infestans* (Hemiptera, Reduvidae)

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Abstract Fine scale patterns of genetic structure and dispersal in *Triatoma infestans* populations from Argentina was analysed. A total of 314 insects from 22 domestic and peridomestic sites from the locality of San Martín (Capayán department, Catamarca province) were typed for 10 polymorphic microsatellite loci. The results confirm subdivision of *T. infestans* populations with restricted dispersal among sampling sites and suggest inbreeding and/or stratification within the different domestic and peridomestic structures. Spatial correlation analysis showed that the scale of structuring is approximately of 400 m, indicating that active dispersal would occur within this distance range. It was detected difference in scale of structuring among sexes, with females dispersing over greater distances than males. This study suggests that insecticide treatment and surveillance should be extended within a radius of 400 m around the infested area, which would help to reduce the probability of reinfestation by covering an area of active dispersal. The inferences made from fine-scale spatial genetic structure analyses of *T. infestans* populations has demonstrated to be important for community-wide control programs, providing a complementary approach to help improve vector control strategies.

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Introduction

Chagas' disease (American trypanosomiasis) is produced by infection with *Trypanosoma cruzi*, parasite transmitted by hematophagous insects of the subfamily Triatominae (Hemiptera: Reduviidae). The disease is a serious public health problem in Latin America, with about 8 million persons infected and around 109 million living in endemic areas (Rassi et al. 2010). *Triatoma infestans* is the most important and widespread vector of Chagas' disease in South America, where has been the target of control programs as part of the Southern Cone Initiative. As a result of the initiative, Chile (Lorca et al. 2001), Brazil (Silveira and Vinhaes 1999), and Uruguay (World Health Organization 1994) have been declared free of Chagas' disease transmission by *T. infestans*. However, vector control has proven to be difficult, in part as a consequence of the variability and extension of endemic areas, and because of the difficulties to implement sustained entomological vigilance to prevent the recovery of treated bug populations. High levels of *T. infestans* reinfestation after spraying were observed in Argentina, Bolivia, and Paraguay (Gürtler et al. 2007). Reinfestation in areas treated with insecticides could be produced from individuals that survive treatment, most likely from peridomiciliary sites, or insect immigration from untreated areas. In agreement with the first hypothesis, Cecere et al. (2002, 2004) observed that peridomestic structures support more abundant populations of *T. infestans* than domestic sites and increase the risk of domiciliary reinfestation. On the other side, it was observed that subsequent infestations after insecticide spraying were

grouped around the initial peridomestic focus at a distance of approximately 400 m (Cecere et al. 2004). Based in these findings, the authors suggested that residual spraying with insecticide should be around 400 m of detected foci. In this regard, the genetic analysis of vector populations may be useful in entomological surveillance of Chagas vector control programs, since it may provide information on the source of the insects in reinfested areas.

The long-term effectiveness of the control campaigns is greatly dependent upon the vector population structure. In this respect, the inferences that can be made from fine-scale spatial genetic structure have significant relevance for control of vector populations; recent researches into spatial genetic structure at this scale (i.e., nonrandom spatial distribution of genetic variation) have proven to be useful to elucidate movement patterns (Peakall et al. 2003; Fredsted et al. 2005). In previous studies of genetic structure in *T. infestans* populations, using microsatellite markers, significant level of genetic differentiation among different localities was detected (Pérez de Rosas et al. 2007, 2008, 2011). These works as well as another studies, which considered different definitions of population (either localities, domestic or peridomestic sites), strongly supported the existence of some type of stratification in the population, e.g. subdivision into breeding units with restricted possibilities of genic exchange (Pérez de Rosas et al. 2007, 2008; Pizarro et al. 2008; Marcet et al. 2008). In this regard, we detected significant level of genetic differentiation among houses within two localities (Pérez de Rosas et al. 2007, 2008). Significant excess of homozygotes was observed within the different dwellings analysed, suggesting the existence of a higher degree of subdivision in the population; however, in these works the limited sample size did not allow to test stratification within each house. The previous studies were not able to determine the actual scale of genetic structure for *T. infestans* populations. Investigations on fine-scale genetic structure would help to understand this issue.

The combination of microsatellite markers and multivariate spatial autocorrelation methods such as the multilocus, multiallele method of Smouse and Peakall (1999) are very sensitive in detecting fine-scale genetic structure. Spatial genetic structure analysis allows to infer the spatial scale over which populations diverge genetically as well as the scale at which gene flow predominates. The characterization of spatial genetic structure has been commonly used for analyses of gene flow between populations (e.g., based in Wright's *F*-statistics), but less frequently applied within a single population. Spatial autocorrelation analysis can be performed at the individual level and has been used to examine spatial genetic structure and infer dispersal within a population, especially sex biased dispersal (Peakall et al. 2003; Double et al. 2005).

Here, we propose a multilocus approach with 10 microsatellite loci (García et al. 2004) to examine fine scale patterns of genetic structure and dispersal in *T. infestans*. We present a genetic study with sufficiently intensive sampling within a geographic area to characterize population structure and gene flow dynamics at inter-individual scales. This study appears to be the first microsatellite-based analysis in *T. infestans* at this scale.

