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REVIEW

Triatoma infestans (Hemiptera: Reduviidae) Population Genetics: What Have We Learned from Microsatellites?

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Abstract

Purpose of Review The genetic structure of insect vectors offers valuable insights for identifying panmictic units, reinfestation sources, and minimal intervention units in vector control programs. This review highlights key findings on the genetic structure of *Triatoma infestans* populations using microsatellites across various geographic scales and landscapes.

Recent Findings Microsatellites have been employed to explore the genetic structure of *T. infestans* across Argentina, Bolivia, Paraguay, Brazil, and Peru. Research has focused on understanding genetic structure, assessing the impacts of short and long-distance migration, identifying sources of reinfestation post-insecticide spraying, evaluating the effects of insecticides on variability, and investigating the potential contribution of sylvatic foci to household infestation.

Summary *Triatoma infestans* populations are highly structured across countries, landscapes, and geographical levels. Although support for the isolation-by-distance migration model is mixed, most studies point to a combination of active and passive dispersal. Insecticide spraying significantly influences genetic structure, intensifying differentiation. Reinfestation is mainly attributed to internal residual foci at the village level. Finally, the contribution of sylvatic populations to (re)infestation varies across geographic areas.

Keywords *Triatoma infestans* · Microsatellites · Population genetics · Genetic structure

Introduction

Since its origin at the beginning of the 1920's population genetics has been a growing field that incorporated more and more sophisticated data and models to understand the patterns and processes affecting the genetic variability in natural populations. Write, Fisher, and Haldane pioneered the mathematical models of evolutionary processes like natural selection, random drift, mutation, and migration [1]. Leading the way to a new era in population genetics, Hubby and Lewontin conducted one of the first studies of molecular variation in natural populations [2]. After the development of the first techniques to obtain DNA sequencies (reviewed in [3]), DNA makers have contributed to an exponential grow of molecular studies of relatedness among individuals and populations, phylogeny, population dynamics, and gene and genome mapping in several insect species [4]. Popular genetic markers include RFLPs, RAPDs, AFLPs, microsatellites, SNPs, and ESTs. These polymorphisms are caused by different mechanisms like point mutations leading to single-nucleotide substitutions, DNA insertions or deletions, and mistakes in DNA replication that are tandemly repeated [5].

Insect vectors of disease pathogens are of particular interest due to their effects over the ecosystem, particularly human health. The analyses of the genetic structure of the populations of insect vectors provide useful approaches to determine panmictic units under different landscapes, sources of reinfestation after insecticide spraying, and minimal units of intervention for vector control programs [6–8].

The insect *Triatoma infestans* (Hemiptera, Reduviidae) is the main vector of *Trypanosoma cruzi* (Trypanosomatida, Trypanosomatidae), the causative parasite of Chagas

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disease, in Argentina and other countries of southern South America. The species is highly adapted to the domestic environment and develops its biological cycle in human dwellings and their surrounding peridomestic structures like corrals, chicken coops, and storerooms among other anthropic constructions [9]. In addition to its high levels of domestication, several wild foci were discovered in different areas from Bolivia [10–12], Chile [13, 14], Paraguay [15], and Argentina [16, 17]. Ever since 1990s, governmental efforts have played a key role to reduce *T. infestans* populations through vector control interventions based on insecticide spraying and community participation [18, 19]. In spite of several major achievements, vectorial transmission of Chagas disease is still important, particularly in the Argentine and Bolivian Gran Chaco regions [20, 21].

Since the pioneering work in isoenzymes of Dujardin and Tybarenc in the 1980s [10, 22], sequencies from different gene regions of the mitochondrial DNA and microsatellites have been used as the principal molecular markers in *T. infestans* populations. Microsatellites are short tandem repeats of usually 1 to 6 nucleotide motifs flanked by regions of nonrepetitive unique sequences of DNA [23]. They are evolutionarily significant due to their abundance and polymorphism: they mutate at rates up to 10 orders of magnitude higher than point mutations [24] and they are suitable to detect differences among closely related populations [25]. This review highlights key findings on the genetic structure of *T. infestans* populations using microsatellites across various geographic scales and landscapes. Results are based on studies comprising domestic, peridomestic, and sylvatic populations from Bolivia, Argentina, Paraguay, Brazil, and Peru and spanning different South American ecoregions (Fig. 1).

