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Active dispersal by wild *Triatoma infestans* in the Bolivian Andes

Wilfrid Richer[1], Pierre Kengne[1], Mirko Rojas Cortez[2], Marie Mathilde Perrineau[1], Anna Cohuet[1], Didier Fontenille[1] and François Noireau[1,3]

1 *Unité de Recherche 016, Institut de Recherche pour le Développement (IRD), Montpellier, France*
2 *Programa Nacional de Control de Chagas (PNCCH), Ministerio de Salud, La Paz, Bolivia*
3 *Centro Universitario de Medicina Tropical, Facultad de Medicina, Universidad Mayor San Simon, Cochabamba, Bolivia*

Summary *Triatoma infestans* is the main vector of Chagas disease and target of control programmes in the Southern Cone countries. So far Bolivia is the only country where true *T. infestans* wild foci are documented. The dispersal ability for wild *T. infestans* was studied at microgeographical scale in Bolivian Andes, to assess the possibility for wild populations to actively recolonize insecticide-treated villages. Nine microsatellite loci were used to detect the extent of gene flow between neighbouring collecting sites. The detection of restricted gene flow between close but distinct sylvatic sites supports the hypothesis that wild *T. infestans* does not disperse by flying at high altitude (2 750 m asl). It gradually disperses over small distances by walking within a 'patch' of continuous land cover. The genetic differentiation detected between sylvatic and domestic populations suggests a limited short-term role of wild insects in the process of recolonization of insecticide-treated houses in the Andes.

keywords Chagas disease, *Triatoma infestans*, dispersal, microsatellite, Bolivia

Introduction

Triatoma infestans (Reduviidae, Triatominae) is the main vector of *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae), the causative agent of Chagas disease, in the Southern Cone countries. Therefore, it is the target of control programmes within this region. The expected success of the elimination programme was particularly based on the assumption that *T. infestans* is an almost exclusive domestic vector (Schmunis *et al.* 1996). Nevertheless, fieldwork revealed that wild populations do exist and have a wide distribution in Bolivia, throughout the Andean valleys and the Chaco, and also in neighbouring countries (Noireau *et al.* 2005). In the high Andean valleys, wild *T. infestans* occurs amongst rock-piles and specimens are morphologically similar to domestic ones. The key question concerns the possibility for sylvatic populations to recolonize insecticide treated villages and thus jeopardize control efforts. In other words, it is essential to assess the dispersal of Andean wild *T. infestans* and the gene flow between sylvatic and domestic populations.

Microsatellites are molecular genetic markers, which are highly polymorphic, neutral, and exhibit Mendelian inheritance and codominance (Jarne & Lagoda 1996). Consequently, they have become tools extensively used for determining population structure. García *et al.* (2004) and Marcet *et al.* (2006) have recently isolated and characterized more than 20 microsatellite loci from *T. infestans*. To assess the dispersal ability of wild Andean *T. infestans* at microgeographical scale, we studied genetic variability at nine microsatellite loci and subsequent population structure and gene flow in vector populations from neighbouring collecting sites.

Methods

Study area

Cotapachi (2 750 m asl.; 17 °26′ S, 66 °17′ W) is a rural area located in the Eastern Andean Cordillera, near the city of Cochabamba, Bolivia (Noireau *et al.* 2005; Cortez *et al.* 2006). It is a small cirque valley open to the east and encircled by three hills (northern, western and southern hills) that overhang a lake and culminate at ≈60 m above it. Some dwellings and cultivated areas (market-gardening, maize) are located in the valley, between hills and lake. The inhabitants belong to the Queshua ethnic group and their dwellings are made of wattle and daub construction, and roofed with tile. Two separate rocky outcrops made of huge blocks occur in the valley. The first is named Inca wall by the local population and the second, close to some houses, is called peridomestic rocks. The

zone located on the foot and slopes of hills is more homogeneous. It is covered by rocky outcrops of different dimensions and displays a low diversity of vegetation dominated by thickets. The climate of the Cochabamba valley is dry and hot during the summer, temperate in winter (mean annual temperature 16.3 °C, mean annual rainfall 362 mm).

