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Research Article

A Microsatellite-Based Analysis of House Infestation With *Triatoma Infestans* (Hemiptera: Reduviidae) After Insecticide Spraying in the Argentine Chaco

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Abstract

Prevention of vector-borne transmission of Chagas disease mainly relies on residual insecticide spraying. Despite significant success at a regional scale, house infestation with *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) still persists in the Gran Chaco ecoregion. One key aspect is the identification of the sources of reinfestant triatomines. After detecting fine-scale genetic structure in two rural villages of Pampa del Indio, Argentine Chaco, we tested hypotheses on the putative origins of the triatomines collected at 4, 8, and 12 mo after insecticide house spraying. We genotyped 10 microsatellite loci in 262 baseline and 83 postspraying triatomines from different houses. Genetic variability was similar between baseline and postspraying populations, but 13 low-frequency alleles were not detected at postspraying. F_{ST}s were not significant between insects collected before and after insecticide spraying at the same house in all but one case, and they clustered together in a neighbor-joining tree. A clustering algorithm detected seven genetic groups, four of them mainly composed of baseline and postspraying insects from the same house. Assignment tests suggested multiple putative sources (including the house of collection) for most postspraying insects but excluded a house located more than 9 km from the study area. The origin of three triatomines was attributed to immigration from other unaccounted sources. Our study is compatible with the hypothesis that house reinfestations in the Argentine Chaco are mostly related to residual foci (i.e., survival of insects within the same community), in agreement with field observations, spatial analysis, and morphometric studies previously published.

Key words: triatomines, microsatellite, reinfestation, residual foci, migration

Prevention of vector-borne diseases is frequently based on suppressing or drastically reducing the population size of arthropod vectors below a defined target level (Rozendaal 2002). Since the 1950s, many countries organized vector control programs to eliminate the transmission of vector-borne pathogens through insecticide spraying of the infested areas and monitoring of the effectiveness of control actions (Rozendaal 2002, Matthews 2011, Mougabure-Cueto and Picollo 2015). In the case of human Chagas disease transmitted by triatomine bugs, an insecticide-based strategy coordinated at a regional level leads to the elimination of *Rhodnius prolixus* (Stål) (Hemiptera: Reduviidae) in Central America (Hashimoto and Schofield 2012) and a significant contraction of the geographic range of *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) in the Southern Cone countries of South America (Schofield et al. 2006, Moncayo and Silveira 2010).

In spite of these achievements, house infestation with *T. infestans* still persists in large tracts of Bolivia, Paraguay and Argentina, especially in the Gran Chaco ecoregion, where approximately 30% of the new vector-mediated cases of Chagas in Latin America were estimated to occur (WHO 2015). High rates of house reinfestation after insecticide application were frequently reported for the Dry (Cecere et al. 2004, 2006, Gürtler et al. 2004, Porcasi et al. 2006) and the Humid Chaco (Arrom et al. 2013, Gurevitz et al. 2013). One key aspect for the design of improved vector control strategies is the identification of the sources of the reinfestant bugs. If the triatomines found after insecticide spraying were local survivors or their offspring, spraying of the persistently infested houses with improved

techniques would be required to suppress the residual infestations (Schofield 2000). Conversely, if triatomines invade and recolonize houses from untreated neighboring areas or from putative sylvatic foci (e.g., immigrants) and the rate of occurrence of these events is sizable, then the geographic coverage of control actions should be expanded and additional management actions may be needed.

Determining the origin of the triatomines that appear in houses after insecticide treatment is challenging. Various tools have been used, including spatial analysis (Manne et al. 2012, Lucero et al. 2013), morphometrics (Dujardin et al. 1997, Dujardin 2008, Gaspe et al. 2013, Hernández et al. 2013), and genetic markers (see Introduction). In the case of *T. infestans* populations in the Argentine Dry Chaco, a spatiotemporal analysis suggested complex patterns of house reinfestation including multiple (internal and external) sources (Cecere et al. 2004, 2006). Head morphometrics and antennal phenotypes suggested that the populations of *T. infestans* found between 3 and 34 mo after insecticide application had a mixed ancestry, with some insects originating from residual foci and others from adjacent habitats (Hernandez et al. 2013).

Genetic markers have proved to be excellent tools to investigate the population structure and gene flow of various triatomine species at different spatial scales (Bargues et al. 2006, Dumonteil et al. 2007, Pérez de Rosas et al. 2007, Fitzpatrick et al. 2008, Piccinali et al. 2009, Brenière et al. 2013, Khatchikian et al. 2015, Stevens et al. 2015). Microsatellites are particularly suitable for studying house reinfestation patterns due to their high sensitivity to detect genetic structure at fine spatial scales—the main requirement for analyzing the sources of postspraying triatomines (Marcet et al. 2008; Pérez de Rosas et al. 2008, 2013; Pizarro et al. 2008). In spite of these favorable features, the application of genetic markers to triatomines collected at the same houses before and after insecticide spraying remains very limited. A pioneering study based on isoenzymes found that *T. infestans* collected in houses from an insecticide-treated village in central Bolivia were unlikely to have originated from neighboring, untreated localities; rather, they most likely were survivors from the local (preintervention) population, that is, residual foci (Dujardin et al. 1996). A similar pattern was found in the Bolivian Chaco where a comparison of *Cyt-b* haplotypes from triatomines collected before and after insecticide spraying suggested a local origin (Quisberth et al. 2011). Pérez de Rosas et al. (2008) analyzed the effects of insecticide spraying on microsatellite variability in one house in Catamarca (Argentina) and found a significant differentiation in allele frequencies 16 mo postspraying (MPS). The appearance of new allelic variants was attributed to immigration from neighboring demes.

A longitudinal research program on the eco-epidemiology and control of *T. infestans* assessed the early and long-term impacts of residual insecticide spraying on house infestation in 13 rural villages of Pampa del Indio, a municipality located in the transition between the Dry and Humid Argentine Chaco (Gurevitz et al. 2012, 2013). This research documented vector control failures partly attributable to moderate insecticide resistance, which most likely lead to residual foci. The hypothesis of residual foci was further examined by using wing geometric morphometry in adult *T. infestans* collected before and 4 MPS (Gaspe et al. 2012, 2013). Most of the postspraying triatomines were not morphologically different from those collected at the same house or at the nearest infested house before insecticide spraying. However, the geographic structure of wing morphological variation showed that triatomines collected up to 4 km were indistinguishable from those collected before insecticide spraying, which precluded the detection of possible migration events at a finer geographic scale. Our microsatellite-based study of *T. infestans* populations in two rural villages of the same area of Pampa del Indio showed the presence of genetic structure at a very fine spatial scale (180–6,300 m), suggesting that this area was suitable for testing hypotheses on the origins of postspraying triatomines (Piccinali and Gürtler 2015). The goal of the present study was to analyze the effects of insecticide spraying on genetic structure and to investigate the putative origins of adults and nymphs collected in domestic and peridomestic sites from the same villages at 4, 8, and 12 MPS.

