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Genetic variability, phylogenetic relationships and gene flow in *Triatoma infestans* dark morphs from the Argentinean Chaco

R.V. Piccinali [a,*], P.L. Marcet [b], L.A. Ceballos [a], U. Kitron [c], R.E. Gürtler [a], E.M. Dotson [b]

[a] Laboratorio de Eco-Epidemiología, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina
[b] Centers for Disease Control and Prevention, Division of Parasitic Diseases and Malaria, Entomology Branch, Atlanta, GA, USA
[c] Department of Environmental Studies, Emory University, Atlanta, GA, USA

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ABSTRACT

The recent discovery of sylvatic populations of *Triatoma infestans* outside the Andean Valleys of Bolivia prompted an evolutionary question about the putative ancestral area of origin and dispersal of the species, and an epidemiological question regarding the possible role of these sylvatic populations in the recolonization process of insecticide-treated houses. The finding of a population of sylvatic melanic *T. infestans* (dark morphs) in the Argentinean dry Chaco at 7 km from a peridomestic bug population of typical coloration gave us the opportunity to test both questions simultaneously by employing phylogenetic and population genetic approaches. For this purpose we analyzed sylvatic and peridomestic bugs using sequence-based mitochondrial and nuclear markers (*mtCOI* and *ITS-1*) and microsatellites. Sylvatic bugs were confirmed to be *T. infestans* and not hybrids, and showed high levels of genetic variability and departures from neutral expectations for *mtCOI* variation. New *ITS-1* and *mtCOI* haplotypes were recorded, as well as haplotypes shared with peridomestic and/or domestic bugs from previous records. The peridomestic population was invariant for *ITS-1* and *mtCOI*, but showed variability for microsatellites and signatures of a population bottleneck, probably due to a limited number of founders. Phylogenetic analyses were consistent with the presence of ancestral haplotypes in sylvatic bugs. According to *F*-statistics and assignment methods there was a significant differentiation between sylvatic and peridomestic bugs and gene flow was low and asymmetric, with more bugs moving from the peridomicile to the sylvatic environment. These results support the hypothesis of the Chaco region as the area of origin of *T. infestans*, and a limited role of sylvatic melanic *T. infestans* in peridomestic infestation in the Argentinean Chaco.

1. Introduction

Recent reports of sylvatic populations of *Triatoma infestans* outside the Andean Valleys of Bolivia included melanic morphs (called "dark morphs") in the Bolivian, and Argentinean Chaco (Noireau et al., 1997, 2000; Ceballos et al., 2009), and typical morphs in the Chilean Metropolitan and Valparaiso regions (Bacigalupo et al., 2006, 2010) and the Argentinean Province of Santiago del Estero (Ceballos, 2010). These findings prompted two questions with evolutionary and epidemiological implications. An important evolutionary question is if these populations are old and can provide more insights on the origin and historical routes of dispersal of *T. infestans*. The second, and epidemiologically relevant question, is the possible role of these sylvatic populations in the recolonization of insecticide-treated houses (Noireau, 2009; Noireau et al., 2005).

The origin of *T. infestans* has been under much debate recently. The traditional hypothesis is that this species originated in the Andean highland valleys in Bolivia and was dispersed through two main routes: a north-western one toward Peru and a southern and eastern one to Chile, Paraguay, Argentina, Uruguay and Brazil (Panzera et al., 2004; Bargues et al., 2006). This model involves an initial step of domiciliation of the vector followed by a passive human-mediated spread (Schofield, 1988). A more recent hypothesis also invokes a Bolivian Andean origin, but considers the Cochabamba Valley as a putative center of dispersal of sylvatic *T. infestans* (Cortez et al., 2010). Spreading of sylvatic *T. infestans* is thought to have occurred through the Incan maize production, storage and distribution system in the region and/or by means of traditional pilgrimages. A third hypothesis (Carcavallo, 1998) suggests that *T. infestans*, or an ancestor, dispersed from the dry

* Corresponding author at: Laboratorio de Eco-Epidemiología, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 2do piso, Pabellón 2, Ciudad Universitaria, Int. Güiraldes 2160, C1429EGA, Ciudad Autónoma de Buenos Aires, Argentina.
Tel.: +54 11 4576 3318; fax: +54 11 4576 3318.
E-mail address: rpicci@ege.fcen.uba.ar (R.V. Piccinali).

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Chaco subtropical forest to the North and Northwest. A prediction from this assumption is that dark morph populations from the dry Chaco could be the most ancient of *T. infestans*.

Regarding the epidemiological importance of sylvatic *T. infestans* populations, studies including sylvatic and peridomestic bugs from the same area were carried mainly in the Cochabamba Valley in Bolivia. Bargues et al. (2006) found two shared *ITS-2* and *ITS-1* composite haplotypes between sylvatic and peridomestic bugs from Quillacollo, while one haplotype was found only in sylvatic bugs. However, analyses based on allozymes (Dujardin et al., 1987), patterns of chromosome C-banding and DNA content (Panzera et al., 2004) and mitochondrial DNA (Monteiro et al., 1999; Piccinali et al., 2009) did not detect differences between sylvatic and domestic populations, suggesting that sylvatic foci could be recent derivatives from nearby peridomestic or domestic populations (or vice versa). Alternatively, a high degree of gene flow between both populations could be invoked. However, a reinfestation study after insecticide spraying based on head morphometrics showed significant differences between domestic and sylvatic nymphs in the Bolivian village of Jumach'Uma (Dujardin et al., 1997). Moreover, reinfesting nymphs were more similar to domestic pre-spraying bugs than to sylvatic bugs lending support to the hypothesis that a residual domestic population survived the insecticide application rather than a recolonization process from the sylvatic habitat. Another study using microsatellites in the Andean locality of Cotapachi, Bolivia, also found significant differences between domestic and sylvatic populations separated by only 300–650 m (Richer et al., 2007). These results were consistent with restricted gene flow between domestic and sylvatic bugs in the Cochabamba Valley. Outside Bolivia, a recent study based on mitochondrial DNA showed no differentiation between peridomestic and sylvatic *T. infestans* from Chile (Torres-Pérez et al., 2010).