Materials and methods

Sampling

New field collections were performed for this study. A total of 314 *T. infestans* specimens were captured in 22 peridomestic and/or domestic sites of 15 houses from the locality of San Martín (Capayán department, Catamarca province, Argentina) in October 2007 (Fig. 1; Table 1). All these houses received the last insecticide treatment 4 years before the sample collection date. Experienced collectors from the National Vector Control Program searched for *T. infestans* specimens in the different capture sites using a dislodging spray. All sites were treated with insecticide after insect collection. Exact geographical coordinates were recorded for each capture site (i.e., coops, corrals, store rooms, etc.) using a GPS. The distances between the different sampling sites ranged from 10 m to 20 km.

DNA isolation and polymerase chain reaction

The DNA was extracted from legs of each bug using a simplified protocol of a phenol–chloroform extraction procedure reported by Werman et al. (1990). The 10 primer pairs used for polymerase chain reactions (PCR) were previously designed and have shown to be very useful for population genetic analyses of *T. infestans* (García et al. 2004; Pérez de Rosas et al. 2007, 2008, 2011; Richer et al. 2007; Pizarro et al. 2008). The forward primer from each primer set was 5′-fluorescent labelled with one of three dyes, 6-FAM, HEX, or NED (Applied Biosystems). PCR amplifications were carried out in a Hybaid thermal cycler (Omnigene) in 25 μl of a solution containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, each dNTP at 200 μM, each primer at 1 μM, genomic DNA (10–50 ng), and 1 U of Amplitaq Gold (Perkin-Elmer Cetus). Thermal profiles consisted of an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of 20 s at 94 °C (denaturation), 30 s at 55 °C (annealing), and 40 s at 72 °C (extension), with a final extension step of 15 min at 72 °C. Reaction products were visualized after electrophoresis on a 3 % agarose gel (MetaPhor). Size of the PCR products was estimated relative to an internal standard (GeneScan-500

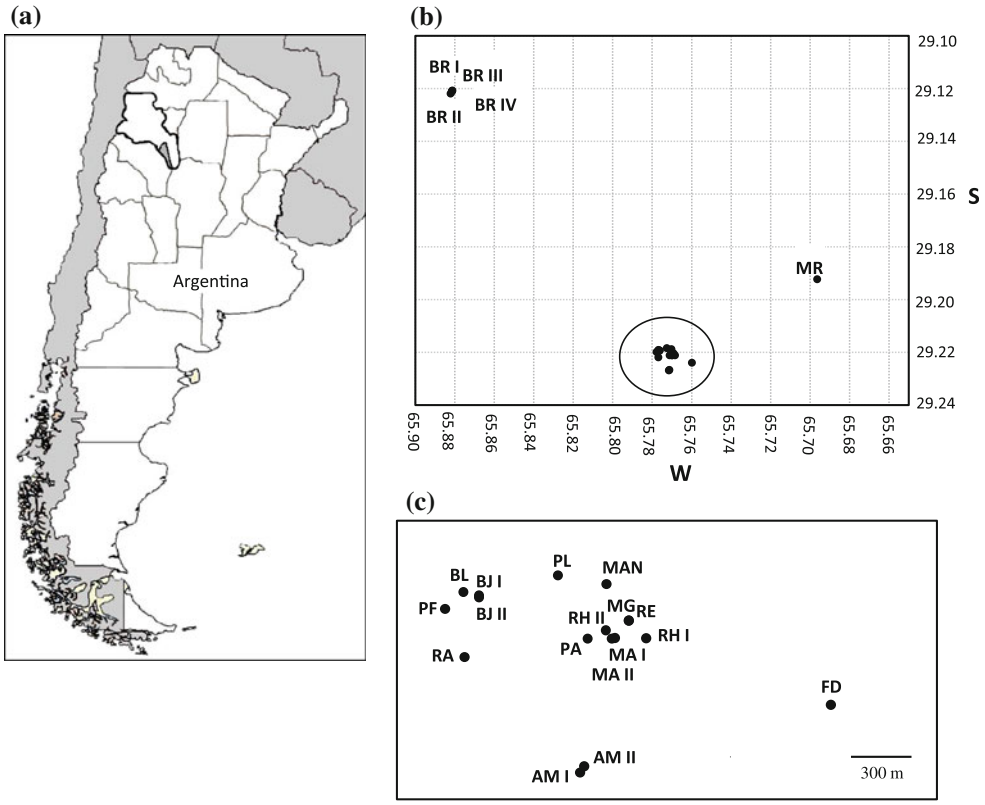

Fig. 1 Map of the area studied. **a** Location of Capayan department (Catamarca province, Argentina); **b** collection sites in the locality of San Martín (longitude and latitude are indicated *below* and to the *right*, respectively); and **c** detail of the area enclosed in a *circle* in **b**) (for more detail, see Table 1)

Table 1 Sampling site and sample size of *T. infestans* from the locality of San Martín (Capayán department, Catamarca province)

House	Capture site	Habitat	Sample size
AM	AM I	Goat corral	42
	AM II	Chiken coop	16
BJ	BJ I	Stable	6
	BJ II	Chiken coop	16
BR	BR I	Domicile	14
	BR II	Goat corral	4
	BR III	Shed	23
	BR IV	Chiken coop	34
BL	BL	Goat corral	4
FD	FD	Chiken coop	4
MA	MA I	Rabbit hutch	10
	MA II	Domicile	5
MAN	MAN	Stable	4
MG	MG	Domicile	23
MR	MR	Chicken coop	4
PF	PF	Goat corral	4
PA	PA	Shed	4
PL	PL	Tree	34
RE	RE	Goat corral	4
RH	RH I	Goat corral	18
	RH II	Shed	14
RA	RA	Tree	27

Numbers indicate different habitats from one dwelling

ROX) in polyacrylamide gels using an ABI PRISM 377 automated DNA sequencer, GENESCAN 3.1 and GENOTYPER 2.5 softwares (all from Perkin-Elmer Applied Biosystems).