Materials and Methods

Study Area

Fieldwork was conducted in a rural section (denominated Area I) of the municipality of Pampa del Indio (25°55′S56°58′W), Department of Libertador General San Martín, Province of Chaco, Argentina. The study area encompassed 13 villages and 327 inhabited house compounds including domiciles and associated peridomestic sites (Gurevitz et al. 2011). Domiciles included human sleeping quarters, whereas peridomestic sites included storerooms, kitchens, corrals, chicken coops, chicken nests, and latrines, among other structures.

Vector Collection

Vector surveys were conducted at baseline (October–December 2007) and at 4, 8, and 12 MPS (Gurevitz et al. 2011, 2013). Timed-manual searches of triatomines were performed by skilled personnel of the Provincial Vector Control Program using 0.2% tetramethrin (Espacial, Argentina) as a dislodging spray. Domiciles were inspected for triatomines by one person for 20 min, whereas peridomestic sites were searched by another person for 15 min. The collected bugs were put in labeled plastic bags, transported to the laboratory for further processing, and identified to species and stage following Lent and Wygodzinsky (1979).

A community-wide spraying with a standard dose (25 mg/m^2) of deltamethrin (K-Othrina, Bayer, Munro, Argentina) of all structures within each house compound was conducted immediately after the baseline survey (Gurevitz et al. 2013). All individual sites found infested with *T. infestans* at 4 or 8 MPS were resprayed with deltamethrin using a standard or double insecticide dose upon completion of the 8 MPS survey. Likewise, sites found infested with *T. infestans* at 12 MPS and other adjacent structures were sprayed with SC beta-cypermethrin (Sipertrin, Chemotecnica, Spegazzini, Argentina) at standard (50 mg/m^2) or double dose.

Vector Sampling

The village of Campo Los Toros and two neighboring houses of Santos Lugares were selected for the present study (Fig. 1) because they had high house infestation rates with *T. infestans* after the first round of community-wide insecticide spraying (Gurevitz et al. 2013). Distances between houses ranged from 180 to 6,300 m, and the total area studied covered 6.32 km^2. Triatomines collected at baseline in a house of 3 Lagunas, located at more than 9 km, were also included for comparison purposes.

The triatomines collected at baseline (N = 262) came from 18 domestic or peridomestic sites at 16 house compounds (Table 1); most of them (228 nymphs and adults) were analyzed in Piccinali and Gürtler (2015). The entire sample comprised most of the houses harboring prespraying high-density triatomine populations in the area (Fig. 1). In total, 83 postspraying triatomines collected in 23 domiciles or peridomestic sites from 13 house compounds were included in the current study: 24 males, 22 females, 27 fifth instars, 5 fourth instars, 2 third instars, and 3 second-instar nymphs collected at 4, 8, and 12 MPS (Table 1 and Supp Table 1 [online]). All

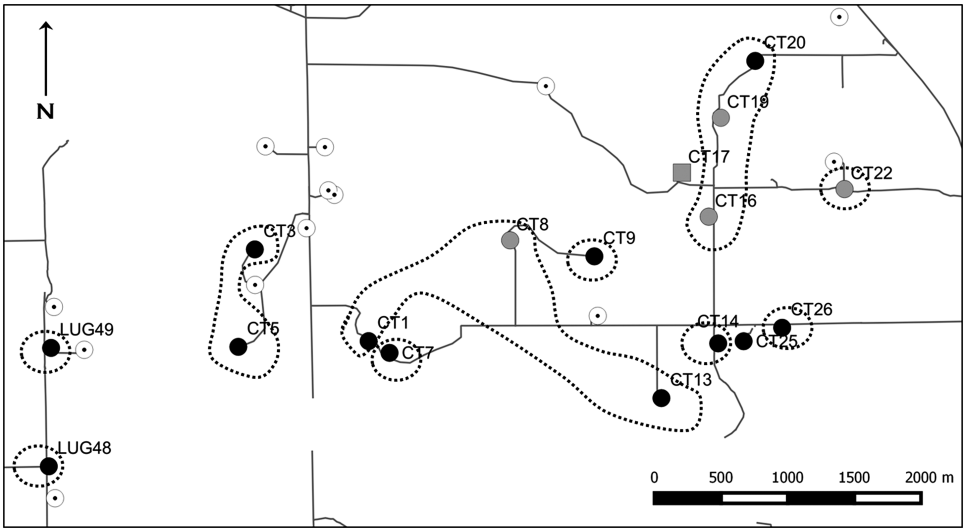

Fig. 1. Location of the study area and house compounds. Black circles, house compounds positive for *Triatoma infestans* before and after insecticide spraying which were included in this study; gray circles, house compounds positive only before spraying and included in this study; gray squares, house compounds positive only after spraying and included in this study; white circles with a dot, house compounds positive before spraying not included in this study; dotted lines mark populations as in Piccinali and Gürtler (2015).

Table 1. Number of *Triatoma infestans* collected at baseline and over postspraying surveys in Pampa del Indio (2007–2008) that were genotyped for the study

			Postspraying surveys		
Population	House	Baseline survey[a]	4 MPS	8 MPS	12 MPS
CT1-8–13	CT1	19	4	5	2
	CT8	7	–	–	–
	CT13	4	–	4	–
CT3-5	CT3	6	–	3	–
	CT5	16	2	–	2
CT7	CT7	18	–	9	–
CT9	CT9	40	9	4	–
CT14	CT14	20	1	2	–
CT16-19–20	CT16	9	–	–	–
	CT19	19	–	–	–
	CT20	14	1	2	–
CT22	CT22	12	–	–	–
CT25	CT25	0[b]	7	1	–
CT26	CT26	20	1	–	–
CT17	CT17	0	–	–	3
LUG48	LUG48	13	–	–	5
LUG49	LUG49	15	13	3	–
3L27	3L27	30	–	–	–
	Total	262	38	33	12

[a]Data from Piccinali and Gürtler (2015) with the exception of CT13 and 3L27.
[b]House positive for *T. infestans* at baseline but with no bugs available for genotyping.

triatomines were found in house compounds positive for *T. infestans* at baseline with the exception of house CT17, which was negative for infestation until 12 MPS.