Triatoma infestans Microsatellite Sets

At present, there are two main sets of *T. infestans* microsatellites, one developed by Garcia et al. [26] and the other by Marcet et al. [27] and Marcet [28]. From the total isolated loci, ten from each study were used in subsequent works, separately or combined (Table 1).

Variability and Genetic Structure at Macrogeographic Scales

Studies comparing *T. infestans* populations from diverse villages across provinces or departments are included in this section. These surveys generally encompass a spectrum from 40 to several hundred km, distances that significantly surpass the range of dispersal of the species.

Argentina

Pérez de Rosas et al. [29••] used microsatellites to analyze 19 Argentine populations with and without insecticide spraying interventions. Most populations showed unique alleles and significant pairwise F_{ST}s estimators, suggesting limited gene flow at this geographic level. Many loci depicted departures from Hardy–Weinberg expectations (HWE) and the authors proposed the existence of subdivision within population samples as the most likely explanation for this pattern (the so-called Wahlund effect) due to the condition of poor flyer of *T. infestans*. F_{ST}s correlated with geographical distances as expected in an isolation by distance model (IBD). IBD is the process by which geographically restricted gene flow produces a genetic structure because random genetic drift occurs locally. It is an important phenomenon in subdivided populations that exchange genes at a distance dependent rate [30]. Levels of genetic diversity in most of the insecticide-treated localities were similar or higher than in untreated areas and no bottlenecks effects were detected, suggesting a limited effect of spraying erasing genetic variability. A latter study [31] confirmed several of these patterns and proposed, as in a previous study based on the mtCOI gene [32], the existence of two colonization events of T. *infestans* in Argentina, one dispersal line through the Andes and the other from non-Andean lowlands.

Bolivia

The high interest in areas like Cochabamba and Sucre-Vallegrande-Potosí comes from the hypothesis than the Andean valleys in Bolivia are the center of origin and dispersal of *T. infestans* throughout South America ([33] and references therein). Pizzarro et al. [34••] conducted one of the first studies using microsatellites in Bolivia. They included 23 Bolivian populations from the Potosí Department and analyzed differentiation between Western, high-altitude regions (2.600 amsl), and Eastern, low-altitude regions (2.300 amsl), as well as among communities at less than 100 km of distance. While Eastern and Western areas exhibited genetic differentiation, there was no discernible trend toward higher or lower diversity at higher altitudes. Localities displayed moderate levels of variability but with highly variable allele frequencies among them. The Structure Bayesian clustering algorithm [35] revealed five different genetic clusters. In contrast to Argentine populations, no IBD was observed. Interestingly, the authors compared R_{ST}s values (a measure of differentiation based on the stepwise mutation model-SMM-), with F_{ST}s (a measure based on the infinite allele mutation model-IAM-). Generally, R_{ST}s were higher than

F_{ST}s. According to Hardy et al. [36], similar values of these measures are expected when mutation rates are negligible compared to genetic drift and migration, and higher values of R_{ST}s compared to F_{ST}s when mutation rates contribute to population differentiation, as seems to be the case of Bolivian *T. infestans* populations.

Breniere et al. [37] analyzed variability among domestic and sylvatic *T. infestans* collected in three localities from Potosí, Cochabamba, and La Paz Departments. The village Thago Thago in Potosí exhibited the highest variability in terms of allelic richness. An AMOVA revealed that 18% of the variation was explained by differences among localities, and 6.7% among subsamples within localities, indicating geographical structuring. According to Structure clustering analysis, two genetic groups could be defined: one comprising samples from Potosí and La Paz and the other consisting of individuals from Cochabamba.