Genetic diversity and population level genetic structure

Allelic richness (number of alleles corrected for sample size), and observed (H_O) and expected (H_E) heterozygosities were assessed at each locus and sampling site. H_E was calculated using the unbiased estimate described by Nei (1987). Linkage disequilibrium was tested between all pairs of loci and for all sites using the program FSTAT version 2.9.3 (Goudet 2001). Genotypic disequilibrium was not apparent for any pair of loci according to a global test for each of the 45 different pairs of loci across all sampling sites based on 900 permutations. Therefore, being linkage between loci unlikely, we proceeded under the assumption of statistical independence between loci.

Departures from Hardy–Weinberg (HW) equilibrium were tested by the inbreeding coefficient F_{IS} (Wright 1951) using the estimator f of Weir and Cockerham (1984). The degree of differentiation between and across all collection sites was determined using Weir and Cockerham (1984) estimator (θ) of Wright's F_{ST}. Single and multilocus f, as well as global and pairwise comparisons of θ were calculated by the program FSTAT version 2.9.3 (Goudet 2001).

The significance of all θ and f estimates was tested using permutation. Standard errors of θ were calculated by jackknifing over populations and loci, and a 95 % confidence interval was generated by bootstrapping over loci. Significance of global θ estimate was further evaluated with an exact G-test after 1,000 randomizations of allele among sites (Goudet et al. 1996). Isolation by distance was examined by plotting the pairwise θ values against the geographical distances (as straight-line distance between all pairs of sites) and was tested using the Mantel test with permutations as implemented in FSTAT version 2.9.3 (Goudet 2001). Hierarchical analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was perfomed using ARLEQUIN 3.01 (Excoffier et al. 2005).

In order to examine any sex differences in dispersal, the program SPAGeDi version 1.2 (Hardy and Vekemans 2002) was used to determine mean values of Kinship coefficient (defined as the probability of identity by descent of the genes compared) for pairwise comparisons among males and among females within each sampling site. In all cases, it was used the Loiselle et al. (1995) estimator of Kinship coefficient, which is especially suitable in cases when low frequency alleles are present. The significance of Kinship coefficient estimates was tested using permutation.

Individual assignment test

A Bayesian approach implemented in the program STRUCTURE version 2 (Pritchard et al. 2000) was used to infer the number of populations (K) in the data set without prior information of the sampling locations. Considering that this study was carried out on a small geographical scale, the admixture model (in which each individual is assumed to have inherited some proportion of its ancestry from each population) and correlated allele frequencies were assumed. To choose an appropriate K value for data analysis, a series of five independent runs for each value of K between 1 and 13 were conducted. In each run, a burn-in period of 100,000 and a 1,000,000 run length were performed.

An assignment analysis was performed to identify possible first-generation migrants as implemented by the program GENECLASS2 (Piry et al. 2004). The program uses multilocus genotypes to identify putative immigrants within each population and the most likely source of these immigrants. The marginal probability of given individual multilocus genotype was compared with the distribution of marginal probabilities of randomly generated multilocus genotypes (10,000 replicates), when the P value was <0.01 the individual was considered not belonging to the population. First-generation migrant test was carried out using Rannala and Mountain (1997) Bayesian individual assignment method and the Monte Carlo resampling method of Paetkau et al. (2004).

Individual spatial autocorrelation analyses

Spatial genetic structure was assessed using an approach to microspatial autocorrelation analysis for multiallelic co-dominat loci such as microsatellites (Smouse and Peakall 1999) implemented in the program GENALEX version 6.2 (Peakall and Smouse 2006). This software calculates the multilocus autocorrelation coefficient r among individual genotypes based on pairwise geographic and genetic distances. The autocorrelation coefficient r is estimated for a specified number of distance classes, and provides a measure of the genetic similarity between pairs of individuals falling within each distance class.

All individuals resident on the same collection site were given identical coordinates. Each distance class must contain enough pairwise comparisons to have sufficient statistical power and the number of pairs should be equal in the distance classes to avoid noise in the confidence limits that can cause unequal sample size (Peakall and Smouse 2006). Therefore, distance classes were selected to make the number of pairwise comparisons in each bin as even as possible; for example the first four distance classes were: (1) class 0–50 m, (2) class 51–300 m, (3) class 301–500 m, (4) class 501–700 m etc. This was necessary because our sampling scheme was not homogeneous in space, but rather concentrated around sampling locations; thus, several distance classes would have no observations otherwise. Spatial genetic autocorrelograms were produced by plotting the calculated r as a function of distance. To test whether the r correlation coefficient was significantly different from the null hypothesis of no spatial genetic structure, we performed 1,000 random permutations to determine upper and lower confidence intervals for the null hypothesis (Peakall et al. 2003).