Populations were defined according to the results of genetic structure analyses in Piccinali and Gürtler (2015) as shown in Table 1 and Figure 1. CT14 was considered a separate population because its clustering was variable across the different analyses performed. CT25 was also treated as a separate population in the absence of information on its clustering at baseline.

Microsatellite Genotyping

DNA extraction from two legs of each insect was made following Miller et al. (1988) with slight modifications. Ten microsatellite loci (Tims3, Tims5, Tims19, Tims22, Tims23, Tims27, Tims42, Tims56, Tims64, and Tims65) were PCR amplified for multilocus genotyping as previously described (Marcet et al. 2006, 2008). DNA fragments were run in an ABI 3130 automated DNA sequencer in Macrogen Inc. (Korea). Allelic sizes and binning were determined with GeneMapper v3.7 (Applied Biosystems) and FlexiBinV2 (Amos et al. 2007).

Data Analysis

Only postspraying populations collected at the same time with five or more insects (CT9, CT25 and LUG49 at 4 MPS, CT1-8–13 and CT7 at 8 MPS and LUG48 at 12 MPS) were considered and compared with baseline populations for genetic variability, DAPC, and F_{ST} because these analyses are for groups. All baseline and postspraying insects were used for the assessment of bottleneck signatures and for performing assignment tests, which are individual-based analyses.

Genetic variability of baseline and postspraying populations was estimated as the mean number of alleles (Na), mean allelic richness based on the smallest sample size (Rs, El Mouskadik and Petit 1996), mean observed heterozygosity (Ho), and mean unbiased expected heterozygosity (He, Nei 1978). Departures from Hardy–Weinberg equilibrium were analyzed with Weir and Cockerham mutilocus F_{IS} (Weir and Cockerham 1984). Calculations were performed with Genealex 6.5 (Peakall and Smouse 2012) and FSTAT 2.9.3 (Goudet 2001).

A K-means clustering algorithm after transforming data with a principal component analysis (Jombart et al. 2010) was applied to prespraying and postspraying populations. The find.cluster function, which finds the number of clusters (K) that maximize variation between groups, was run sequentially with increasing K values up to a maximum of 15. The optimal clustering solution was selected using the Bayesian Information Criterion (BIC). A discriminant analysis of principal components (DAPC, Jombart et al. 2010) was applied to clusters to estimate how well they were defined. Thirty-five principal components (99.4% of total variance) and four linear discriminants were retained for the analysis. Calculations and graphs were performed with the package adegenet (Jombart 2008) of the R software environment version 3.4.1 (R Core Team 2017). Pairwise Weir and Cockerham F_{STs} were calculated with FSTAT 2.9.3 (Goudet 2001). The neighbor-joining tree was built with MEGA 6 (Tamura et al. 2013). Bootstrap values and the degree of fit of the tree to the F_{ST} matrix (R^2 statistic) were calculated with TreeFit (Kalinowski 2009).

BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) was used to detect signals of bottlenecks in postspraying populations. A Wilcoxon's test was used to determine whether microsatellite loci had a heterozygosity excess as expected in bottlenecked populations (Piry et al. 1999). The two-phase model with 95% of single-step mutations (TPM) and the stepwise mutation model (SMM) were used as recommended for microsatellites (Cornuet and Luikart 1996). Private alleles for the total prespraying and postspraying samples were quantified with Genealex 6.5 (Peakall and Smouse 2012).

Assignment or exclusion of postspraying insects to baseline reference populations was made by applying the Bayesian method of Rannala and Mountain (1997) and the Monte Carlo resampling method of Paetkau et al. (2004). All calculations were run in GENECLASS 2.0 (Piry et al. 2004).

Results

Genetic Variability

Mean Na varied between 2.5 and 4.1 and mean Rs between 2.8 and 2.9; these values were very similar among sites and samples (Table 2). Mean Ho was more variable (between 0.38 and 0.58), and increased after insecticide spraying in houses CT9, CT7, and LUG48, and decreased in CT1-8–13 and LUG49. F_{IS} values were positive for all populations with the exception of postspraying CT25, CT7, and LUG48 (Table 2). F_{IS} values decreased at postpraying for all populations but LUG49. CT25 had an excess of observed heterozygotes and a negative F_{IS}. However, none of these values was statistically different from zero.

Genetic Structure

Pairwise F_{ST}s between baseline and postspraying populations varied from 0 to 0.15, and they were statistically different from zero in most cases (Table 3). Interestingly, no genetic differentiation was found between populations CT9, CT1-8–13, LUG48, and LUG49 before and after insecticide spraying. The neighbor-joining tree (R^2 = 0.841) showed that the closest populations to postspraying populations were the same houses at baseline, with the exception of CT7, which was closer to CT1-8–13 (Fig. 2). The bootstrap support of those groups was high (>90%) for CT9 and intermediate (>70%) for LUG48 y LUG49.

The optimal K value that described the data was 7 according to the BIC (Supp Fig. 1). The DAPC showed that the proportion of successful reassignment (based on the discriminant functions) of individuals to their original clusters was 93.41%. The most loose clusters were 1 (86.36% of successful assignments), 5 (88%), and 2 (89.66%). The bestdefined clusters were 3 and 4 (100% of successful assignments each). None of the clusters included individuals from only one house or group of houses (CT1-8–13; Fig. 3; Supp Table 2 [online only]). However, for four clusters there were more than 50% of the individuals from the same house. Clusters 6 and 7 had 55 and 78% of their individuals from CT9 (including both baseline and postspraying insects), respectively. Cluster 2 had 65% of the total

Table 2. Genetic variability and Hardy–Weinberg equilibrium estimators for 10 microsatellite loci in *Triatoma infestans* populations from Pampa del Indio before and after insecticide spraying

Population	Sample	N	Na	Rs (5)	Ho	uHe	F_{IS}
CT9	Baseline	39.80	3.50	2.42	0.381	0.415	0.083
	4 MPS	9.00	2.80	2.51	0.456	0.456	0.002
LUG49	Baseline	14.80	3.10	2.59	0.483	0.506	0.047
	4 MPS	13.00	3.20	2.64	0.462	0.498	0.077
CT25	4 MPS	8.00	3.00	2.93	0.575	0.523	–0.108
CT7	Baseline	17.90	3.60	2.81	0.506	0.536	0.058
	8 MPS	9.00	3.10	2.75	0.544	0.531	–0.028
CT1-8–13	Baseline	30.00	4.10	2.83	0.483	0.521	0.073
	8 MPS	8.90	3.00	2.68	0.469	0.486	0.037
LUG48	Baseline	12.90	3.50	2.71	0.454	0.471	0.037
	12 MPS	5.00	2.50	2.50	0.500	0.469	–0.075

N, mean number of individuals genotyped per locus; Na, mean number of alleles; Rs, mean allelic richness based on the smallest sample size (in parenthesis); Ho, mean observed heterozygosity; uHe, mean unbiased expected heterozygosity; F_{IS}, Weir and Cockerham mulilocus F_{IS}.