Brazil

T. infestans arrived late to Brazil compared to other South American countries, probably associated to human-mediated transportation. The species was likely introduced from the south, reaching Sao Paulo and other southern states during the XIXth century expansion of coffee plantations [38] and from there expanded to north reaching the state of Bahia among others. Belisario et al. [39] analyzed four residual foci from Brazil located in the States of Bahia, São Paulo, and Río Grande do Sul. Using the same microsatellite set, average allelic richness (a measure of variability independent of the sample size) was similar to the values found in the Argentine Humid Chaco [40], but lower than in the Argentine Dry Chaco [41••]. Moreover, high structuring was found among the Brazilian foci, corresponding each to a different genetic cluster except for the focus in Santa Rosa (Río Grande do Sul) where two genetic groups were detected. This shows that proximity between populations does not necessarily result in greater genetic similarity. The authors propose that the observed trends may indicate a genetic simplification in introduced *T. infestans* populations, accompanied by minimal gene flow and a dispersal pattern influenced by human activities.

Variability, Genetic Structure, and Reinfestation at Microgeographic Scales

This section explores studies comparing intimately connected populations. These populations include insects from villages separated by less than 20–30 km or different houses within a village, usually separated by less than 2 km—the range of active dispersal of the species.

Genetic structure of domestic and peridomestic populations

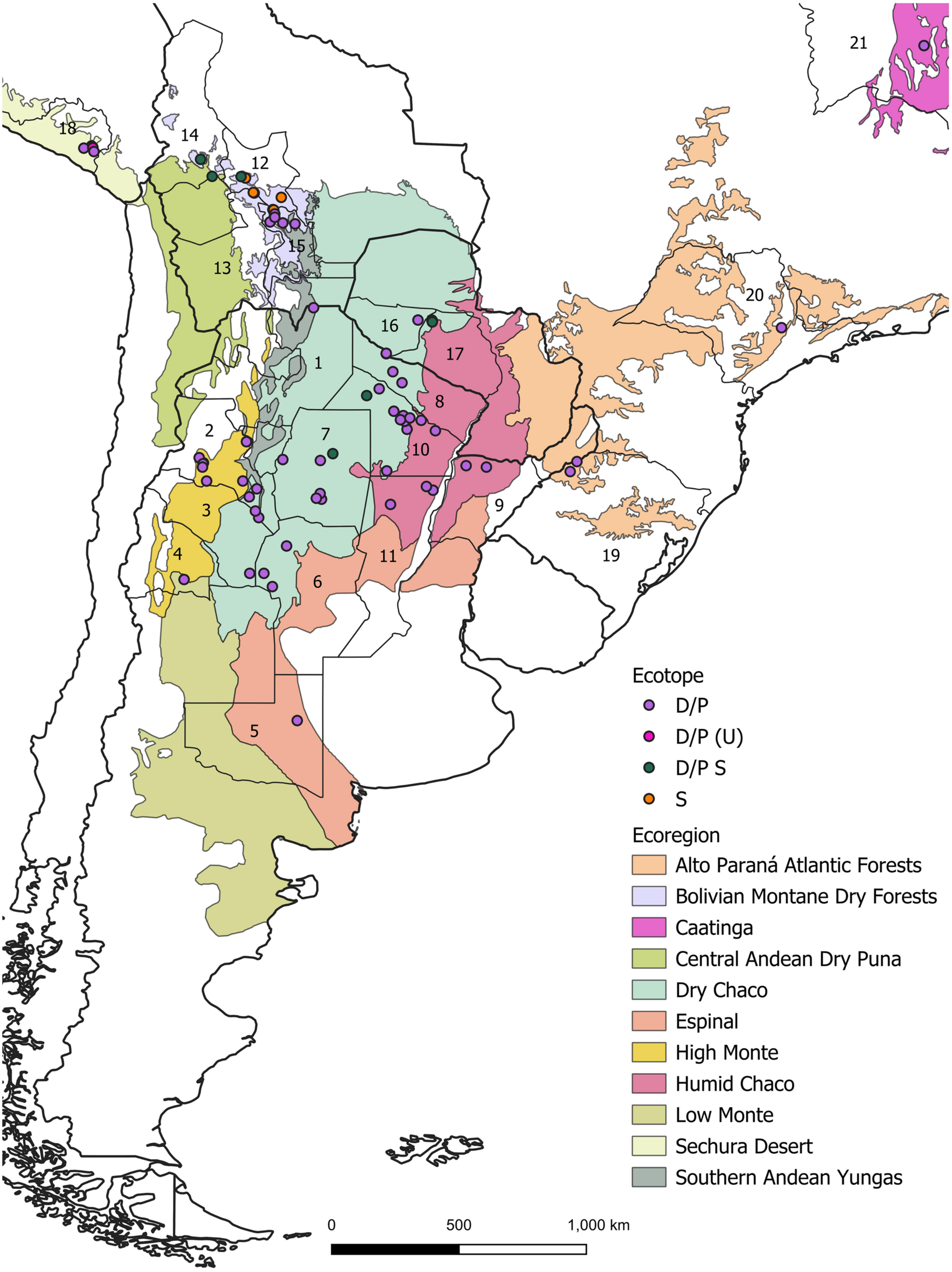
Many studies at this level have focused on investigating the genetic structure of domestic and peridomestic populations. The main interest in this topic is related to the causes of the incomplete elimination of *T. infestans* in rural communities that have been subjected to insecticide treatments in both domiciliary and peridomiciliary environments.