Collection of triatomines

Five sylvatic sites were investigated in June 2005: southern, western and northern hills, Inca wall and peridomestic rocks (Figure 1). *Triatoma infestans* was searched for in its natural habitat of cracks between rocks and shelters of small mammals located under the rocks (Noireau *et al.* 2005). Baited traps as described by Noireau *et al.* (1999) were used to capture the insects. Collections were also made inside two dwellings of Cotapachi. Legs from triatomines were stored individually at −20 °C in ethanol 70%. Analysed samples are described in Table 1. As geographical outgroup, we used 13 domestic *T. infestans* collected at Mataral, a locality situated approximately 200 km southwest of Cotapachi.

DNA extraction

Genomic DNA was extracted from legs of each individual triatomine following a slightly modified version of the protocol of Edwards (1998).

Microsatellite amplification, genotyping and analysis

Ten microsatellite loci (TiA02, TiC02, TiC08, TiC09, TiD09, TiE02, TiE12, TiF03, TiF11 and TiG03) were selected from published *T. infestans* sequence data (García *et al.* 2004) and amplified by polymerase chain reaction (PCR). Two reaction mixtures were used. For TiA02, TiC02, TiD09, TiE02, TiE12, TiF03, TiF11 and TiG03, PCR amplification was carried out in 25 μl reaction volume of 10 ng of template DNA. Reaction mixture contained 10X PCR buffer with 0.2 mM dNTP, 1.5 mM MgCl2, each primer at 10 pm, and 0.5 U Taq Polymerase (QIAgen, Courtaboeuf, France). For TiC08 and TiC09, the reaction mixture contained 10X PCR buffer with 0.2 mM dNTP, 2.5 mM MgCl2, 10 ρm of each primer, and 0.75 U Taq Polymerase. Amplification was performed under the following conditions: an initial denaturation step at 94 °C for 3 min followed by 35 cycles of 30 s at 94 °C, 49 °C for 30 s and 72 °C for 30 s followed by a 72-°C final extension for 10 min. The forward primer of each locus that produced the expected size was 5′-fluorescent labelled with VIC, PET, FAM or NED dyes (Applied Biosystems, Warrington, UK). PCR products obtained with labelled primers were diluted at 1:30 (A02, C09, E02, F11 and G03 loci) or 1:60 (other loci). Pools consisting of 3.5 μl aliquots of PCR reactions, 0.4 μl of size standard (Applied Biosystems) and 16.1 μl dHi-Di formamide (Applied Biosystems) were loaded on the Applied Biosystems 3130XL Genetic Analyzer. Alleles were sized relative

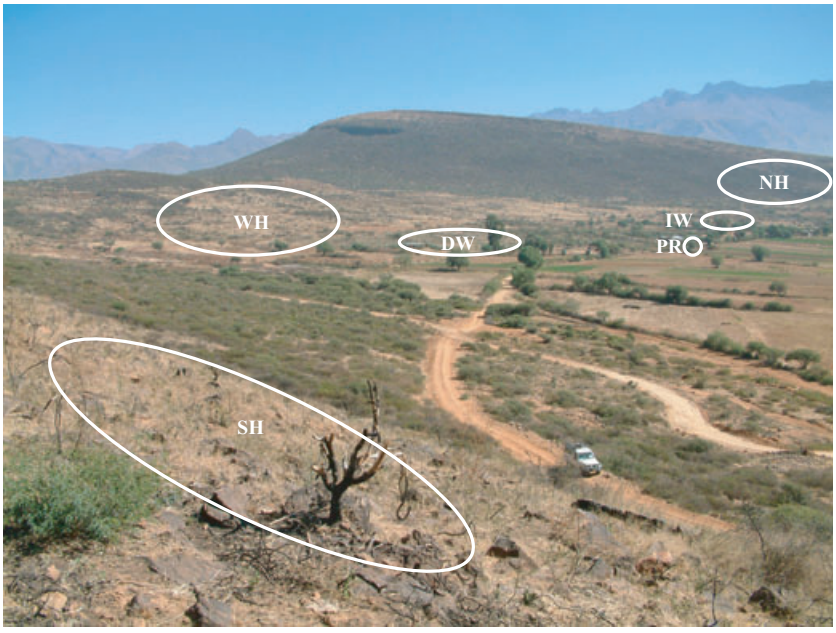

Figure 1 Collecting sites at Cotapachi, Cochabamba valley, Bolivia, 2 750 m asl. (NH = northern hill; WH = western hill; SH = southern hill; IW = Inca wall; PR = peridomestic rocks and DW = dwellings). The distances between the collecting sites are shown in Table 4.