Results

A total of 314 insects from 22 domestic or peridomestic sites of 15 houses were typed for 10 polymorphic microsatellite loci (Table 1). In 10 of these sampled sites <10 insects were captured; these samples were included for individual-level analyses such as autocorrelation and individual assignment test, but were excluded for the analyses of genetic diversity and population level genetic structure because they depend on sample size.

Genetic diversity and population level genetic structure

A total of 258 alleles at 10 microsatellite loci were detected across the 271 individuals captured from 12 sampled locations in which the sample size ranged from 10 to 42 (Table 1). All loci were polymorphic with the number of

Table 2 Summary of genetic variation at 10 microsatellite loci at 12 collection sites for *Triatoma infestans*

Capture site	A_R^a	H_O^b	H_E^c	F_{IS}^d
AM I	4.36	0.583	0.855	0.321***
AM II	4.06	0.600	0.812	0.267***
BJ II	3.55	0.563	0.724	0.229***
BR I	4.18	0.600	0.817	0.273***
BR III	3.90	0.558	0.784	0.292***
BR IV	4.14	0.619	0.827	0.254***
MA I	3.68	0.570	0.767	0.268***
MG	4.16	0.698	0.829	0.162***
PL	3.99	0.549	0.801	0.318***
RH I	4.56	0.623	0.877	0.298***
RH II	3.77	0.678	0.791	0.148***
RA	3.61	0.513	0.739	0.310***

The significance of all heterozygote deficits was tested using permutation: *** $P < 0.001$

[a] Allelic richness

[b] Observed heterozygosity

[c] Unbiased expected heterozygosity

[d] Inbreeding coefficient

distinct alleles per locus ranging from 17 (locus TiC08) to 46 (locus TiG03). The 10 microsatellite loci examined were polymorphic within the 12 sampled sites. Each locus presented exclusive alleles in one of the sites. These private alleles were found in all sampling locations, except in BR I, and suggest limited current levels of genetic exchange.

Relatively high genetic diversity levels were detected in the 12 examined sites (Table 2). The mean allelic richness value (A_R) per population ranged from 3.55 in BJ II to 4.56 in RH I. The average observed heterozygosities (H_O) per sampling site ranged from 0.513 to 0.698 in the samples from RA and MG, respectively. The average expected heterozygosities (H_E) per sampling site ranged from 0.724 to 0.877, with the lowest values in the sample from BJ II and the highest values in the sample from RH I.

Significant departures from Hardy–Weinberg equilibrium were observed in some of the 10 microsatellite loci in the 12 samples analysed. Deviations from Hardy–Weinberg expectations were associated with positive F_{IS} indicating excess of homozygotes. Multilocus F_{IS} analyses revealed a significant multilocus heterozygote deficiency in all sampling sites ($P < 0.001$) (Table 2).

Levels of genetic differentiation among the 12 sampled locations were examined using overall and pairwise comparisons of multilocus F_{ST} (using the estimator θ; Table 3). The overall θ value of 0.086 is significantly different from zero ($P = 0.001$), as are all pairwise θ values ($P < 0.05$, $P < 0.01$, and $P < 0.001$ with FSTAT permutation procedure); θ values ranged from 0.014 between the samples of BJ II and RA to 0.154 for the comparison between the populations of BR I and RA.

There was a significant association between geographical distance and genetic differentiation (θ) among sites (Mantel $r = 0.25$, $P = 0.013$). However, the scatterplot does not show a consistent pattern with a isolation by distance model due to the lack of pairwise comparisons between populations separated by 1 km up to 15 km. Analyses performed with populations separated by <1 km indicated a significant association between geographical distance and genetic differentiation (Mantel $r = 0.35$, $P = 0.014$) (Fig. 2a). In this case, the scatterplot shows moderate level of divergence with little scatter of the pairwise points among populations separated up to 400 m of distance and greater degree of divergence and more scatter over longer geographic distances of separation.

Table 3 Pairwise matrix of genetic differentiation (θ, upper diagonal) and geographical distance (in km, lower diagonal)

	AM I	AM II	BJ II	BR I	BR III	BR IV	MA I	MG	PL	RH I	RH II	RA
AM I	0	0.032	0.102	*0.025*	0.057	0.038	0.110	0.074	0.058	0.101	0.042	0.124
AM II	0.035	0	0.130	0.036	0.077	0.067	0.132	0.107	0.066	0.127	0.076	0.143
BJ II	0.954	0.937	0	0.142	0.118	0.112	0.092	0.035	0.132	0.087	0.077	0.014
BR I	15.890	15.880	14.970	0	0.042	0.048	0.145	0.104	0.050	0.130	0.077	0.154
BR III	15.870	15.860	14.950	0.022	0	0.053	0.135	0.098	0.089	0.133	0.078	0.134
BR IV	15.890	15.890	14.970	0.024	0.039	0	0.115	0.078	0.076	0.111	0.044	0.128
MA I	0.657	0.624	0.629	15.520	15.510	15.530	0	0.058	0.153	**0.045**	**0.066**	**0.098**
MG	0.761	0.727	0.683	15.520	15.490	15.520	0.116	0	0.113	*0.059*	0.042	0.039
PL	0.950	0.923	0.370	15.140	15.120	15.140	0.389	0.386	0	0.142	0.084	0.149
RH I	0.691	0.659	0.591	15.480	15.450	15.480	0.048	0.114	0.340	0	0.041	0.085
RH II	0.709	0.674	0.776	15.630	15.610	15.630	0.154	0.115	0.498	0.185	0	0.092
RA	0.759	0.751	0.293	15.130	15.110	15.140	0.668	0.759	0.432	0.821	0.647	0