In 2006, a dark morph population was discovered in the Argentinean dry Chaco (Ceballos et al., 2009). This population was found at only 7 km from a peridomestic population of typical coloration bugs. Because *T. infestans* may easily fly >550 m (Schofield et al., 1992) or reach 1500 m (Schweigmann et al., 1988) in an open field, and it may sustain tethered flights for >20 min at speeds of 2 m/s (Ward and Baker, 1982), the study of dark morphs together with peridomesctic bugs from the same area gave us the opportunity to test not only questions about the origin of *T. infestans* but also the putative role of a sylvatic population in the process of house (re)infestation by using phylogenetic and population genetic approaches. The specific goals of this study were: (1) to confirm the taxonomic status of melanic sylvatic *T. infestans* and to exclude the existence of hybrids. For this purpose we used a nuclear marker, the first internal transcribed spacer region (*ITS-1*) of the ribosomal cistron; (2) to analyze genetic variability and search for signatures of population expansions or bottlenecks in sylvatic and peridomestic bugs using the mitochondrial gene cytochrome oxidase I (*mtCOI*) and microsatellites; (3) to compare *mtCOI* haplotypes of sylvatic bugs with previously reported sylvatic, domestic and peridomestic haplotypes from this and other geographical areas, and analyze their phylogenetic relationships; and (4) to estimate the degree of gene flow between sylvatic and peridomestic bugs in the Argentinean dry Chaco with *mtCOI* and microsatellites.

2. Materials and methods

2.1. Collection area

Field work was conducted 40 km south of Fuerte Esperanza (25°30′S, 61°50′W), General Güemes Department, Chaco Province, Argentina. This area is an extended and old hardwood forest called "El Impenetrable" and is located in the dry Chaco ecoregion. Fifteen sylvatic melanic bugs (7 males, 3 fourth instars, 4 fifth instars and 1 third instar) were collected with mouse-baited traps or fumigant canisters in hollow trees and in blue-fronted parrot (*Amazonia aestiva*) and blue-crowned conure (*Aratinga acuticaudata*) nests (Ceballos, 2010; Ceballos et al., 2009).

Fifteen peridomestic bugs (2 males, 7 females and 6 fifth instars) were collected by manual searches with a dislodging agent (0.2% tetramethrin) in a single peridomestic storeroom at 7 km from the nearest site where a sylvatic dark morph *T. infestans* was found (Ceballos et al., 2009). All peridomestic bugs had the typical coloration of *T. infestans*.

2.2. Mitochondrial and nuclear sequences

A 661-bp fragment of *mtCOI* was PCR-amplified and sequenced from 15 peridomestic bugs and 4 of the sylvatic bugs for this paper as described elsewhere (Cortez et al., 2007; Piccinali et al., 2009). Data from the remaining 10 sylvatic bugs were published in two previous studies (Ceballos et al., 2009; Piccinali et al., 2010). The amplification and sequencing of a 544 bp fragment of the nuclear region *ITS-1* was made with the conditions and primers described in Rodriguero (2009). Sequences were aligned manually or with MEGA 4.1 software (Kumar et al., 2008).

2.3. Microsatellites

Ten microsatellite loci, Tims3, Tims5, Tims19, Tims22, Tims23, Tims27, Tims42, Tims56, Tims64 and Tims65 were used for multilocus genotyping of sylvatic and peridomestic bugs (Marcet et al., 2006, 2008). Multiplex PCR were carried out in pairs of loci with the same annealing temperature and primers marked with different dye colors (Marcet, 2009). DNA fragment analysis was performed in an ABI 3130 automated DNA sequencer and allele sizes were determined with GeneMapper v3.7 (Applied Biosystems).

2.4. Data analyses

2.4.1. mtCOI and ITS-1

Nucleotide diversity was estimated according to Watterson's estimator θ_W (Watterson, 1975) and Tajima's estimator π (Tajima, 1983). Haplotype variability was described as the total number of haplotypes (h) and the haplotype diversity (Hd). D_T (Tajima, 1989), F_S (Fu, 1997), and R_2 (Ramos-Onsins and Rozas, 2002) neutrality tests were applied to determine whether populations of sylvatic and peridomestic *T. infestans* were in mutation-drift equilibrium. All analyses were performed with DNASP v5.10 (Librado and Rozas, 2009).

Phylogenetic relationships between sylvatic and peridomestic *T. infestans mtCOI* haplotypes from this and two previous studies (Piccinali et al., 2009, 2010) were performed with a Bayesian approach. *Triatoma delpontei* (Genbank accession number FJ439768) was used as an outgroup due to its close relationship to *T. infestans*. The model of evolution (HKY+I+Gamma) was chosen with MRMODELTEST 2.3 using four alternative hierarchical likelihood ratio tests and the Akaike information criterion (Nylander, 2004). MRBAYES 3.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) default priors were assumed and the analysis was run for four million generations (ngen = 4,000,000) with a heating scheme (temp = 0.05). Trees were sampled each 100th generation (samplefreq = 100) and the first 10,000 topologies were discarded (burnin = 10,000) to compute the 50% majority rule consensus tree. Convergence of the Markov Chain Monte Carlo analyses was investigated with the standard deviation of the split frequencies and the diagnostics

implemented in AWTY (Nylander et al., 2008). The phylogram was drawn with MRENT 2.2 (Zuccon and Zuccon, 2010). A statistical parsimony network (Templeton et al., 1992) of a subset of the total haplotypes was built with TCS 1.21 (Clement et al., 2000).

Historical migration rates between sylvatic and peridomestic *T. infestans* from the Argentinean Chaco were estimated with the Bayesian coalescence-based approach implemented in LAMARC 2.1.3 (Kuhner, 2006). Only *mtCOI* sequences were used because LAMARC does not consider gap polymorphisms, as those found in *ITS-1*. Base frequencies and transition/transversion rate were set at values estimated by MRMODELTEST 2.3 (Nylander, 2004). The search strategy was based on two independent runs of a long chain of 40,000 samples, a sampling interval of 40 and a burnin phase of 10,000 (25%) with two sub-runs. The performance of each Markov Chain Monte Carlo search was evaluated with TRACER 1.5 (Rambout and Drummond, 2009).

2.4.2. Microsatellite loci

The presence of null alleles and allelic dropouts was investigated with MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004). Allele number (Na) per locus and population, expected (He) and observed heterozygosity (Ho), fit to Hardy–Weinberg equilibrium (HWE) and Weir and Cockerham F_{ST} statistic (F_{STWC}) were computed with ARLEQUIN 3.5 (Excoffier and Lischer, 2010). Linkage disequilibrium (LD) exact tests between microsatellite loci pairs were calculated with GENEPOP 4.0 (Rousset, 2008). Bonferroni correction for multiple comparisons was applied when needed (Rice, 1989).