Table 3. Pairwise F_{ST}s between baseline and postspraying populations of *T. infestans*

	CT9	CT7	CT1-8–13	LUG49	LUG48	CT9 P	CT7 P	CT1-8–13 P	LUG49 P	LUG48 P
CT7	**0.0869*** **									
CT1-8–13	**0.0948*** **	0.0251*								
LUG49	**0.1272*** **	**0.0931*** **	**0.0684*** **							
LUG48	**0.1490*** **	**0.0611*** **	**0.0846*** **	**0.0831*** **						
CT9 P	0.0000	0.0376	0.0422*	0.0692***	**0.0982*** **					
CT7 P	**0.1176*** **	0.0278*	0.0192	**0.1372*** **	**0.1493*** **	0.057*				
CT1-8–13 P	**0.1270*** **	0.0343	0.0000	0.0933**	0.0998***	0.0767**	0.0289			
LUG49 P	**0.1360*** **	**0.0838*** **	0.0686***	0.0021	0.0712**	0.0734**	**0.1406*** **	0.1154**		
LUG48 P	**0.1569*** **	0.0644*	0.0729**	**0.1020*** **	0.0299	0.0978**	0.1434**	0.0948*	0.0721**	
CT25 P	**0.0946*** **	**0.0919*** **	**0.0796*** **	0.0635***	0.0976**	0.0674**	0.089*	0.1162***	0.0651**	0.1162*

Bolded values, significant after Bonferroni's correction.
P, postspraying sample.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

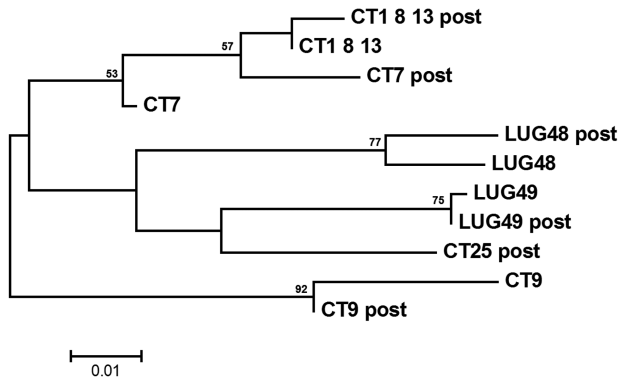

Fig. 2. F_{ST}-based neighbor-joining tree. Post, postspraying sample.

individuals from CT1-8–13. Cluster 3 had 73% of the individuals from LUG49 and Cluster 7 32% from LUG48. In general, the most frequent cluster at baseline in one house was also the most frequent cluster at postspraying in the same house (CT9, CT1-8–13, and LUG49). There were also some cases of postspraying bugs assigned to clusters not detected at baseline at the same house (Cluster 3 in CT9, Cluster 5 in LUG49, and Cluster 6 in LUG48).

Bottlenecks

Wilcoxon's tests for the total postspraying sample as well as for each population were not statistically significant for both the TPM and the SMM evolution models (results not shown). However, the analysis of private alleles indicated that 13 alleles were found only in the baseline sample, whereas no private alleles were present in the postspraying sample. These exclusive alleles belonged to 7 (Tims 3, 19, 22, 27, 42, 56, and 65) of the 10 loci, and their frequencies were below 5% (total sample) in three cases and below 1% in the rest.

Assignment Tests

The results of the assignment tests are shown in Table 4. Time, site, and house of collection and insect life stage are indicated. Only 3 of the 83 postspraying triatomines that were genotyped were excluded from any reference population: one male captured at the CT9 kitchen; one female collected at the LUG49 storeroom at 4 MPS, and a second-instar nymph collected at a granary in house CT1 at 12 MPS. Two postspraying insects were assigned to a single baseline population: two males found in a chicken house in CT5 at 12 MPS. The origin of these triatomines was attributed to the same population (domicile CT3 and domicile CT5) and most probably originated at the same house. The rest of the insects were assigned to two or more baseline reference populations. The house where the bug was collected was included as a possible source in 62 cases (Tables 3 and 4). Conversely, the house of bug collection was excluded as a possible origin in five cases: two-fifth instars collected at CT7 (8MPS), one-fifth instar found at CT1 (8 MPS), one-fifth instar at LUG49 (8 MPS), and a male collected at CT9 (4 MPS). In the case of house CT7, despite that individual triatomines were assigned to multiple sources, the only baseline population common to the two of them was CT1-CT8-CT13. CT1 was separated from CT7 by only 250 m, whereas CT8 and CT13 were located at 1,300 and 2,100 m from CT7, respectively.

The putative origin of the three triatomines from CT17 (the only house negative at baseline and positive at 12 MPS) could not be fully elucidated because several sources were possible. However, if the three bugs originated at the same site, the sources were restricted to CT16-CT19-CT20 and CT14, all of them within 500 to 1,400 m from house CT17.

Postspraying insects collected at CT25 could not be tested against the same population before insecticide spraying. However, if all CT25 insects were considered together, the only population from which they were not excluded was CT14, suggesting that CT25 was genetically close to CT14, the insects may have immigrated from there, or both. The distance between these two houses was 250 m.

The most distant house of the village of 3 Lagunas was excluded as a possible source of postspraying insects in all but four cases (Table 4).