Italic values are significant at the 5 % level. Bold values are significant at the 1 % level. All other estimates of θ are significant at the 0.1 % level

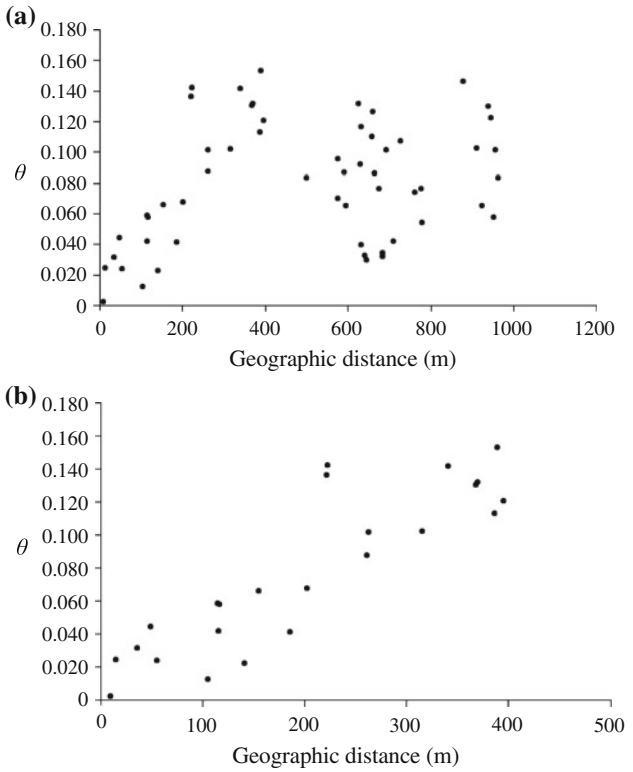

Fig. 2 Isolation by distance among sampling sites. Pairwise estimates of F_{ST} (θ) are plotted against the corresponding straight-line geographical distances between sites. **a** Sampling site pairs at distances ranging from 0 to 1000 m (Mantel r = 0.35, $P = 0.014$). **b** Sampling site pairs at distances ranging from 0 to 400 m (Mantel r = 0.81, $P = 0.0005$)

When the populations separated by more than 400 m were excluded, a higher significant association was detected (Mantel r = 0.81, $P = 0.0005$) (Fig. 2b).

The mean values of Kinship coefficient (defined as the probability of identity by descent of the genes compared) for pairwise comparisons among males and among females were calculated from the total of individuals captured in seven of the houses examined (AM, BJ, BR, MG, PL, RA, and RH), where the number of males and females collected was enough to analyse them independently. These mean values ranged from 0.197 to 0.426 for males and from 0.028 to 0.311 for females. All mean values of Kinship coefficient estimated for each of the seven houses analysed were significant, except in the female sample from MG (Kinship coefficient = 0.028, $P = 0.552$); however, in all cases the females presented lower values than the males.

Individual assignment test

Using the program STRUCTURE version 2 (Pritchard et al. 2000), three different genetic clusters could be identified from the 22 sampled locations examined (Fig. 3). Thirteen of these sampled locations correspond to one almost homogeneous cluster. Samples from the house AM (AM I and AM II) form a separate cluster with bugs collected in the distant house BR (BR I, BR II, BR III, and BR IV). The sample from PL also forms a distinct, nearly homogeneous cluster. The majority of the different sites analysed in each house belonged to the same cluster. On the contrary, the individuals from RH I were found to consist of a mixture of all the three different clusters and the individuals from PF correspond to two distinct clusters.

The groups of individuals (genetic clusters) detected by Bayesian analysis were used in the hierarchical analysis of molecular variance (AMOVA). All variance components tested by AMOVA (among cluster, among houses/sampling sites within clusters and among individuals within houses/sampling sites) were significantly different from zero, indicating considerable components partitioning of genetic variation (Table 4). In agreement with the significant positive F_{IS} values obtained, the highest variance component was detected at intra-house and intra-sampling site levels (95.57 and 91.34 % of the total variance, respectively).

The first-generation migrant test identifies immigrants within each population and the most likely source of these immigrants. A total of 30 putative immigrants were identified originating from 15 of the sampled sites and migrating to 17 of the sites. Two out of the 15 sites, AM I and BR I, were the source of the highest number of the immigrants detected (10 putative immigrants). The sampling sites AM I and PL received more immigrants than all other sites (5 and 4 putative immigrants, respectively).