BOTTLENECK 1.2.02 (Cornuet and Luikart, 1996) was used to detect signals of past bottlenecks or population expansions. This analysis relies on the comparison between the heterozygosity from observed data to heterozygosity in a simulated population in mutation-drift equilibrium. Bottlenecked populations are predicted to show an excess of heterozygosity, whereas the opposite pattern is expected in an expanding population (Cornuet and Luikart, 1996). A sign test was used to determine whether a significant majority of loci had a heterozygosity excess or defect using the infinite allele model (IAM), the two phase model with 70% and 90% of single-step mutations (TPM), and the stepwise mutation model (SMM).

Population structure was examined using the Bayesian model-based approach of Pritchard et al. (2000) implemented in STRUCTURE 2.3.3. This method is based on a model in which there are *K* populations (where *K* may be unknown), each of which is characterized by a set of allele frequencies at each locus. Individuals in the sample are probabilistically assigned to one population, or jointly to two or more populations if their genotypes indicate that they are admixed. It is assumed that loci are at HWE and in linkage equilibrium within each population. The number of clusters evaluated (=*K*) ranged from 1 to 5. The analysis was performed using ten replicate runs per *K* value, a burn-in period length of 100,000 and a run length of 500,000. No prior information on the origin of the individuals was used to define the clusters. All the analyses were run under both the independent and the correlated allele frequency models to test their performance in case of the occurrence of subtle population structure. The selection of the *K* value that better recovered the structure of data was performed comparing the rate of change in the log-probability of the data between successive *K* values (Evanno et al., 2005) implemented in STRUCTURE HARVESTER 0.56.4 (Earl, 2010).

The detection of first-generation migrants was carried out with the Bayesian individual assignment method of Rannala and Mountain (1997) and the Monte Carlo resampling method of Paetkau et al. (2004) implemented in GENECLASS 2 (Piry et al., 2004). This approach is based on the comparison of the marginal probability of a given individual multilocus genotype to the distribution of marginal probabilities of randomly generated multilocus genotypes (10,000 replicates). If the *P*-value obtained was below 0.01, the individual was considered not to belong to the reference population.

3. Results

3.1. ITS-1 and mtCOI

Comparison of the *mtCOI* and the *ITS*-1 sequences with published sequences (Piccinali et al., 2009; Bargues et al., 2006) confirmed that all sylvatic bugs except one were *T. infestans*. One sylvatic fourth-instar nymph for which the *mtCOI* could not be amplified (Ceballos et al., 2009) had a high similarity (98%) with *Triatoma sordida ITS-1* sequence (Genbank accession number AJ576063); therefore it was excluded from the study.

The sylvatic population exhibited more genetic variability for both markers (Table 1). Twelve *mtCOI* haplotypes were found in sylvatic bugs and all variable sites were synonymous transitions. Eight haplotypes were private to this population, while the remaining four were previously recorded (Piccinali et al., 2009) in peridomestic or domestic *T. infestans* from Argentina (*d*, *r*, *t*, and *w*) and Uruguay (*d*) (Table 2). No shared mitochondrial haplotypes were found between Argentinean dark morphs and dark morphs or sylvatic non-melanic *T. infestans* from Bolivia (Piccinali et al., 2009).

Three *ITS-1* haplotypes were found in sylvatic bugs. One was the same as haplotype A from Bargues et al. (2006) and the other two were new variants named D and E. They only differed from haplotype A by one insertion each (Table 3).

No variability was found in the peridomestic population, neither for nuclear nor mitochondrial gene regions (Table 1). The only mitochondrial haplotype found was the most widespread haplotype (*c*) reported for *T. infestans* in Argentina, which has also been recorded in Uruguay and the Bolivian Department of Tarija (Table 2, Piccinali et al., 2009, 2010). All peridomestic bugs had *ITS-1* sequences with haplotype A (Bargues et al., 2006), which has been recorded in Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay (Bargues et al., 2006).

A significant departure from neutral expectations in *mtCOI* variability was found in the sylvatic population using the haplotype-based *Fs* test statistic (Table 1).

Table 1
Nucleotide variability and neutrality tests.

Molecular marker	Population	*N*	θ_W	π	*h*	*Hd*	D_T	F_S	R_2
mtCOI	Sylvatic	14	0.0119	0.0106	12	0.978	−0.459	−3.987[a]	0.114
	Peridomestic	15	0.0000	0.0000	0	0.000	–	–	–
nITS-1	Sylvatic	14	0.0012	0.0005	3	0.538	−0.200	−0.207	0.169
	Peridomestic	15	0.0000	0.0000	0	0.000	–	–	–

N: number of sequences; θ_W: Watterson's estimator; π: Tajima's estimator; *h*: number of haplotypes; *Hd*: haplotype diversity; D_T: Tajima's test. F_S: Fu's test. R_2: Ramos-Onsins and Rozas' test.

[a] $P < 0.05$.

Table 2
mtCOI haplotypes found in peridomestic and sylvatic *T. infestans* from Chaco, Argentina.

Haplotype	Nucleotide position																									N	Ecotope	Previous records	Genbank
	1	1	1	1	1	1	1	1	1	2	2	2	2	3	3	4	4	4	5	5	5	5	5	5					
	8	0	0	1	1	2	3	3	4	4	6	3	4	6	6	6	8	1	7	8	1	2	4	5	6	8			
	8	3	6	2	3	4	3	9	2	5	3	5	4	2	6	7	5	9	6	7	4	6	7	0	6	9			
c	G	G	C	T	C	T	C	A	G	T	C	G	A	T	C	C	A	T	T	C	C	G	A	A	C	C	15	P	Arg (9 Prov.), Bol (1 Dpto.), Uru (1 Dpto.)	EF451012[a]
d	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	S	Arg (3 Prov.), Uru (1 Dpto.)	EF451013[a]
r	.	A	.	C	.	C	.	G	A	.	T	.	.	.	T	.	.	.	.	.	.	A	G	.	.	.	2	S	Arg (1 Prov.)	EF451027[a]
t	.	A	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	S	Arg (2 Prov.)	EF451029[a]
w	.	A	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	1	S	Arg (1 Prov.)	EF451032[a]
am	.	A	T	C	.	C	T	.	A	.	T	.	.	.	.	.	.	C	.	.	.	A	.	G	.	.	2	S		FJ811845[b]
ar	.	A	.	.	.	.	.	.	A	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	1	S		FJ811846[b]
as	.	A	T	.	.	.	.	.	A	.	.	A	.	.	.	.	.	.	C	.	T	.	.	.	.	T	1	S		FJ811847[b]
aw	.	A	.	C	.	C	.	G	A	.	T	.	.	.	T	T	.	.	.	.	.	A	G	.	.	.	1	S		FJ811848[b]
aaa	.	A	.	C	T	C	.	G	A	.	T	.	.	.	T	.	.	.	.	.	.	A	G	.	.	.	1	S		GQ479004[c]
aab	.	A	.	.	.	.	.	.	A	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	T	.	1	S		GQ479005[c]
aac	A	A	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	S		HQ437703[d]
aad	.	A	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	1	S		HQ437704[d]