Bolivia

Pizarro et al. [34••] analyzed genetic structure among households within a diameter of 750 m in the locality of Zurima and a single corral in Jackota, Chuquisaca. Most insects were collected up to 6 months after insecticide spraying. Households were significantly differentiated according to R_{ST} and F_{ST} values, particularly peridomestic versus domestic samples. Contrarily to the pattern observed at a higher geographic scale in the same area, R_{ST} and F_{ST} were not different, suggesting that mutation does not play a role in differentiation at this level. Five genetic clusters were detected among the 7 households, but only two of them were composed mostly of insects from the same household. Combining relatedness indexes and insects' life stage distributions, the authors inferred reinfestation patterns associated with household colonization from several sources, multiple colonization from a single source and the recrudescence of full sibs and unrelated eggs.

Argentina

Marcet et al. [41••] examined genetic structure and immigrant sources in *T. infestans* populations from two neighboring rural areas in Santiago del Estero, in the Argentine Dry Chaco. These areas had been under different frequencies of residual insecticide spraying over the previous 10 years. The called core area was subjected to recurrent supervised vector control actions and the peripheral area had unsupervised insecticide applications irregularly conducted. Peridomestic insects were collected in 21 households from 11 villages with geographic distances between 100 m and 30 km. Levels of genetic variability were similar among households and departures from HWE were detected in both areas. Pairwise F_{ST}s were significant among most villages and households, indicating high degrees of structuring of genetic variability. Interestingly, average F_{ST}s were higher between villages from the core area compared to villages from the peripheral area. These results agree with the hypothesis that insecticide spraying increases the genetic structure in *T. infestans* populations [29••]. No IBD was found at this geographic scale and the authors propose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Ecotope
D/P
D/P (U)
D/P S
S
Ecoregion
Alto Paraná Atlantic Forests
Bolivian Montane Dry Forests
Caatinga
Central Andean Dry Puna
Dry Chaco
Espinal
High Monte
Humid Chaco
Low Monte
Sechura Desert
Southern Andean Yungas
0
500
1,000 km

◂**Fig. 1** Map of *Triatoma infestans* populations studied using microsatellites. Each dot represents a village or city with at least five genotyped insects. D/P: domestic and peridomestic rural. D/P (U): domestic and peridomestic urban. D/P S: domestic, peridomestic and sylvatic. S: sylvatic. Argentina (provinces): 1. Salta. 2. Catamarca. 3. La Rioja. 4. San Juan. 5. La Pampa. 6. Córdoba. 7. Santiago del Estero. 8. Formosa. 9. Corrientes. 10. Chaco. 11: Santa Fé. Bolivia (departments): 12. Cochabamba. 13. Potosi. 14. La Paz. 15. Chuquisaca. Paraguay (departments): 16. Boquerón. 17. Presidente Hayes. Peru (departments): 18. Arequipa. Brazil (states): 19. Río Grande do Sul. 20. São Paulo. 21. Bahía. The map was created for this review with QGIS 3.32.1 (QGIS Development Team 2023. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org)

that bug dispersal between sites might be determined by other factors than distance such as host availability or microclimatic conditions.