Individual spatial autocorrelation analyses

Spatial autocorrelation analysis for the 314 individuals from 22 sampling sites indicated that significantly positive autocorrelations were found in the first (50 m, r = 0.081, 95 % CI 0.085, 0.078) and second distance classes (300 m, r = 0.039, 95 % CI 0.043, 0.034), with an x-intercept at 405 m (Fig. 4a). Similar results were obtained when the analysis was restricted to the 115 females captured (50 m, r = 0.102, 95 % CI 0.112, 0.091; 300 m, r = 0.047, 95 % CI 0.060, 0.034), with positive r values up to 402 m (Fig. 4b). When the analysis was restricted to males, spatial autocorrelation analysis of the 136 individuals showed significantly positive *r* values only in the first distance class (50 m, r = 0.107, 95 % CI 0.117, 0.097), with an x-intercept at 332 m (Fig. 4c). The correlation at the shortest distance class (≤50 m) was high and similar in females and males, but at the second distance class males showed significantly lower r values (r = 0.006, 95 % CI 0.016, −0.003) than females.

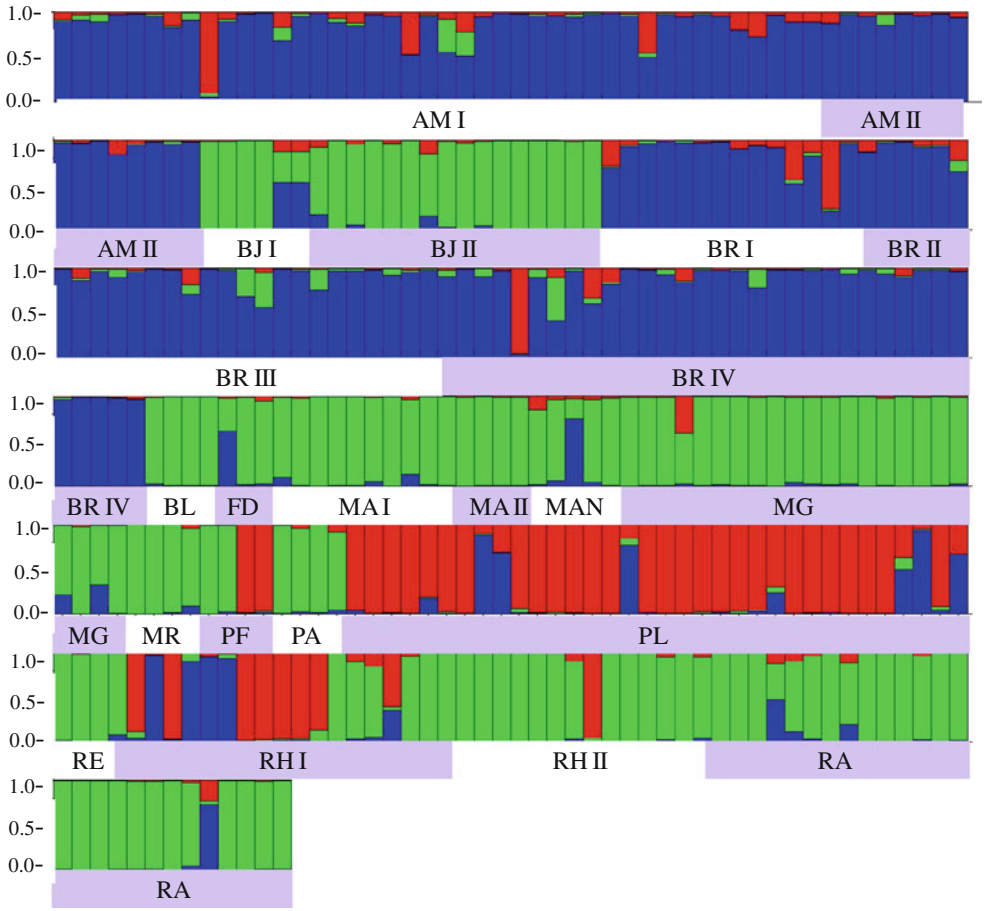

Fig. 3 Results of STRUCTURE analyses for the sampled locations from San Martín (Capayán department, Catamarca province). A model of K = 3 was most supported. Each column represents an individual where cluster membership assignment is on the y-axis

Table 4 Results of hierarchical analysis of molecular variance (AMOVA) at 10 microsatellite loci

Source of variation	Degree of freedom	Percentage of variation	*P* value
Among clusters	2	1.99	<0.050
Among houses within clusters	4	2.45	<0.001
Within houses	501	95.57	<0.000
Among clusters	2	4.33	<0.001
Among sampling sites within clusters	9	4.32	<0.000
Within sampling sites*	488	91.34	<0.000

Significance levels based on 10,000 permutations

* Domestic or peridomestic sites

Discussion

Significant levels of genetic differentiation were detected among all collection sites, including the different sampled sites within the same house (Table 3). These results confirm a high degree of subdivision in populations of *T. infestans* (Pérez de Rosas et al. 2007, 2008; Marcet et al. 2008; Pizarro et al. 2008). Moreover, significant excess of homozygotes within domestic and peridomestic sites was detected (Table 2). The existence of null alleles (alleles that are never amplified because of nonmatching primer sequences) usually leads to an underestimation of heterozygotes for the locus affected. They can be suspected for microsatellite loci, especially when using heterologous primers. The primers used in this study were specifically defined for *T. infestans* (García et al. 2004); however, some amplifications were unsuccessful after three attempts. Although this occurred only in a few cases out of 3,410 amplifications, the existence of null alleles cannot be ruled out. Another explanation for the heterozygote deficiency observed is the existence of inbreeding and/or a high degree of subdivision in the population into breeding units with restricted possibilities of genic exchange. Although recently has been detected sylvatic foci of *T. infestans* connected to domestic and peridomestic conspecifics in the Argentinean Chaco (Ceballos et al. 2011), it has been