Discussion

Residual Foci

Our genetic analyses, combined with evidence from field observations, spatial analysis, and wing geometric morphometrics (Gaspe et al. 2013; Gurevitz et al. 2012, 2013; Provecho et al. 2017), point to residual foci as the main process explaining the origin of reinfestant triatomines in rural communities of Pampa del Indio. The assignment of most specimens to two or more baseline populations and rejection of a more distant source is consistent with the notion

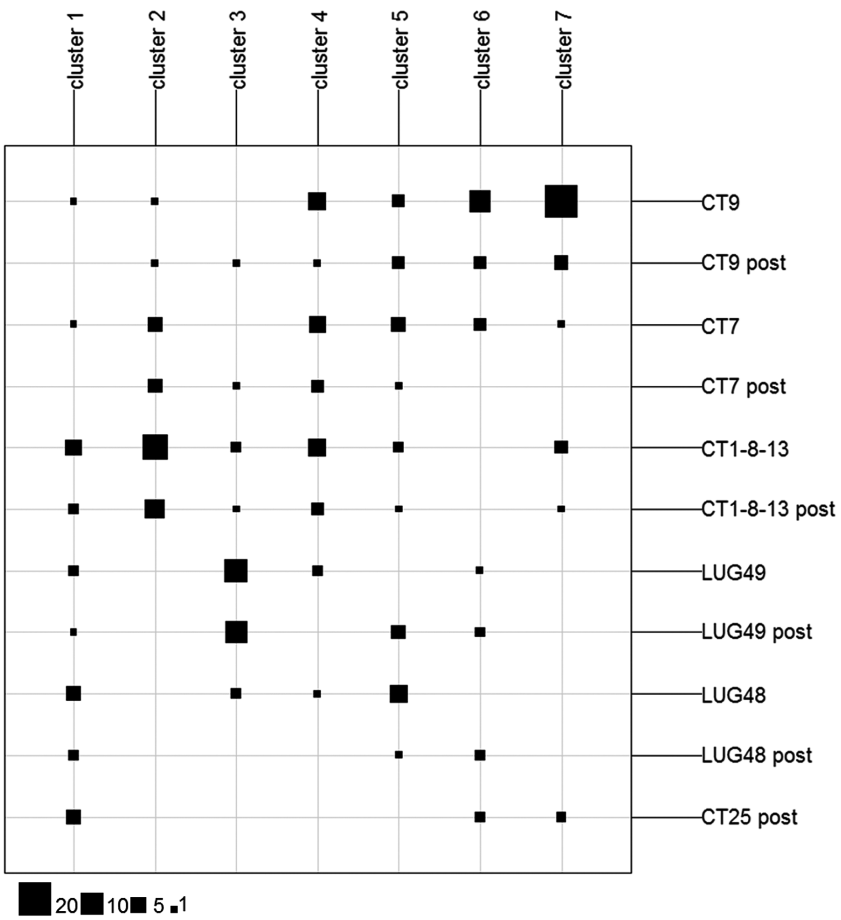

Fig. 3. Assignment of baseline and postspraying *Triatoma infestans* to the genetic clusters found with adegenet. The size of each square is proportional to the number of individuals. Post, postspraying.

that reinfestants (i.e., adults and late-stage nymphs collected at 4 MPS) or its progeny (i.e., early-stage nymphs collected at 8 or 12 MPS) most likely survived the initial community-wide insecticide spraying or the focal sprays conducted immediately after the 8 MPS survey. In most cases, the house of bug collection and neighboring populations were included as putative sources of postspraying triatomines, suggesting that some of the reinfestant insects may have dispersed from their preintervention collection site to other structures within the same house compound or to other nearby houses. Although a clear distinction between both scenarios could not be achieved for any collected triatomine, some results clearly show the existence of both processes in our survey area. On one hand, in five cases including houses CT9, CT7, CT1, and LUG49, the insects were excluded from the collection site but not from neighboring houses, indicating local migration. On the other hand, two insects collected in CT3-5 had this population as their only possible source. In addition, F_{ST} analyses showed that for CT9 and LUG49 at 4 MPS and for LUG48 at 12 MPS, the genetically closest population was the same at baseline. These results are in agreement with survival within the same house and dispersal to other structures within the whole house compound, at least for the majority of the postspraying individuals from those samples. The K-means cluster algorithm and posterior DAPC, despite the good percentage of correctly assigned individuals to clusters, did not provide a good resolution at house level due to the assignment of bugs from the same house to multiple clusters, probably because of important degrees of admixture in our data (Piccinali and Gürtler 2015). However, the fact that some houses (e.g., CT9, LUG49, and CT1-8–13) had most bugs from clusters that were infrequent in other houses both at baseline and postspraying also suggest the presence of bugs that survived the spraying at the same site. The finding of postspraying bugs from some clusters not registered at baseline might reflect migration from other sources, but sampling errors could not be excluded due to the limited number of postspraying individuals and the low resolution achieved.

These results agree with the hypothesis of Gurevitz et al. (2013) that local postspraying infestations were in part explained by frequent flight or walking dispersal of *T. infestans* during spring-summer (Vazquez-Prokopec et al. 2006). Pyrethroid insecticides exert excito-repellent effects and may affect walking and flight dispersal of triatomines (Diotaiuti et al. 2000). Triatomines hidden deep in wall cracks or thatched roofs may escape from direct contact with pyrethroid molecules and may eventually outmigrate from the house without uptaking a lethal dose of insecticide. The fate of the escapees most likely depends on the availability of suitable habitats free from insecticide within their effective dispersal range. In the specific context of our study, this mechanism may explain why focal insecticide spraying of infested sites only (not the entire house compound) performed poorly as a vector control tactic (Diotaiuti et al. 2000, Gurevitz et al. 2013).

Wing geometric morphometry identified significant shape differences in triatomines collected at 4 km of distance or more within the same section of Pampa del Indio (Gaspe et al. 2012).

Table 4. Site and date of collection, life stage, and probability of postspraying *Triatoma infestans* assigned to each baseline population