An analysis of first-generation migrants detected more putative migrants in the core than in the peripheral area. This apparent contradiction with higher structuring in the core area could be due to the higher probability of detecting migrants when sources are more differentiated [42]. Sex-bias dispersal tests suggested higher female dispersal rates.

A temporal analysis of only 3 houses in the Medanitos locality, Catamarca, Argentina, compared the genetic variability in samples collected before and 16 months after a round of insecticide spraying [43]. Houses separated by the Abaucán river belonged to distinct genetic groups. In addition, putative migrants with (3.8%) and without (4.7%) a sampled source were identified. The levels of genetic variability of the only housed infested after insecticide spraying was not affected by the treatment. However, the allelic composition of the population changed: low frequency alleles were lost, the estimated effective population size diminished, and several new allelic variants were detected. These results suggest an important role of migration restoring the variability after insecticide spraying in the Dry Chaco. In a latter study, Pérez de Rosas et al. [44] examined insects collected in 22 domestic or peridomestic sites from 15 houses in the San Martin locality (Catamarca). Distances among houses ranged between 10 m and 20 km. All the houses had received insecticide spraying 4 years before the study. All the houses showed departures from HWE, particularly an excess of homozygotes. The authors discuss the possibility of null alleles, sub-structuring, and/or inbreeding. Of the collected insects, 9.6% were identified as first-generation migrants. These insects putatively originated in 15 of the 22 studied sites and moved to 17 sites. Despite this, differentiation among sites was significant, even when two sites belonged to the same household. An IBD analysis revealed a mild correlation between genetic and geographic distances (Mantel test $r=0.35$, $p=0.014$). However, when considering only houses separated up to 400 m, the correlation significantly strengthened (Mantel $r=0.81$, $p=0.0005$). The authors speculate that these differences indicate a significant role of migration in structuring diversity at shorter distances while genetic drift seems to prevail at higher geographic distances. Consistent with the findings of Marcet et al. [41••] dispersal seemed to be female-biased, as indicated by spatial autocorrelation and kinship analysis.

The previous studies in Argentina were conducted in the Dry Chaco and in communities under long-term vector interventions or sprayed with insecticides 3–5 years before. In contrast, no information was available for the Humid Chaco and communities under no systemic vigilance for a long time. Piccinali and Gürtler [40] analyzed genetic structure in a small area of 6.32 km^2 near the city of Pampa del Indio (Chaco) without insecticide interventions in the last 12 years. Houses were separated by distances ranging from 180 m to 6300 km. Insects were collected from 16 domestic and peridomestic sites in 14 houses. Despite the fact that they used the same set of microsatellites, variability was lower in this area than in the work of Marcet et al. [41••]. Interestingly, a few sites showed departures from HWE, contrary to what was observed in Catamarca [43, 44] and Santiago del Estero [41••]. These results support the hypothesis of Breniere et al. [37] that in *T. infestans* sites are a close proxy to a deme. The authors found significant differentiation among sites and a better performance of clustering algorithms that include spatial information. In addition, they proposed the hierarchical island model with stratified migration better explain the patterns of variability found in the area. This migration model has demes arranged into groups and different migration rates between demes within and between groups [45].

High rates of house reinfestation after the application of insecticide have been frequently reported for *T. infestans* [46–49]. Identifying the sources of reinfesting bugs is a crucial element in implementing improved vector control strategies. If the insects collected after insecticide spraying are local survivors, it is advisable to employ enhanced techniques for spraying persistently infested houses [50]. Piccinali et al. [51•] analyzed insects collected after 4, 8, and 12 months of insecticide spraying in the same houses surveyed in [40]. Genetic variability exhibited comparable levels between the baseline and post-spraying populations; however, 13 low-frequency alleles remained undetected in the post-spraying sample, indicating a loss of genetic variants. Most F_{ST} values were non-significant between insects collected before and after the insecticide application at the same site. Moreover, baseline and post-spraying populations clustered together in a neighbor-joining tree, and some of them in a discriminant analysis of principal components (DAPC). Assignment tests suggested multiple putative sources (including the house of collection) for