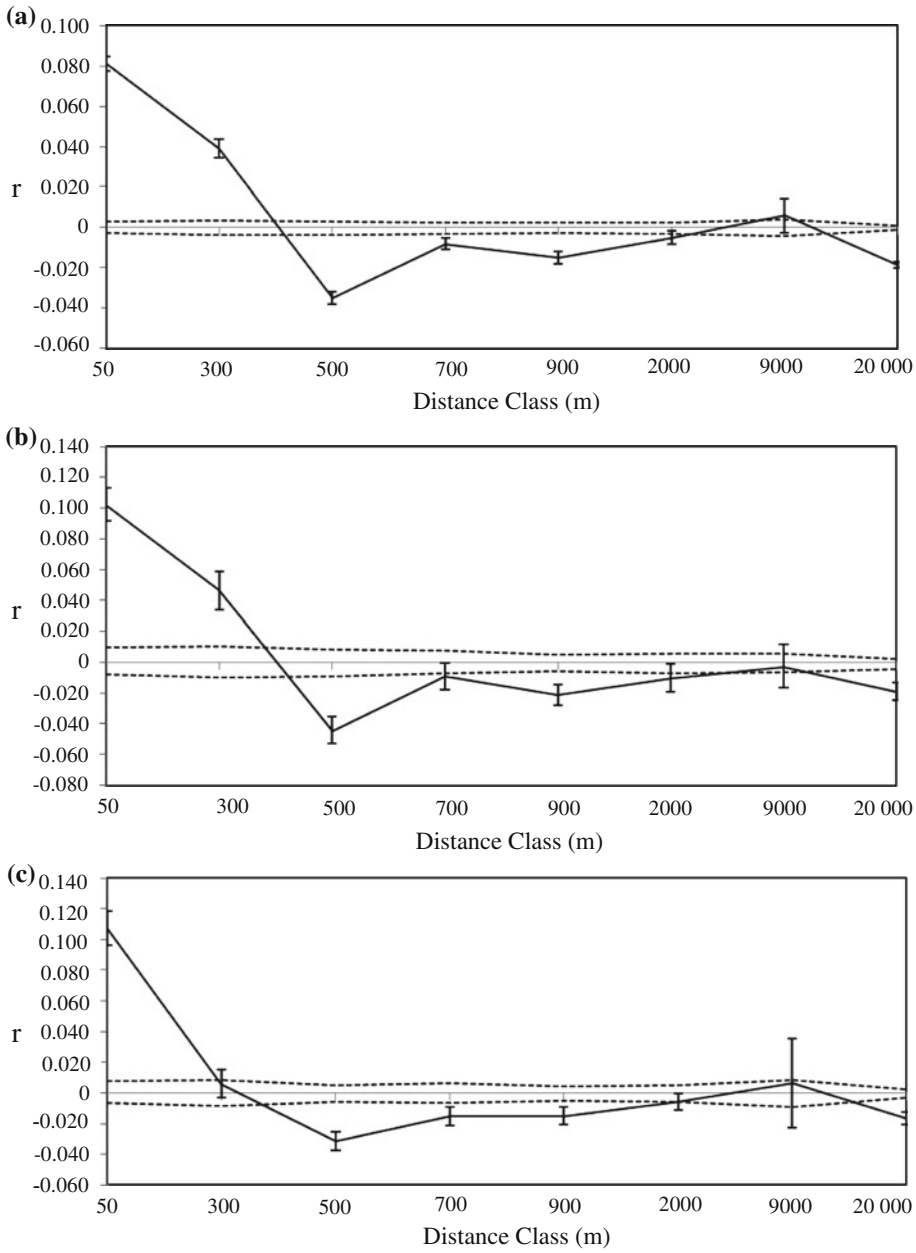

Fig. 4 Autocorrelograms plots of the genetic correlation coefficient (r) as a function of distance for all individuals (**a**), females only (**b**), males only (**c**). The permuted 95 % confidence intervals (*dashed lines*) and bootstrapped 95 % confidence error bars are also showed

considered that *T. infestans* is primarily restricted to domestic and peridomestic environments (such as chicken coops and pig or goat corrals) particularly in rural areas and usually remains in the same house or in its immediate vicinity during its lifetime.

Restricted gene flow resulting from restricted movement of individuals can lead to the nonrandom spatial distribution of individuals with respect to their genetic similarity. Isolation by distance is the most commonly tested model to analyse the relative influences of gene flow and drift on the spatial distribution of genetic variability. There was significant isolation by distance among sampling sites in the different geographic scales examined in this study. When distances ranges between population pairs smaller than 1 km were considered, the scatterplot shows moderate divergence with little scatter over shorter geographic distances (up to 400 m) and greater divergence and more scatter over longer geographic distances of separation (Fig. 2a). This pattern suggests the differing roles of gene flow and drift over differing spatial scales; gene flow more effective at shorter distances of geographic separation and drift more influential at greater distances of geographic separation. When the populations separated by more than 400 m were excluded, a higher significant association between geographical distance and genetic differentiation among sites (Mantel $r = 0.81$, $P = 0.0005$) was detected, with a scatterplot consistent with isolation by distance across the scale analysed (Fig. 2b).