Dot: identity with nucleotide in first haplotype; *N*: number of sequences; P: peridomestic; S: sylvatic; Arg: Argentina; Bol: Bolivia; Uru: Uruguay. In parenthesis: number of Provinces (Prov.) or Departments (Dpto.) where the haplotype was found.
[a] Piccinali et al. (2009).
[b] Ceballos et al. (2009).
[c] Piccinali et al. (2010).
[d] Present work.

A Bayesian fifty-percent majority rule consensus tree was built with *T. infestans mtCOI* haplotypes from this and previous studies (Piccinali et al., 2009, 2010) (Fig. 1). Argentinean sylvatic haplotypes (regardless of whether they were shared or not with peridomestic bugs) did not form a clade. Three of them were located in the base of the tree; haplotype *aaa* was the sister group of all the remaining haplotypes, whereas haplotypes *aw* and *r* formed a polytomy with the clade of Bolivian plus Argentinean haplotypes. The remaining nine haplotypes from the sylvatic bugs of this study were part of the monophyletic group that clusters most Argentinean variants. Within this group, haplotype *am* formed a well supported clade with haplotype *b*, and haplotypes *d*, *t*, *w*, *ar*, *as*, *aab*, *aac* and *aad* were part of a bigger clade that comprises 37 haplotypes, including two shared haplotypes between Argentina, Uruguay and Bolivia and a Bolivian haplotype only found at Tarija. Relationships within this clade were not resolved or had low clade credibility values (53–61%). Because intraspecific phylogenies are sometimes better represented by a network due to persistent ancestral haplotypes in the populations (Posada and Crandall, 2001), a haplotype network was built with these 37 haplotypes. This large and mainly Argentinean clade was star-shaped and had several ambiguous connections, suggesting the presence of homoplasy in the data (Fig. 2). Haplotypes found in sylvatic bugs tended to be located on the tips of the network, a pattern expected for newly arisen variants. However, the most central haplotype (i.e. the one with more connections to other haplotypes) was *d* which is shared between sylvatic and peridomestic bugs.

Estimates of historical gene flow between the Argentinean Chaco peridomestic and sylvatic populations based on *mtCOI* were consistent between two independent runs of LAMARC (Table 4). The number of female migrants was close to one per generation from the peridomicile to the sylvatic habitat and one per 100 generations in the opposite direction.

Table 3
nITS-1 haplotypes found in peridomestic and sylvatic *T. infestans* from Chaco, Argentina.

Haplotype	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
	6	6	7	9	9	9	9	9	1	1	2	2	2	2	2	2	2	2	2	2
	3	4	8	0	1	2	3	4	8	9	0	1	2	3	4	5	6	7	8	9
A	A	T	–	A	T	A	A	A	–	–	–	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	–	–	T	A	A	A	T	A	A	A	A	T	A	A
C	.	.	–	.	.	.	.	.	T	A	A	A	T	A	A	A	A	T	A	A
D	.	.	–	.	.	.	.	.	–	–	–	–	–	–	–	–	–	–	–	–
E	.	.	A	.	.	.	.	.	–	–	–	–	–	–	–	–	–	–	–	–

Haplotype	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	5	N	Ecotope	Genbank
	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	1	1			
	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	6	8			
A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	–	24	P, S	AJ576051[a]
B	A	A	A	G	C	C	G	C	A	A	A	G	A	C	C	–	–			AJ582024[a]
C	A	A	A	G	C	C	G	C	A	A	A	G	A	C	C	–	–			AJ582025[a]
D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	.	A	1	S	HQ437705[b]
E	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	.	–	4	S	HQ437706[b]

Dot: identity with nucleotide in first haplotype; dash: gap; *N*: number of sequences; P: peridomestic; S: sylvatic. Haplotypes B and C are only shown for comparison purposes.
[a] Bargues et al. (2006).
[b] Present work.

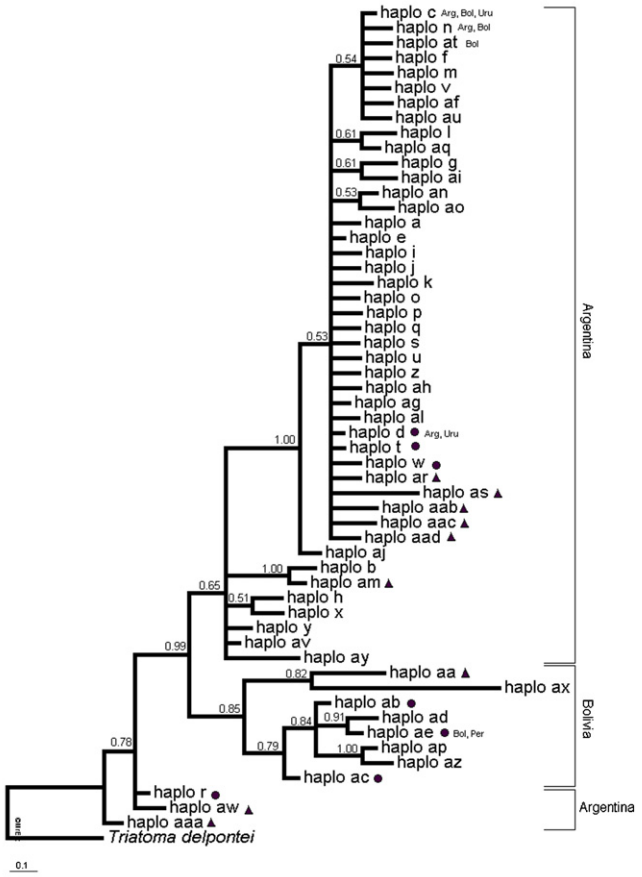

Fig. 1. Phylogenetic relationships between *Triatoma infestans mtCOI* haplotypes. Numbers above branches are clade credibility values higher than 50%. Triangle: sylvatic haplotype; circle: shared haplotype between peridomestic and sylvatic bugs. The remaining haplotypes are all domestic and/or peridomestic (Piccinali et al., 2009, 2010). Arg: Argentina; Bol: Bolivia; Per: Peru; Uru: Uruguay. The scale bar indicates nucleotide substitutions per site.