Table 1 Microsatellites used for population genetic studies in *T. infestans*

Locus	Repeat array	Clone size	Allele size range	Na
TiA02[a]	$(GT)_{6}G(GT)_{8}AT(GT)_{3}$	197	171–226	13
TiC02[a]	$(GT)_{10}$	159	157–211	14
TiC08[a]	$(GT)_{11}ATGTATT(GT)_{2}$	203	197–227	7
TiC09[a]	$(GT)_{9}$	139	135–159	5
TiD09[a]	$(GT)_{14}(ATGT)_{3}$	208	188–230	14
TiE02[a]	$(GT)_{13}CTGCCT(GTGC)_{2}$	147	147–167	8
TiE12[a]	$(GT)_{9}GGGA(GT)_{9}$	301	301–323	7
TiF03[a]	$(GT)_{17}$	176	162–216	19
TiF11[a]	$(GT)_{10}$	194	197–239	8
TiG03[a]	$(TA)_{10}CATAT(GT)_{11}GC(GT)_{4}AT(GT)_{2}$	236	200–254	12
Tinfest_ms3[b]	$(GT)_{10}G(GT)_{2}GC(GT)_{2}GG(GT)_{2}$	200	160–204	8
Tinfest_ms5[b]	$(CA)_{25}$	396	364–396	15
Tinfest_ms22[b]	$(TG)_{9}$	177	162–196	13
Tinfest_ms23[b]	$(CA)_{5}CC(CA)_{5}AA(CA)_{2}$	161	148–177	5
Tinfest_ms27[b]	$(CT)_{13}$	299	290–314	11
Tinfest_ms42[b]	$(CA)_{16}$	216	206–246	15
Tinfest_ms56[b]	$(CA)_{8}\ T(AC)_{3}$	163	156–170	7
Tinfest_ms64[b]	$(CT)_{6}\ (GC)_{4}CT(CA)_{13}$	160	146 – 162	9
Tinfest_ms65[b]	$(GA)_{17}$	231	222–266	17
Tinfest_ms19[c]	$(CA)_{15}$	348	334–375	19

[a] [26]

[b] [27]

[c] [28]

Na number of alleles

most post-spraying insects, with only 3.6% individuals attributed to immigration from other unaccounted sources. These results are compatible with the belief that house reinfestations in the Argentine Chaco are mostly explained by survival of insects within the same community (i.e., residual foci).

Finally, in a recent study in Pampa del Indio, microsatellite genotypes and wing size and shape were integrated to assess *T. infestans* populations in the Argentine Chaco [52]. Results showed significant genetic structure at a small spatial scale (2.2–15 km), partially aligning with morphological groups. Three main genetic groups were found with varying levels of admixture, as well as extensive sub-structuring, supporting once more the hierarchical island model of migration in this area [45]. Females displayed greater number of microsatellite alleles than males, which showed signs of IBD. These results are consistent with previous ones [41••, 44] and suggest higher dispersal capacity in females. While genetic groups differed in wing size, no differences in wing shape were noted. Among potential migrants (8% of sampled individuals), no differences were observed in sex, ecotope, wing shape, or size compared to non-migrants. However, migrant males exhibited lower weight-to-length ratios, suggesting a poorer nutritional state.

Role of Sylvatic Foci in Reinfestation

After the discovery of several sylvatic foci of *T. infestans*, there was a growing interest in analyzing the possible role of these populations in the reinfestation process. Noireau et al. [11] reported the first melanic sylvatic *T. infestans* individuals, also known as "dark morphs," in the Bolivian Chaco. Later, a dark morph population was found in the Argentine Chaco associated with parrot nests in an extended and old hardwood forest called "El Impenetrable" [16]. When compared to the closest peridomestic population, situated 7 km from the collection site, microsatellites showed significant differentiation between sylvatic and peridomestic bugs and a low and asymmetric gene flow, with more bugs moving from the peridomicile to the sylvatic environment [53].