					CT9	CT26	CT7	CT14	CT1-8–13	CT16-19–20	CT3-5	LUG49	LUG48	CT22	3L27
Individual	House	Site	MPS	Stage	C	S	D	D, K	C, D	D, K, C	D	C	S	D	C
CHia08_556	**CT9**	**L**	**4**	**M**	**0.046**	**0.003**	0.743	0.245	0.394	0.081	0.181	0.118	0.163	**0.000**	**0.000**
CHia08_562	CT9	L	4	F	0.378	**0.019**	0.265	0.571	0.253	0.107	0.622	0.580	0.198	**0.006**	**0.001**
CHia08_575	CT9	C	4	F	0.296	**0.008**	0.485	0.350	0.114	0.448	0.175	0.139	0.165	**0.039**	**0.012**
CHia08_661	CT9	C	4	M	0.433	**0.014**	0.612	0.263	0.487	0.103	0.064	**0.022**	0.059	**0.006**	**0.001**
CHia08_663	CT9	C	4	M	0.492	0.214	0.851	0.674	0.655	0.616	0.823	0.375	0.457	0.110	**0.012**
CHia08_665	CT9	C	4	F	0.956	0.083	0.905	0.651	0.830	0.913	0.725	0.700	0.345	0.529	0.212
CHia08_677	CT9	C	4	N V	0.395	**0.047**	0.473	0.459	0.111	0.543	0.184	**0.034**	0.319	0.366	**0.001**
CHia08_686	CT9	C	4	N V	0.191	0.301	0.340	0.736	0.418	0.325	0.665	0.148	**0.048**	**0.028**	**0.001**
CHia08_702	CT9	C	4	N V	0.783	0.075	0.704	0.945	0.910	0.774	0.792	0.668	0.239	0.230	**0.017**
CHig08_268	CT9	C	8	M	0.364	0.074	0.686	0.353	0.644	0.283	0.087	0.081	0.118	**0.038**	**0.015**
CHig08_271	CT9	C	8	M	0.364	0.074	0.686	0.353	0.644	0.283	0.087	0.081	0.118	**0.038**	**0.015**
CHig08_297	**CT9**	**K**	**8**	**M**	**0.000**	**0.000**	**0.001**	**0.005**	**0.001**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**
CHig08_497	CT9	D	8	F	0.854	0.334	0.875	0.983	0.990	0.972	0.993	0.961	0.514	0.361	**0.046**
CHia08_897	CT26	S	4	N V	0.675	0.131	0.962	0.811	0.852	0.865	0.860	0.439	0.637	0.293	0.052
CHig08_179	CT7	K	8	N V	**0.045**	0.218	0.675	0.446	0.692	0.230	0.150	**0.005**	**0.014**	**0.005**	**0.001**
CHig08_189	CT7	K	8	N V	**0.013**	**0.004**	0.184	**0.049**	0.125	**0.007**	0.099	**0.003**	**0.002**	**0.000**	**0.000**
CHig08_192	CT7	K	8	N IV	0.054	**0.047**	0.226	0.321	0.492	0.571	0.269	**0.012**	0.082	**0.041**	**0.000**
CHig08_198	CT7	K	8	N II	0.344	0.095	0.198	0.444	0.203	0.115	0.377	**0.005**	**0.018**	**0.009**	**0.001**
CHig08_202	**CT7**	**K**	**8**	**N V**	**0.013**	**0.013**	**0.042**	0.063	0.162	**0.020**	0.077	**0.031**	**0.048**	**0.002**	**0.000**
CHig08_203	**CT7**	**K**	**8**	**N V**	**0.002**	**0.000**	**0.006**	**0.002**	0.071	0.086	**0.015**	**0.005**	**0.018**	**0.000**	**0.000**
CHig08_206	CT7	K	8	N V	**0.026**	0.079	0.645	0.280	0.467	**0.047**	0.154	**0.034**	**0.020**	**0.000**	**0.000**
CHig08_207	CT7	K	8	N IV	0.087	**0.003**	0.160	0.149	0.241	0.316	0.441	0.258	0.283	**0.002**	**0.001**
CHig08_219	CT7	K	8	N IV	0.259	0.957	0.882	0.918	0.948	0.692	0.847	0.086	0.164	0.071	**0.003**
CHia08_544	CT14	C	4	F	**0.002**	0.199	**0.006**	0.221	**0.013**	**0.013**	0.282	**0.002**	0.078	**0.003**	**0.000**
CHig08_542	CT14	M	8	M	**0.035**	**0.001**	0.278	0.308	0.450	0.352	0.086	0.814	0.096	**0.013**	**0.000**
CHig08_551	CT14	M	8	N II	0.471	0.320	0.998	0.687	0.848	0.878	0.628	0.332	0.611	0.380	0.112
CHia08_407	CT1	C	4	M	**0.025**	0.077	0.675	0.258	0.667	0.072	0.189	**0.029**	**0.022**	**0.005**	**0.000**
CHia08_408	CT1	C	4	F	**0.019**	**0.015**	0.197	0.243	0.737	0.097	0.297	**0.022**	0.087	**0.009**	**0.000**
CHia08_436	CT1	C	4	M	**0.021**	0.262	0.873	0.261	0.652	0.097	0.150	**0.020**	0.059	**0.006**	**0.001**
CHia08_437	CT1	C	4	F	0.267	0.113	0.691	0.892	0.892	0.510	0.417	0.391	0.158	**0.002**	**0.000**
CHig08	**CT1**	**U**	**8**	**N V**	**0.002**	**0.003**	0.268	0.069	**0.043**	**0.034**	0.148	**0.022**	0.062	**0.000**	**0.000**
CHig08_169	CT1	C	8	N IV	0.394	0.104	0.682	0.923	0.923	0.807	0.435	0.792	0.262	**0.022**	**0.000**
CHig08_172	CT1	C	8	N V	**0.019**	0.245	0.761	0.161	0.508	0.113	0.161	**0.012**	0.066	**0.011**	**0.005**
CHig08_332	CT1	C	8	N V	0.353	0.421	0.826	0.942	0.915	0.583	0.971	0.434	0.323	0.101	**0.003**
CHig08_333	CT1	C	8	N V	**0.007**	**0.008**	0.405	0.186	0.433	0.092	0.223	**0.023**	0.055	**0.002**	**0.000**
CHin08_8	CT1	G	12	N V	0.111	0.051	0.719	0.390	0.406	0.320	0.439	**0.046**	0.353	**0.036**	**0.003**
CHin08_10	**CT1**	**G**	**12**	**N II**	**0.000**	**0.014**	**0.019**	**0.007**	**0.020**	**0.000**	**0.012**	**0.000**	**0.005**	**0.000**	**0.000**
CHig08_315	CT13	S	8	F	0.167	0.685	0.715	0.752	0.852	0.830	0.727	0.487	0.325	0.200	**0.005**
CHig08_316	CT13	S	8	F	**0.003**	**0.042**	0.062	0.651	0.402	0.071	0.189	**0.013**	0.096	**0.000**	**0.000**
CHig08_318	CT13	S	8	N III	**0.032**	0.249	0.217	0.791	0.828	0.362	0.257	0.080	0.064	**0.000**	**0.000**
CHig08_322	CT13	C	8	N III	**0.038**	**0.003**	0.115	0.235	0.638	0.363	0.493	0.271	0.065	0.230	**0.000**
CHia08_770	CT20	C	4	N V	0.102	0.062	0.063	0.446	0.577	0.534	0.255	0.050	0.105	**0.024**	**0.001**
CHig08	CT20	C	8	F	0.364	0.588	0.830	0.867	0.969	0.934	0.716	0.805	0.397	0.384	**0.001**
CHig08_335	CT20	G	8	F	0.319	0.510	0.487	0.453	0.594	0.768	0.352	0.285	0.122	0.447	**0.048**
CHig08_176	CT3	S	8	F	**0.011**	0.161	0.183	0.237	0.367	0.058	0.101	**0.005**	0.074	**0.000**	**0.000**
CHig08_177	CT3	S	8	M	0.064	0.315	0.830	0.613	0.744	0.690	0.723	0.486	0.289	**0.046**	**0.012**
CHig08_178	CT3	S	8	F	0.059	0.150	0.363	0.913	0.833	0.495	0.962	0.364	0.545	0.107	**0.000**
CHia08_543	CT5	D	4	F	0.193	**0.008**	0.847	0.400	0.633	0.595	0.102	0.495	0.606	**0.030**	**0.000**
CHia08_435	CT5	D	4	F	0.103	**0.008**	0.365	0.319	0.108	0.107	0.374	**0.029**	0.309	0.065	**0.001**
CHin08_36	CT5	P	12	M	**0.002**	**0.000**	**0.000**	**0.002**	**0.028**	**0.033**	0.115	**0.020**	**0.011**	**0.000**	**0.001**
CHin08_37	CT5	P	12	M	**0.007**	**0.004**	**0.011**	**0.040**	**0.028**	**0.018**	0.135	**0.034**	**0.005**	**0.000**	**0.001**
CHia08_460	LUG49	S	4	N V	0.130	**0.019**	0.218	0.337	0.324	0.422	0.208	0.843	0.260	0.109	**0.000**
CHia08_463	LUG49	S	4	N V	0.147	**0.001**	0.286	0.214	0.232	0.262	0.125	0.941	0.471	0.104	**0.000**
CHia08_465	LUG49	S	4	N V	0.125	**0.009**	0.212	0.313	0.445	0.254	0.284	0.941	0.127	**0.042**	**0.001**
CHia08_472	LUG49	S	4	M	**0.000**	**0.000**	**0.003**	**0.004**	**0.009**	**0.000**	**0.002**	0.157	0.096	**0.000**	**0.000**
CHia08_473	LUG49	S	4	M	**0.012**	**0.003**	**0.029**	**0.031**	0.222	0.061	**0.013**	0.457	0.168	**0.009**	**0.000**
CHia08_474	LUG49	S	4	M	**0.038**	**0.005**	0.371	0.192	0.175	0.361	0.190	0.139	0.431	0.138	**0.001**
CHia08_475	LUG49	S	4	M	**0.030**	**0.001**	0.059	0.074	0.122	**0.049**	**0.025**	0.840	0.059	**0.001**	**0.000**
CHia08_477	LUG49	S	4	F	0.057	**0.004**	0.144	0.085	0.176	0.260	0.213	0.091	0.303	**0.005**	**0.000**
CHia08_480	LUG49	S	4	F	0.526	0.097	0.688	0.696	0.813	0.939	0.407	0.743	0.224	0.202	0.052
CHia08_481	LUG49	S	4	F	**0.034**	**0.003**	0.182	0.183	0.317	0.189	**0.040**	0.878	0.131	**0.006**	**0.000**
CHia08_482	LUG49	S	4	F	0.081	**0.009**	0.711	0.472	0.715	0.439	0.123	0.553	0.249	**0.035**	**0.000**