3.2. Microsatellite loci

In contrast with *mtCOI* and *ITS-1* analyses, both sylvatic and peridomestic populations exhibited variability for 10 microsatellite loci. No evidence of linkage disequilibrium was found between any pair of loci (results not shown). The number of alleles in the sylvatic population ranged from 3 to 15, and in the peridomestic population from 3 to 10. In general, sylvatic bugs exhibited higher number of alleles per loci, with the exception of Tims3, Tims56 and Tims64 (Table 5). Observed heterozygosities varied from 0.18 to 1 in the sylvatic population, and between 0.08 and 0.80 in peridomestic bugs. Loci Tims64 and Tims65 depicted strong departures from HWE in both populations, and loci Tims19 and Tims22 in sylvatic bugs only. The analyses performed with MICRO-CHECKER were consistent with the presence of null alleles at these four loci, as suggested by a general excess of homozygotes for most allele size classes. Estimated genotypic frequencies for individuals carrying a null allele in the peridomestic population were around 65% for loci Tims64 and Tims65, and in the sylvatic population around 55% for loci Tims64, and 10% for loci Tims19, Tims22 and Tims65. Because the number of loci seems to have greater effects on the accuracy of assignment tests than does the presence of null alleles (Carlsson, 2008), we excluded from the remaining analyses only loci Tims64 and Tims65 which exhibited higher frequencies of null alleles.

Most individual loci as the global multilocus F_{STWC} showed a significant differentiation between sylvatic and peridomestic bugs (Table 5). The Bayesian analysis of population structure also supported this differentiation, and indicated that two genetic clusters ($K = 2$) better recovered the genetic structure of data, both for the correlated and the independent allele frequency models. All sylvatic bugs clustered with high probability in one group, with the exception of one fifth-instar nymph, that had a higher probability to belong to the peridomestic cluster (70%) than to the sylvatic group (30%) (Fig. 3). The GENECLASS analysis also was consistent with this result, being this bug the only one excluded to its reference population and considered a first-generation migrant.

Sign tests showed that the peridomestic population had more loci exhibiting an excess of heterozygosity for the IAM and TPM mutational models than expected by chance (Table 6).

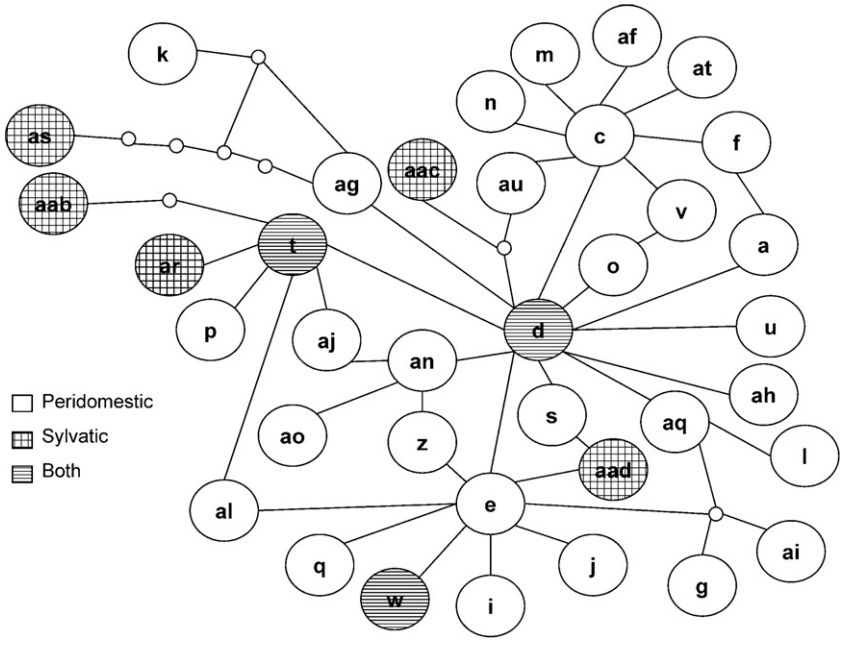

Fig. 2. Statistical parsimony haplotype network. Each connection represents a mutational step. Empty small circles are missing (not found) haplotypes.

Table 4
LAMARC gene flow estimation between sylvatic and peridomestic *Triatoma infestans* from Chaco.

	M_{PS}			M_{SP}			$2N_F\mu_{PS}$			$2N_F\mu_{SP}$		
	MIN	MPE	MAX	MIN	MPE	MAX	MIN	MPE	MAX	MIN	MPE	MAX
RUN 1	0.84	40.1	151.48	0.018	534.3	1349.52	0.0079	0.787	6.229	$2 \times E^{-07}$	0.0083	0.908
RUN 2	0.91	37.8	156.51	0.019	554.2	1336.77	0.0086	0.699	6.381	$2 \times E^{-07}$	0.0079	0.817

M_{PS}: migration rate to mutation rate ratio from the peridomestic to the sylvatic habitat; M_{SP}: migration rate to mutation rate ratio from the sylvatic to the peridomestic habitat; $2N_F\mu_{PS}$: female-based number of migrants per generation to the sylvatic habitat; $2N_F\mu_{SP}$: female-based number of migrants per generation to the peridomestic habitat; MPE: most probable estimator; MIN: minimal value; MAX: maximum value; N_F: female effective population size; μ: neutral mutation rate per generation.

Table 5
Microsatellite genetic variability, HWE departures and genetic differentiation in peridomestic and sylvatic *T. infestans* from Chaco, Argentina.