Later, non-melanic sylvatic foci of *T. infestans* were found close to the villages studied in [41••], in selectively deforested areas and in a secondary forest [17]. A neighbor-joining tree based on microsatellite genotypes indicated that sylvatic bugs clustered among domestic and peridomestic individuals with no discontinuities. In addition, a statistical parsimony network using mtCOI and mtCytb haplotypes revealed two sylvatic variants shared with domestic and peridomestic bugs, while the remaining variants were distributed throughout the network without forming distinct clusters. Sylvatic sites were colonized by up to 5 different females and microsatellite sibship analyses indicated that bugs from the same site with the same mitochondrial haplotype were always full or half sibs. Spatial analysis also supported an association of some sylvatic foci with the infestation in neighboring domestic and peridomestic houses. The authors inquired into the viability of these small populations and if they could perdure without immigration from domestic and peridomestic sites or if they function as temporary refugees. Besides these two options, they suggest that vector control programs should consider the potential occurrence of sylvatic foci around the target communities.

In a separate study conducted in rural Cotapachi, Cochabamba, Bolivia, Richer et al. [54] examined the genetic variability of *T. infestans* collected from the northern, western, and southern hills, two distinct rocky outcrops known as Inca Wall and peridomestic rocks, and two peridomestic sites. The geographic distances between these sites ranged from 250 to 1100 m. No IBD pattern was observed, and no differences were detected among the insects from the 3 hills. Bugs from the peridomestic rocks and houses were different from the ones from the surrounding sylvatic sites. The pattern of restricted gene flow between some of these close sites supports the hypothesis that *T. infestans* does not disperse by flying but primary by walking in this high-altitude valley of Cochabamba. In addition, the significant genetic differentiation detected between domestic and sylvatic populations suggest a limited short-term role of sylvatic *T. infestans* in the process of reinfestation of insecticide-treated houses of that Andean area.

Breniere et al. [37] conducted an analysis of the genetic connectivity between domestic and peridomestic *T. infestans* and sylvatic bugs collected in the surroundings. No differentiation was observed between sylvatic and domestic/peridomestic populations in Sapini (La Paz) and Quillacollo (Cochabamba), as determined by AMOVAs and Structure analyses. In Thago Thago (Potosi), different genetic clusters were found in sylvatic and domestic/peridomestic populations. Putative first-generation migrant rates were 5.3% in Sapini, 2.6% in Quillacollo, and 7.4% in Thago Thago, including males, females, and nymphs. These findings support the hypothesis of bug dispersion between ecotopes, both by flying and walking, and suggest a putative sylvatic origin for reinfesting bugs, at least in some localities from Bolivia.

More recently, Rojas de Arias et al. [55•] studied four communities in the Paraguayan Chaco before and after a residual spraying campaign. The post spraying sample included sylvatic individuals collected with a trained dog at distances ranging from 230 m to 3.6 km from the nearest household. In this study, all the bugs collected in different houses were considered a single population, opposite to previous microgeographic studies where the site of the house was considered a population. All baseline samples were differentiated according to F_{ST}s, and the two reinfesting samples did not differ from their respective baseline samples. Regarding the role of sylvatic individuals, in Campo Largo village, the reinfesting population was likely founded only by domestic survivor foci. However, in 12 de Junio village, reinfesting bugs were likely derived from baseline survivors and sylvatic bugs, pointing to a different role of sylvatic foci in reinfestation depending on the locality.

Genetic Structure of Insecticide-Resistant Populations

Toxicological studies have confirmed several mechanisms of pyrethroid resistance in *T. infestans*, which have challenged the primary method for Chagas disease control [56]. Fronza et al. [57] reported an area in the Argentine Chaco with the highest levels of deltamethrin resistance found so far. Piccinali et al. [58] applied microsatellites to study the genetic structure of population samples with high, low, and no insecticide resistance in this area. The most diverse populations were those susceptible or with low resistance degrees while the high resistance populations had lower observed heterozygosity (Ho) and some monomorphic loci. These results were confirmed by a negative association between Ho and the resistant ratio, in agreement with a loss of neutral variation in these populations, possibly associated with selective sweeps for resistant variants. A DAPC differentiated all but the three high resistance populations while a Structure analysis showed that these populations shared a genetic group in high proportion, almost not present in the other populations. These results suggest a common origin of the three high resistance foci.