Table 1 Samples analysed according to the collecting site at Cotapachi

Site of collection	Sort of triatomine population	Sample size
Northern hill (NH)	Sylvatic	6
Western hill (WH)	Sylvatic	20
Southern hill (SH)	Sylvatic	12
Inca wall (IW)	Sylvatic	31
Peridomestic rocks (PR)	Sylvatic†	32
Dwellings (DW)‡	Domestic	10
Total		111

†This population was considered as sylvatic because it feeds on small wild mammals and its habitat is natural and not artificial.
‡Triatomines collected from both dwellings were pooled.

to the internal size standard with GeneMapper 3.7 software. For each microsatellite locus, goodness-of-fit with Hardy-Weinberg expectation was tested in each location and overall. Statistical significance for linkage disequilibrium between pairs of microsatellite loci within each population and in the pooled population was computed by exact tests using Genepop 3.4. Genetic differentiation between geographical populations was examined by *F* statistics calculated according to Weir and Cockerham (1984) using *F*stat 2.9.3.2 software (Goudet 2002). Significance of tests was expressed with Bonferroni-adjusted *P*-values (Rice 1989). To test for isolation by distance, the correlation between genetic and geographical distances was assessed by the regression of *Fst*/(1–*Fst*) on the logarithm (ln) of geographical distance (Rousset 1997) and tested using the Mantel test available in GENEPOP.

Results

Genotypes at nine microsatellite loci were determined. The locus TiC08 was rejected because of the absence of amplification product. Except for the locus TiF11, all loci were highly polymorphic (Table 2). At Cotapachi, the mean number of alleles per locus was 7.3 (range 2–12) and expected heterozygosity ranged from 0.34 to 0.85. Similar levels of variability were observed in all populations.

At the level of each population of Cotapachi, only slight deviations from Hardy-Weinberg expectations occurred but they were never significant when the Bonferroni procedure was applied (Table 3). When all specimens of Cotapachi were considered as belonging to one single population, Hardy-Weinberg equilibrium expectation was significantly rejected only for TiF11 ($P = 0.0061$). This locus specific deviation from Hardy-Weinberg expectations could be because of null alleles or selection effect. The TiF11 locus, therefore, was discarded for further analyses. The deficit of heterozygotes across all loci was significant ($P < 0.05$) even when this locus was removed from the analysis, suggesting a subdivision in whole population of Cotapachi (Table 3). A similar result was obtained for the outgroup (Mataral). Linkage disequilibrium measures the non-random association of alleles at different gene loci in a population. None of the 168 pairwise tests performed within populations was significant at the 5% level after Bonferroni-type correction. Linkage disequilibrium within the whole population was detected at the 5% level for only two tests out of 28 after applying the Bonferroni correction.

Pairwise *Fst* estimates between populations and across all eight loci are shown in Table 4. *Fst* values between the six Cotapachi populations and outgroup (Mataral) were

Table 2 Microsatellite variation (number of alleles) at nine loci for *T. infestans* populations from the different collecting sites at Cotapachi (*n*: sample size; He: expected heterozygosity; Ho: observed heterozygosity)

	Populations								
Locus	NH $n = 6$	WH $n = 20$	SH $n = 12$	IW† $n = 31$	PR‡ $n = 32$	DW $n = 10$	Total§ $n = 111$	Outgroup¶ $n = 13$	He/Ho Cotapachi
TiA02	4	6	4	4	4	4	7	6	0.68/0.63
TiC02	4	7	8	7	6	5	12	0	0.56/0.56
TiC09	3	4	5	5	5	3	5	5	0.58/0.53
TiD09	6	8	6	8	6	7	11	10	0.80/0.82
TiE02	5	3	5	5	5	4	5	6	0.65/0.72
TiE12	4	7	6	6	7	6	8	5	0.77/0.70
TiF03	5	10	9	9	7	9	11	9	0.85/0.80
TiF11	2	2	2	2	2	2	2	1	0.34/0.15
TiG03	4	5	4	4	4	4	5	8	0.67/0.66

At the locus TiC02, the sample size varies for populations: †$n = 29$; ‡$n = 26$; §$n = 103$; ¶ $n = 0$.