		Tims3	Tims5	Tims19	Tims22	Tims23	Tims27	Tims42	Tims56	Tims64	Tims65	Mean
Sylvatic	N	13	13	11	13	13	13	13	13	11	13	12.6
	Na	3	13	9	9	6	12	15	4	4	12	8.7
	Ho	0.23	0.85	0.45	0.54	0.69	0.85	1.00	0.77	0.18	0.62	0.62
	He	0.22	0.94	0.91	0.90	0.77	0.93	0.95	0.66	0.74	0.94	0.79
	F_{IS}	−0.06	0.10	0.50**	0.40**	0.10	0.09	−0.05	−0.17	0.75***	0.35**	0.19*** (0.12**)
Peridomestic	N	15	15	15	15	15	15	15	15	12	12	14.4
	Na	4	10	7	3	3	4	7	4	4	5	5.1
	Ho	0.53	0.80	0.80	0.80	0.27	0.53	0.87	0.60	0.08	0.17	0.55
	He	0.61	0.90	0.81	0.66	0.25	0.65	0.81	0.76	0.66	0.69	0.67
	F_{IS}	0.13	0.11	0.01	−0.22	−0.09	0.18	−0.07	0.21	0.87***	0.76***	0.14** (0.04)
	F_{STWC}	0.32***	0.015	0.08*	0.04	0.185**	0.10**	0.08**	0.11**	0.06	0.113*	0.10***(0.10***)

N: number of bugs; Na: number of alleles; Ho: observed heterozygosity; He: expected heterozygosity. F_{IS}: inbreeding coefficient; F_{STWC}: variance of allele frequencies between population according to Weir and Cockerham (1984). In parenthesis: mean values after removing loci Tims64 and Tims65.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

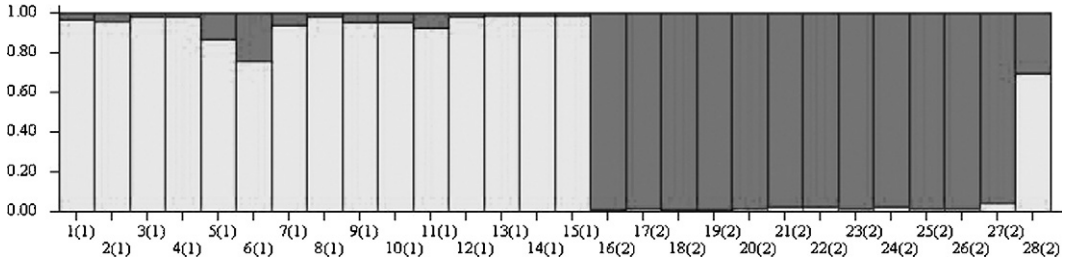

Fig. 3. Population structure in sylvatic and peridomestic *T. infestans* from Chaco. Each bar represents an individual and the two colors of grey the probability of belonging to the peridomestic (pale grey) or sylvatic (dark grey) cluster. Each number represents a particular individual and the number in parenthesis is the ecotope where it was collected. (1) Peridomestic storeroom and (2) hollow trees or parrot nests.

Table 6
Sign test for detection of population bottlenecks or expansions.

Population	Hexc:Hdef			
	IAM	TPM (70%)	TPM (90%)	SMM
Sylvatic	7:1	6:2	6:2	6:2
Peridomestic	8:0**	8:0*	8:0*	7:1

Hexc:Hdef: ratio of loci showing heterozygosity excess to heterozygosity deficiency. The expected value for a population in mutation-drift equilibrium is approximately 1:1. IAM: infinite allele model; TPM: two-phase mutation model; in brackets: percentage of single-steps mutations; SMM: stepwise mutation model.
* $P < 0.05$.
** $P < 0.001$.

4. Discussion

4.1. Hybridization

Hybridization between closely related species of Triatominae has long been described and the combined study of mitochondrial and nuclear DNA sequences provides a powerful tool for hybrids detection (Mas-Coma and Bargues, 2009). Experimental crosses between the species of the infestans subcomplex have shown variable degrees of reproductive isolation: while *T. infestans* and *T. delpontei* hybrids died as nymphs, *T. infestans* and *Triatoma platensis* and *T. platensis* and *T. delpontei* produced fertile progeny capable of crossing with the parental species (Pérez et al., 2005). For this reason, and due to the particular environment where sylvatic dark morphs were caught in comparison with Argentinean peridomestic *T. infestans*, we decided to confirm previous results based only on *mtCOI* (Ceballos et al., 2009) by analyzing the *ITS-1* sequence of sylvatic bugs. Our results corroborate that all the Argentinean dark morphs belong to *T. infestans* and are not hybrids.

4.2. Genetic variability and population history

Sylvatic melanic *T. infestans* from Argentina exhibited high levels of mitochondrial DNA nucleotide and haplotype diversity. A comparison with a previous study mostly based on peridomestic and domestic *T. infestans* populations from Argentina, shows that variability in these sylvatic bugs estimated by θ_W was very similar ($N = 14$, $\theta_W = 0.0119$) to the total Argentinean sample ($N = 207$, $\theta_W = 0.0102$) and to the two more variable populations of Santiago

del Estero (θ_W = 0.0077) and La Rioja (θ_W = 0.0067), whereas π (0.0106) was almost twice the reported value for those populations (Argentina = 0.0059, Santiago del Estero = 0.0042 and La Rioja = 0.0039) (Piccinali et al., 2009). These observations may be explained by two main hypotheses. One is that the Argentinean dark morph population has had a large historical population size. Because expected neutral variability is proportional to effective population size, larger populations are expected to carry higher levels of variability. The second hypothesis is that this dark morph population is older than others and therefore, more time has allowed the accumulation of a high number of mutations. Additional evidence that supports the second hypothesis is the occurrence of sylvatic haplotypes at the base of the phylogenetic tree (haplotypes *aaa*, *aw* and *r*, which diverged early from the remaining Bolivian and Argentinean sylvatic, peridomestic and domestic haplotypes) and in the center of the network of the main Argentinean clade (haplotype *d*, which is the most central haplotype in the network and is connected to several haplotypes). The finding of basal haplotypes in the Argentinean Chaco region supports Carcavallo's hypothesis that the dry Chaco is the area of origin of *T. infestans*. Also, the departure from neutral expectations (Fu's statistic) in sylvatic bugs is consistent with a process of diversification, including the occurrence of an important population expansion. The fact that nuclear microsatellites do not recover such a pattern can be explained by the higher mutation rate of these molecular markers, which may have returned more quickly to the equilibrium after the demographic expansion. Microsatellite mutation rates are about 10^{-2} to 10^{-6} mutations per generation (Schlötterer, 2000) in comparison with the 2.4% of divergence values per million years expected for mitochondrial DNA (Monteiro et al., 2003); this equals 6×10^{-9} mutations per generation if two generations are produced per year.