Table 4. Continued

Individual	House	Site	MPS	Stage	CT9 C	CT26 S	CT7 D	CT14 D, K	CT1-8–13 C, D	CT16-19–20 D, K, C	CT3-5 D	LUG49 C	LUG48 S	CT22 D	3L27 C
CHia08_483	**LUG49**	**S**	**4**	**F**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	**0.021**	**0.001**	**0.042**	**0.000**	**0.000**
CHia08_485	LUG49	S	4	N V	0.096	0.610	0.383	0.619	0.751	0.688	0.794	0.652	0.326	0.247	**0.001**
CHig08_436	LUG49	S	8	N V	0.501	0.242	0.598	0.942	0.858	0.994	0.749	0.832	0.620	0.814	**0.025**
CHig08_438	**LUG49**	**S**	**8**	**N V**	0.074	**0.009**	0.218	0.149	0.071	0.340	0.249	**0.015**	0.256	**0.036**	**0.001**
CHig08_440	LUG49	S	8	N IV	**0.002**	**0.001**	**0.006**	**0.038**	0.075	**0.011**	0.052	0.409	0.055	**0.000**	**0.000**
CHin08_95	LUG48	S	12	N V	0.058	**0.015**	0.194	0.453	0.196	0.242	0.593	0.091	0.717	**0.010**	**0.012**
CHin08_96	LUG48	S	12	N V	**0.013**	0.332	0.440	0.459	0.512	0.423	0.578	0.212	0.435	0.181	**0.017**
CHin08_97	LUG48	S	12	M	**0.046**	**0.009**	0.627	0.245	0.132	0.235	0.241	**0.021**	0.242	**0.021**	**0.026**
CHin08_105	LUG48	S	12	M	**0.020**	**0.005**	0.271	0.169	**0.028**	0.176	0.123	**0.007**	0.374	**0.016**	**0.059**
CHin08_107	LUG48	S	12	F	**0.021**	0.145	0.372	0.444	0.555	0.341	0.375	0.111	0.149	**0.030**	**0.002**
CHin08_29	CT17	K	12	N V	0.219	0.245	0.493	0.347	0.689	0.564	0.549	0.373	0.155	0.438	**0.001**
CHin08_30	CT17	K	12	N V	**0.017**	**0.009**	**0.024**	0.234	**0.044**	0.058	**0.040**	**0.015**	**0.049**	**0.032**	**0.001**
CHin08_33	CT17	K	12	N V	0.261	**0.015**	0.236	0.403	0.763	0.859	0.930	0.887	0.508	0.224	**0.001**
CHia08_749	CT25	D	4	M	**0.013**	**0.016**	**0.037**	0.050	0.243	0.055	0.172	0.085	0.134	**0.000**	**0.000**
CHia08_750	CT25	D	4	M	**0.021**	**0.005**	**0.018**	0.193	0.136	0.057	0.098	**0.047**	0.394	**0.000**	**0.001**
CHia08_751	CT25	D	4	M	0.334	0.093	0.638	0.511	0.509	0.610	0.474	0.692	0.529	**0.016**	**0.001**
CHia08_752	CT25	D	4	M	0.075	**0.001**	**0.024**	0.063	0.226	0.104	0.158	0.292	0.235	**0.000**	**0.000**
CHia08_753	CT25	D	4	M	**0.035**	**0.016**	**0.005**	0.194	**0.021**	0.063	**0.026**	**0.003**	**0.020**	**0.001**	**0.000**
CHia08_754	CT25	D	4	F	**0.039**	0.143	0.144	0.311	0.544	0.122	0.336	0.438	0.268	**0.006**	**0.001**
CHia08_755	CT25	D	4	N V	0.074	**0.003**	**0.005**	0.085	**0.008**	**0.028**	**0.019**	**0.005**	**0.009**	0.009	**0.000**
CHig08_325	CT25	D	8	M	**0.038**	0.104	0.222	0.379	0.314	**0.037**	0.171	**0.013**	0.228	**0.000**	**0.001**

Bold and dark shaded values, populations that were excluded as sources of the bugs; in bold and light gray, cases where the house of collection was excluded as a putative source.