Marcet et al. [59] analyzed genetic structure of one domestic and four sylvatic populations in Bolivia with different pyrethroid insecticide susceptibility. Four populations belonged to the Cochabamba Department and the remaining one to the Potosí Department. As in Argentinean populations, the authors found high differentiation according to a Bayesian cluster analysis, F_{ST}s indices, and a pattern of IBD, with a correlation coefficient close to the one reported by Pérez de Rosas et al. ($R_{XY} = 0.413$; $p < 0.01$). Despite their decreased susceptibility to deltamethrin, only one sylvatic individual carried a *kdr* mutation (a mutation in the *Vgsc* gene that confers DDT and pyrethroid resistance in insects) and high genetic variability was found in the five populations, particularly in the locality of Mataral. HWE departures were attributed to the Wahlund effect or inbreeding because samples came from laboratory strains.

Genetic Structure in Urban Environments

All the previous examples come from the rural environment, the historical area of incidence of vectorial transmission of Chagas disease. However, the rapid urbanization across southern South America has given place to the phenomenon of urban Chagas [60]. Cities create new suitable habitats for the establishment of *T. infestans* populations, and some authors have investigated how the dispersal patterns of the species may be altered in this new scenario. Foley et al. [61] tried to disentangle if in the urban–rural interface of Arequipa, in Peru, *T. infestans* dispersal is mostly actively (by flying or walking) or passively (human assisted) mediated. The compared urban, periurban, and rural districts separated by 1.8 to 41 km. Levels of variability were similar across districts and departures from HWE were found in all of them. Despite the fact that not private alleles were found, significant genetic differentiation was detected according to F_{ST}s, PCA, and Structure. The IBD model was tested at several geographic scales, and significant spatial autocorrelation was found only at distances less than 5 km suggesting that human activities are an important factor in the displacement of *T. infestans* in Arequipa.

Posteriorly, Khatchikian et al. [62] made a more detailed study across a transect in the urban district of Mariano Melgar, Arequipa. The authors founded a very fine spatial structuring pattern of genetic variability. Most city blocks had the same genetic group with a patchy distribution across the transect and genetic distances were higher between individuals from different blocks than between insects from the same block. The genetic neighborhood (a measure of the autocorrelation of genotypes across a range of distance classes) was estimated in 225 m and the western (older) located blocks had higher heterozygosity than eastern (newer) blocks. These results pointed to a demographic history of *T. infestans* in Arequipa characterized by persistent local dispersal and sporadic long-distance migration events. Local dispersal mainly involves movements between households on the same city block with rare dispersal to houses across a city street. According to these conclusions, urban streets act mainly as barriers to *T. infestans* dispersal, and strategies of vector control mediated by insecticide spraying should be targeted to an initial application of insecticide on the city block where the infestation was detected.

Conclusions

Microsatellites have proven to be suitable molecular markers to study the dynamics of *T. infestans*. *T. infestans* populations are highly structured across various countries, landscapes, and geographical levels. While the IBD model has received mixed support, most studies point to a combination of short-distance active dispersal and long-distance passive dispersal. This structured pattern is evident in both rural and urban settings, where demes are well represented by sites and city blocks. The impact of insecticide spraying on genetic structure is significant, acting as an agent that enhances differentiation. Genetic tests reveal no severe bottlenecks affecting heterozygosity after spraying, yet certain allelic classes are lost. At the village level, reinfestation appears primarily driven by internal residual foci. However, when considering higher geographic scales, a significant number of insects are detected as external immigrants. Finally, the role of sylvatic populations in (re)infestation varies across different geographic areas, with sylvatic foci demonstrating heterogeneous contributions to domestic and peridomestic populations.

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Declarations

Conflict of Interest The author declares no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the author.

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Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

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