Table 3 *Fis* coefficient according to the locus and collecting site

	NH	WH	SH	IW	PR	DW	Total†	Outgroup
TiA02	0.231	0.076	0.149	0.025	0.027	−0.068	0.074	0.406
TiC02	−0.351	−0.090	0.246	0.195	0.167	−0.220	0.108	–
TiC09	0.750	0.231	−0.215	0.176	0.071	−0.516	0.205	0.459
TiD09	−0.200	−0.022	0.141	−0.022	0.050	−0.125	0.030	−0.095
TiE02	−0.042	−0.249	0.089	0.041	−0.029	−0.417	−0.045	0.185
TiE12	0.184	−0.010	0.218	−0.003	0.193	0.007	0.090	0.256
TiF03	−0.064	0.205	0.083	0.120	−0.007	−0.013	0.106	0.226
TiF11	0.706	0.782	0.585	0.077	0.000	0.757	0.455*	–
TiG03	−0.190	0.052	0.054	0.042	0.065	0.045	0.126	0.246
Across 8 loci‡	0.030	0.039	0.096	−0.143	0.065	0.065	0.097*	0.226*

†All specimens from Cotapachi were considered as belonging to one single panmictic population; ‡Locus Tiff11 was excluded from the analysis (see text for details).
*$P < 0.05$ assessed through Fisher exact test after Bonferroni correction.

Table 4 Genetic differentiation (*Fst*) and geographical distance among *T. infestans* populations from the different collecting sites

	NH	WH	SH	IW	PR	DW
NH	–	550	1 100	250	350	650
WH	0.0227		1 000	600	600	600
SH	0.0051	0.0086		850	750	450
IW	0.0134	**0.0108**	0.0019		100	450
PR	**0.0539**	**0.1101**	**0.0503**	**0.1048**		300
DW	***0.0434***	**0.0720**	***0.0260***	**0.0475**	**0.0695**	
Outgroup	**0.1613**	**0.1635**	**0.1276**	**0.1509**	**0.1634**	**0.1422**

Fst is below the diagonal. Distances in metres are above the diagonal. Statistical significance of *Fst* was assessed through Fisher exact test after Bonferroni correction (*Fst* with $0.01 < P < 0.05$ in bold-italic; *Fst* with $P < 0.01$ in bold).

high and highly significant, suggesting restricted gene flow between *T. infestans* populations collected 200 km apart. At the level of the sylvatic area at Cotapachi, the PR population showed a significant genetic differentiation with all other populations (*Fst* estimates ranging from 0.0503 to 0.1101). Another significant genetic differentiation was detected between IW and WH. On the contrary, no differentiation was detected between *T. infestans* populations from the three hills ($P = 0.1383$). Finally, restricted gene flow was observed between the domestic population (DW; populations of both dwellings pooled) and all sylvatic populations. Isolation by distance was tested and no significant correlation between genetic (pairwise *Fst*) and geographic distance was detected ($r = 0.28$; $P = 0.75$).

Discussion

To our knowledge, this is the first study investigating population genetics of wild *T. infestans*, the main Chagas disease vector, using microsatellite markers. The studied populations come from an Andean mesothermic valley of Bolivia regarded as the geographical centre of origin for *T. infestans*. From the 10 microsatellite loci tested, eight provided PCR product and were highly polymorphic in a sample of sylvatic *T. infestans* from the Bolivian Andes. The lack of linkage disequilibrium detected among 168 tests suggests the absence of statistical linkage between loci and that the loci provide independent information on the population genetics study. Microsatellite markers did not show significant deviations from Hardy-Weinberg expectations at the population level. Finally, although it was small, this sampling was suitable for genetic population study.

Differentiation level clearly demonstrates that the populations from the three hills (NH, WH and SH) belong to the same reproductive unit, when the population collected from PR belongs to a separate reproductive unit. The detection of restricted gene flow between these close but distinct sylvatic sites supports the hypothesis that the vector does not disperse by flying in the high altitude valley of Cochabamba. Some studies dedicated to *T. infestans* flying ability under more favourable conditions (lowlands of the Chaco) pointed out that this species shows a flight

potential on a village-wide scale and in sylvatic environment (Schweigmann *et al.* 1988; Schofield *et al.* 1992; Noireau *et al.* 2000; Gurevitz *et al.* 2006; Vazquez-Prokopec *et al.* 2006).