The existence of a genetic structure due to limited gene dispersal was also evaluated through the analysis of spatial autocorrelation. The autocorrelation coefficient (r) reflects the tendency of individuals in particular distance classes to covary in the same, opposite or in random directions from the genetic mean. If gene flow is limited by distance at the spatial scale of the study, a positive correlation (r) is expected at the shorter distance classes, but this correlation will become zero at distance classes where drift predominates over gene flow. In this respect, the scale of structuring detected in the spatial autocorrelation analyses suggests that dispersal typically occurs on the scale of approximately 400 m (Fig. 4a). The results of the Mantel test described above support this finding. It has been indicated that this species fly up to 1,500 m depending on nutritional and temperature conditions (Schweigmann et al. 1988; Schofield et al. 1992; Lehane et al. 1992); the active dispersal inferred for *T. infestans* by autocorrelation is within the flight range indicated for this species.

Spatial correlation analysis of the 115 females and 136 males collected in the 22 sampling sites showed difference in the scale of structuring among sexes. We detected more extensive genetic structure with relatively larger dispersal distance for females than for males (approximately 400 and 330 m, respectively) (Fig. 4b, c). If exist significant sex differences in dispersal, members of the dispersing sex should display lower levels of Kinship coefficient than the more philopatric sex. In this regard, in agreement with the sex biased dispersal detected by autocorrelation analysis, the Kinship coefficient values obtained for pairwise comparisons among females were lower than among males. To this respect, Gurevitz et al. (2007) reported larger flight muscle mass in females, which would imply that females have higher capacity than males to have longer and broader flight ranges.

Three different genetic clusters could be identified (Fig. 3). The majority of the sampled locations correspond to one almost homogeneous cluster. Samples from the house AM (AM I and AM II) form a separate cluster with bugs collected in the distant house BR (BR I, BR II, BR III, and BR IV) ($\sim$16 km apart). Relatively moderate pairwise θ values between BR and AM sampling sites were observed; they ranged from 0.025 between the samples from BR I and AM I to 0.077 for the comparison between the populations of BR III and AM II (Table 3). Moreover, it is important to point out that the highest numbers of migrant was detected between the AM and BR dwellings (data not shown). *T. infestans* can disperse passively through accidental carriage by humans and their belongings. In this sense, the human movement would allow to maintain a higher genetic exchange between distant houses; for example, through visits or exchange of goods.

Insects from the house PL were collected from a tree inhabited by chickens. These insects form a distinct, nearly homogeneous cluster, where a total of nine private alleles were detected. This result is compatible with an independent origin of these insects.

Although the level of genetic differentiation was significant among collection places and hierarchical AMOVA showed high levels of genetic structuring, it is important to point out that almost all the different sites analysed in each house belonged to the same cluster. Among them, the four different capture sites of the house BR were the most representatives, with almost all the individuals from these domestic and peridomestic sites sharing ancestry. Similarly, the individuals from domicile and peridomicile of MA (MA I and MA II) share ancestry as well as the specimens from the domicile MG with those from nearby peridomestic sites of different houses. These findings support the hypothesis that peridomestic structures may be involved in the reinfestation process (Cecere et al. 2002, 2004).

In contrast with the rest of samples analysed, individuals from RH I and PF were found to consist of a mixture of different clusters, suggesting the existence of genetic exchange among individuals from these places and other sampling sites. In this regard, one immigrant was detected within RH I and two putative immigrants were identified originating from this collection site. The insects captured in RH I and PF belonged to goat corrals, in which insecticide spraying has limited effectiveness and may be key sources of *T. infestans* during the reinfestation process (Cecere et al. 2004). To this respect, we observed that the goat corral AM I was one of the two origin places of the highest number of immigrants detected. Animal corrals have been known to support abundant population of *T. infestans* before and after residual spraying insecticides than others peridomestic environments and may increase the risk of reinfestation in the whole area (Cecere et al. 2002, 2004; Gürtler et al. 2004).

Implications in the Chagas' disease vector control

The inferences made from fine-scale spatial genetic structure analyses of *T. infestans* populations have relevance for community-wide control programs. The scale of structuring detected indicates that active insect dispersal could occur on the distance of approximately 400 m, with females dispersing over greater distances than males. These results suggest that in order to reach a higher vector control effectiveness, insecticide treatment and surveillance should be extended within a radius of 400 m around the infested area, which would prevent the propagation and reinfestation process after insecticide spraying. This is consistent with a previous spatio-temporal study carried out by Cecere et al. (2004), where spatial tools were used to analyse the reinfestation process. Moreover, our results reinforce the

hypothesis that peridomestic structures may be involved in the reinfestation of insecticide treated areas.

In summary, the results obtained in this study indicate that the understanding of the spatial genetic structure of populations at fine scale can provide insight into the dynamic population and evolutionary process of the *T. infestans*, and a complementary approach to help improve vector control strategies.

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