There are two additional explanations for the presence of basal haplotypes in the Argentinean dark morph population that deserve further discussion. One is that these haplotypes may have originated in the peridomestic environment and migrated into the sylvatic environment secondarily. This idea is supported by the presence of ancient haplotypes shared between sylvatic and peridomestic bugs (haplotypes *r* and *d*). Despite that this matter is not simple to answer, it is interesting to note that haplotypes *r* and *d* were found mainly in peridomestic bugs from Santiago del Estero, an area which also belongs to the Chaco region. Therefore, if the Argentinean dark morph population is not more ancient that others, at least it bears ancient variants that most probably have originated in the Chaco region. A second explanation that cannot be completely excluded is the possibility that basal haplotypes are not ancient and result from old introgression events between *T. infestans* and *T. delpontei*. Even though all the *T. infestans* and *T. delpontei* bugs used in the present study were confirmed by *ITS-1* sequences, there are recent reports based on the *mtND1* gene of individuals with *T. infestans* genes and *T. delpontei* and *T. platensis* ITS-1 and ITS-2 sequences in Argentina, Uruguay and Bolivia (Mas-Coma and Bargues, 2009). The possibility that *T. infestans*, *T. delpontei* and *T. platensis* are polyphyletic species (Funk and Omland, 2003) and its causes is an interesting evolutionary matter that deserves to be investigated in more detail.

A surprising result of this analysis was that melanic sylvatic haplotypes found in Argentina were not closely related to Bolivian dark morphs. However, a common origin of the two populations cannot be completely ruled out, because results can also be explained by allopatric divergence after habitat fragmentation. A recent study suggested that dark morphs were also present in the Paraguayan Chaco (Yeo et al., 2005), which implies that dark morph populations had a broader distribution range in the past. Analyses including more Bolivian and Paraguayan dark morph populations are needed to shed light on this matter.

4.3. Historical and current gene flow between sylvatic and peridomestic bugs

Mitochondrial DNA is an excellent marker for indirect estimation of gene flow (Lowe et al., 2004). The Bayesian coalescence analysis agrees with a low, asymmetric historical gene flow between sylvatic and peridomestic bugs in the Argentinean Chaco. Despite the fact that mitochondrial analyses only estimate female-based gene flow, analyses of nuclear microsatellites are in agreement with these results. Peridomestic and sylvatic bugs had a high degree of differentiation and mostly belonged to two different genetic clusters. The only putative migrant was a sylvatic fifth-instar nymph found in a hollow tree, which later molted to a *T. infestans* dark morph female in the laboratory. The intriguing fact about this bug was its coloration: had it been a first-generation immigrant from the peridomestic population into the trees, it should have had the typical coloration of *T. infestans* as all the peridomestic bugs. One possible explanation for such a discrepancy is that perhaps this bug was not a first-generation migrant, but a descendant of a migrant and a local sylvatic bug. This could explain its lower assignment probability to the peridomestic population than other peridomestic bugs (70% vs. 86% or more, consistent with the expected pattern of an admixed origin) and for not carrying the only mitochondrial haplotype found in peridomestic bugs in the area. Crosses between dark morphs and regular *T. infestans* are successful (Noireau et al., 2000) and suggest that the phenotype is recessive (Noireau, personal communication). This means that this nymph might not be an F_1 progeny, but it could be an F_2 or the descendent of a backcross between an F_1 and a dark morph, where the melanic coloration is expected to be expressed again.

The evidence of peridomestic bugs emigrating to the sylvatic environment suggested by *mtCOI* and microsatellites prompts the question of why recessive melanic alleles are not replaced by typical coloration variants in the sylvatic population. One possibility is that, because gene flow is low, typical coloration alleles would have low frequencies in the sylvatic population and eventually could be lost by genetic drift. The second alternative is that melanic coloration is a local adaptation to the sylvatic environment and thus, melanic individuals have higher fitness than typical coloration morphs. Selective advantages involving melanic alleles have been reported for several insect taxa and include phenomena such as increased crypsis, thermoregulation, and ultraviolet light, abrasion, pathogen and desiccation resistance (True, 2003).

Microsatellite-based results demonstrate that the closest peridomestic population of *T. infestans* (located 7 km from the area where sylvatic bugs were caught), was not derived from sylvatic bugs and that it has originated from a different source, probably from human-assisted passive transportation, a common proposed mechanism of dispersal in this species (WHO, 2002). The heterozygosity pattern found at microsatellites is consistent with a bottleneck in the peridomestic population and suggests that this population originated from a reduced number of insects. The lack of variability of mitochondrial DNA also supports this hypothesis. The fact that the only mitochondrial haplotype segregating in peridomestic bugs is the most widespread *T. infestans* haplotype precludes any further speculation about the possible geographic origin of these bugs.

In summary, the Argentinean dark morph population seems to have a limited role in infestation of surrounding peridomestic ecotopes, as is also the case of the typical *T. infestans* sylvatic population of Cotapachi, Bolivia (Richer et al., 2007). However, the discovery of new sylvatic foci of *T. infestans* in thorny hills, prickly pears, rock-pile boundary walls (Buitrago et al., 2010), bromeliads (Bacigalupo et al., 2006) and fallen trees (Ceballos, 2010) at variable distances of surrounding human settings, shows not only

the great heterogeneity of suitable habitats for the establishment of sylvatic *T. infestans* but also the heterogeneity of possible reinfestation sources. More population genetic studies including sylvatic and peridomestic bugs from other areas are needed before making any generalization about the epidemiological relevance of sylvatic *T. infestans* populations in the house reinfestation process.

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References

Bacigalupo, A., Segura, J.A., Garcia, A., Hidalgo, J., Galuppo, S., Cattan, P.E., 2006. First finding of Chagas disease vectors associated with wild bushes in the Metropolitan Region of Chile. Rev. Med. Chil. 134 (10), 1230–1236.

Bacigalupo, A., Torres-Perez, F., Segovia, V., Garcia, A., Correa, J.P., Moreno, L., Arroyo, P., Cattan, P.E., 2010. Sylvatic foci of the Chagas disease vector *Triatoma infestans* in Chile: description of a new focus and challenges for control programs. Mem. Inst. Oswaldo Cruz 105 (5), 633–641.