Results rather suggests that, at 2 700 m asl in the Andes, the wild *T. infestans* gradually disperses over small distance by walking within a 'patch' that might be characterized as a continuous land cover, with all necessary resources for the persistence of triatomine population (Gustafson & Gardner 1996). On the contrary, when the land cover is disrupted by man made activities (building of dwelling and peridomestic structures, land or livestock farming etc.), the triatomine bugs bump into an unsuitable environment and cannot spread to separate patch by walking. At Cotapachi, the land cover located between the PR and the other natural structures investigated (hills and Inca wall) may be considered as such disrupted area that makes difficult the walking of triatomines.

Cotapachi area included seven dwellings. Before the chemical control performed in 2004, all the dwellings were infested by triatomines (Cortez, unpublished observation). In June 2005, 1 year after the control, two houses contained *T. infestans*. This low domestic infestation rate, as well as significant genetic differentiation detected between domestic and sylvatic populations (despite the small size of domestic sample) strongly suggest restricted gene flow and a limited short-term role of wild insects in the process of recolonization of insecticide-treated houses of this Andean area.

The results of this study proceed from the Cotapachi area but they may most likely be extended to other high altitude sylvatic foci of *T. infestans*. However, *T. infestans* wild populations are not restricted to the high Andean Valleys in Bolivia. They also occur in the Andes of middle altitude and particularly in the subtropical Chaco forest in South-eastern Bolivia, Paraguay and the North of Argentina (Noireau *et al.* 2005; Ceballos *et al.* unpublished observation). Because of the consequent ecological variety of the sylvatic foci, it is essential to determine the role that wild *T. infestans* populations may play as potential source of reinfestation in the different ecosystems where they occur.

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Corresponding Author **François Noireau**, Unité de Recherche 016 Caractérisation et Contrôle des Populations de Vecteurs, Institut de Recherche pour le Développement (IRD), 911 Av. Agropolis, 34394 Montpellier Cedex 5, France. Tel.: +33 4 67416178; Fax: +33 4 67542044; E-mail: francois.noireau@ird.fr

Dispersion active de *Triatoma infestans* sylvestre dans les Andes boliviennes

Triatoma infestans est le principal vecteur de la maladie de Chagas et la cible des programmes de contrôle dans les pays du Cône Sud. Jusqu'à ce jour, la Bolivie est le seul pays où de vrais foyers sylvestres de *T. infestans* sont documentés. La capacité de dispersion du vecteur sylvestre a été étudiée à échelle micro-géographique dans les Andes boliviennes, afin d'évaluer la possibilité pour les vecteurs sauvages de recoloniser activement les villages traités par insecticide. Neuf loci microsatellites ont été utilisés pour mesurer l'importance du flux de gènes entre des sites de collecte voisins. L'observation de flux de gènes restreints entre des sites sylvestres proches mais distincts soutient l'hypothèse que les populations sauvages de *T. infestans* ne se dispersent pas par le vol dans une telle région d'altitude élevée (2 750 m). Ils se disperseraient de proche en proche, sur de petites distances, en migrant sur un terrain à la structure continu. La différentiation génétique détectée entre les populations sylvestres et domestiques suggère un rôle limité des insectes sauvages dans le processus de recolonisation à court terme des maisons traitées par insecticide dans les Andes.

mots clés Maladie de Chagas, *Triatoma infestans*, dispersion, microsatellite, Bolivie

Dispersión activa de *Triatoma infestans* silvestre en los Andes Bolivianos

Triatoma infestans es elprincipal vector de la enfermedad de Chagas y el blanco de los programas de control en los países del Cono Sur. Hasta ahora, Bolivia es el único país donde focos silvestres de *T. infestans* están documentados. La capacidad de dispersión de *T. infestans* silvestre ha sido estudiada a escala micro-geográfica en los Andes Bolivianos, con el fin de evaluar la posibilidad de que vectores silvestres recolonicen activamente poblados tratados con insecticida. Se utilizaron nueve loci microsatélite para detectar el nivel de flujo génico entre sitios de colecta vecinos. La detección de un flujo génico restringido entre sitios cercanos apoya la hipótesis de que *T. infestans* silvestre no se dispersa volando en esta altitud (2 750 msnm). Se dispersaría gradualmente, en pequeñas distancias, caminando dentro de una parcela con una cubierta vegetal continua. La diferenciación genética detectada entre poblaciones silvestres y domésticas sugiere un papel limitado de los insectos silvestres en el proceso de recolonización, a corto plazo, de casas rociadas con insecticida en los Andes.

palabras clave Enfermedad de Chagas, *Triatoma infestans*, dispersión, microsatélite, Bolivia