D, domicile; K, kitchen; S, storeroom; L, latrine; G, granary; P, poultry house; C, chicken coop; M, piled materials; U, unknown; F, female; M, male; N, nymph, roman numbers indicate nymphal stage; MPS, months postspraying.

This approach was naturally restricted to adult triatomines and was appropriate to reveal long-distance dispersal events compatible with passive bug transportation. By contrast, our present study area comprised the estimated flight range of *T. infestans*, which likely exceeds 2,400 m (Gürtler et al. 2014 and several references therein), and the tested specimens included both nymphs and adults. Despite the limited microgeographic scale and subtle genetic differentiation between triatomine populations, we detected migration events in *T. infestans* after two insecticide applications. Five triatomines had unlikely genotypes in comparison with baseline insects at the same house; four of them were fifth instar nymphs which may have immigrated from neighboring populations. Both flight and walking dispersal have been documented in *T. infestans* (e.g., Schofield et al. 1992, Vazquez-Prokopec et al. 2006). Walking dispersal apparently plays an important role in the local movement of triatomines at house-compound level or between neighboring houses, including gravid females and late-instar nymphs (Abrahan et al. 2011).

Our results qualitatively agree with the outcome of studies conducted in other rural villages of the Argentine Dry Chaco suggesting that most of the *T. infestans* collected from 1 up to 5 yr after spraying with pyrethroids originated from within the study communities (Cecere et al. 2002, 2013; Gürtler et al. 2004). These studies were based on the spatial patterns of house reinfestation, the relative stage composition of triatomine populations, and the limited effectiveness of pyrethroids outdoors. Microsatellites applied at a house-compound level in the same area also provided evidence showing that half of the postspraying triatomines would have survived to insecticide spraying (i.e., residual foci), and the remainder were putative migrants between communities (Marcet 2009).

Migrants From Unidentified Sources

Three triatomines collected after insecticide spraying were excluded from any site potentially considered as a source population. Two pieces of evidence strongly suggest that these insects originated from sources not accounted for in our surveys: the low degree of differentiation between many local triatomine populations (Piccinali and Gürtler 2015), and the fact that all other triatomines were assigned to more than one reference population. However, whether these three insects had an internal (within-village) origin cannot be completely ruled out because we did not genotype all preintervention triatomine populations (i.e., low-density colonies were excluded a priori because they would provide sparse information). Passive transportation of triatomines from another village or area, a well-documented mode of dispersal in *T. infestans* (Forattini et al. 1971, WHO 2002, Bayer et al. 2009, Piccinali et al. 2010), may explain the origin of the three triatomines. Another possibility is flight dispersal from nearby sylvatic foci. Although extensive searches failed to detect sylvatic foci of *T. infestans* in two rural areas of Pampa del Indio municipality (Alvarado-Otegui et al. 2012, Provecho 2015), their occurrence elsewhere in the Gran Chaco (Ceballos et al. 2011, Rolón et al. 2011, Waleckx et al. 2012) suggest they cannot be fully ruled out as sources.

Genetic Variability

Insecticide spraying and other human activities that increase habitat fragmentation are expected to reduce the effective population sizes of insects. In consequence, populations would become more isolated and subdivided into smaller demes and their susceptibility to stochastic effects would increase (Van Dongen et al. 1998). Genetic drift is expected to decrease variability within demes and increase variability among subpopulations. Our results after two insecticide applications over 1 yr partially fit to these predictions. Although allelic richness

before and after spraying remained at very similar levels, several low-frequency alleles were not detected in the total postspraying sample, suggesting a loss of genetic variability in the study area.

Limitations

The interpretation of our results is limited by technical and sampling issues. The power of assignment tests is affected by the number of loci and their degree of polymorphism, the model of evolution of the analyzed genetic regions, the number of insects sampled in each reference population, and the degree of genetic differentiation between populations (Cornuet et al. 1999). The fact that several reinfestant insects were not excluded from many putative source sites as well as the pooling of triatomines from different houses in the same genetic clusters using a K mean algorithm and a DAPC may be related to different causes. These results could be attributed to the low number of microsatellite loci available for *T. infestans*, the low number of alleles per loci in the study area (mean Na per sample 2.50–4.10), the limited sample sizes for many collection sites in spite of the considerable search effort invested, and the low differentiation between some triatomine populations (Piccinali and Gürtler 2015). On the flip side, the intense sampling effort and follow-up at a very fine geographic scale (250–6,000 m) are unprecedented for domestic Triatominae. This scale, however, enhances the probability of migration between populations and the probability of admixture, which decreases the performance of assignment tests and the clustering algorithms.

Implications for Vector Control

Our study has implications for improved insecticide-based control of *T. infestans* and other domestic triatomines. The microsatellite-based analysis of bug populations (surveyed at a geographic scale relevant to the active dispersal of *T. infestans*) supports that: First, most triatomines collected after control interventions most likely originated from residual foci and then dispersed to other sites within the village. This pattern appears to be quite general throughout the Gran Chaco. House compounds and the surrounding houses thus emerge as the minimal units for vector control, up to a distance ranging from 250 to 2,400 m from highly infested sites, as suggested by our microsatellite study, spatiotemporal analyses of house reinfestation (Cecere et al. 2004, 2006), and estimated flight ranges. These units should be monitored for (re) infestation and appropriately sprayed with insecticides. Second, there are other entry pathways of postspraying triatomines that remain unaccounted for. The detection of multiple residual foci after insecticide spraying highlights the importance of effective vector surveillance and response, routine screening of triatomine populations for pyrethroid resistance (Mougabure-Cueto and Picollo 2015), enhanced treatment and coverage of key vector habitats that may function as (super)productive sources (Gürtler et al. 2014), application of appropriate insecticide doses (Cecere et al. 2013), and improved spraying technique.

Supplementary Material

Supplementary data are available at *Journal of Medical Entomology* online.

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