Bargues, M.D., Klisiowicz, D.R., Panzera, F., Noireau, F., Marcilla, A., Perez, R., Rojas, M.G., O'Connor, J.E., Gonzalez-Candelas, F., Galvaõ, C., Jurberg, J., Carcavallo, R.U., Dujardin, J.P., Mas-Coma, S., 2006. Origin and phylogeography of the Chagas disease main vector *Triatoma infestans* based on nuclear rDNA sequences and genome size. Infect. Genet. Evol. 6 (1), 46–62.

Buitrago, R., Waleckx, E., Bosseno, M.F., Zoveda, F., Vidaurre, P., Salas, R., Mamani, E., Noireau, F., Breniere, S.F., 2010. First report of widespread wild populations of *Triatoma infestans* (Reduviidae, Triatominae) in the valleys of La Paz, Bolivia. Am. J. Trop. Med. Hyg. 82 (4), 574–579.

Carcavallo, R.U., 1998. Phylogeny of Triatominae. The *Triatoma infestans* complex. Mem. Inst. Oswaldo Cruz 93 (Suppl. 2), 68–70.

Carlsson, J., 2008. Effects of microsatellite null alleles on assignment testing. J. Hered. 99 (6), 616–623.

Ceballos, L.A., 2010. Ciclo silvestre de transmisión de *Trypanosoma cruzi* en el noroeste de Argentina. Doctoral Thesis. Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina, p. 176.

Ceballos, L.A., Piccinali, R.V., Berkunsky, I., Kitron, U., Gürtler, R.E., 2009. First finding of melanic sylvatic *Triatoma infestans* (Hemiptera: Reduviidae) colonies in the Argentine Chaco. J. Med. Entomol. 46 (5), 1195–1202.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9 (10), 1657–1659.

Cornuet, J.M., Luikart, G., 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144 (4), 2001–2014.

Cortez, M.R., Emperaire, L., Piccinali, R.V., Gurtler, R.E., Torrico, F., Jansen, A.M., Noireau, F., 2007. Sylvatic Triatoma infestans (Reduviidae Triatominae) in the Andean valleys of Bolivia. Acta Trop. 102, 47–54.

Cortez, M.R., Monteiro, F.A., Noireau, F., 2010. New insights on the spread of *Triatoma infestans* from Bolivia—implications for Chagas disease emergence in the southern cone. Infect. Genet. Evol. 10 (2), 350–353.

Dujardin, J.P., Bermudez, H., Schofield, C.J., 1997. The use of morphometrics in entomological surveillance of sylvatic foci of *Triatoma infestans* in Bolivia. Acta Trop. 66 (3), 145–153.

Dujardin, J.P., Tibayrenc, M., Venegas, E., Maldonado, L., Desjeux, P., Ayala, F.J., 1987. Isozyme evidence of lack of speciation between wild and domestic *Triatoma infestans* (Heteroptera: Reduviidae) in Bolivia. J. Med. Entomol. 24 (19), 40–45.

Earl, D.A., 2010. Structure Harvester v0.56.4. Program available from website: http://taylor0.biology.ucla.edu/struct_harvest (last accessed 31.08.10).

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14 (8), 2611–2620.

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. 10 (3), 564–567.

Fu, Y.-X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147 (2), 915–925.

Funk, D.J., Omland, K.E., 2003. Species-level paraphyly and polyphyly: frequency, causes and consequences, with insights from animal mitochondrial DNA. Annu. Rev. Ecol. Evol. Syst. 34, 397–423.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17 (8), 754–755.

Kuhner, M.K., 2006. LAMARC 2.0: maximum likelihood and Bayesian estimation of population parameters. Bioinformatics 22 (6), 768–770.

Kumar, S., Nei, M., Dudley, J., Tamura, K., 2008. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief. Bioinform. 9 (4), 299–306.

Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25 (11), 1451–1452.

Lowe, A., Harris, S., Ashton, P., 2004. Ecological Genetics. Design, Analysis, and Application. Blackwell Publishing, Malden, MA.

Marcet, P.L., 2009. Estructura genético-poblacional de *Triatoma infestans* en comunidades rurales del Noroeste Argentino. Doctoral Thesis. Departmento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina, p. 141.

Marcet, P.L., Lehmann, T., Groner, G., Gürtler, R.E., Kitron, U., Dotson, E.M., 2006. Identification and characterization of microsatellite markers in the Chagas disease vector *Triatoma infestans* (Heteroptera: Reduviidae). Infect. Genet. Evol. 6 (1), 32–37.

Marcet, P.L., Mora, M.S., Cutrera, A.P., Jones, L., Gürtler, R.E., Kitron, U., Dotson, E.M., 2008. Genetic structure of *Triatoma infestans* populations in rural communities of Santiago del Estero, northern Argentina. Infect. Genet. Evol. 8 (6), 835–846.

Mas-Coma, S., Bargues, M.D., 2009. Populations, hybrids and the systematic concepts of species and subspecies in Chagas disease triatomine vectors inferred from nuclear ribosomal and mitochondrial DNA. Acta Trop. 110 (2–3), 112–136.

Monteiro, F.A., Barrett, T.V., Fitzpatrick, S., Cordon-Rosales, C., Feliciangeli, D., Beard, C.B., 2003. Molecular phylogeography of the Amazonian Chagas disease vectors *Rhodnius prolixus* and *R. robustus*. Mol. Ecol. 12 (4), 997–1006.

Monteiro, F.A., Perez, R., Panzera, F., Dujardin, J.P., Galvao, C., Rocha, D., Noireau, F., Schofield, C., Beard, C.B., 1999. Mitochondrial DNA variation of *Triatoma infestans* populations and its implication on the specific status of *T. melanosoma*. Mem. Inst. Oswaldo Cruz 94 (Suppl. 1), 229–238.

Noireau, F., 2009. Wild *Triatoma infestans*, a potential threat that needs to be monitored. Mem. Inst. Oswaldo Cruz 104 (Suppl. 1), 60–64.

Noireau, F., Flores, R., Gutierrez, T., Dujardin, J.P., 1997. Detection of sylvatic dark morphs of *Triatoma infestans* in the Bolivian Chaco. Mem. Inst. Oswaldo Cruz 92 (59), 583–584.

Noireau, F., Bastrenta, B., Catala, S., Dujardin, J.P., Panzera, F., Torres, M., Perez, R., Galvao, C., Jurberg, J., 2000. Sylvatic population of *Triatoma infestans* from the Bolivian Chaco: from field collection to characterization. Mem. Inst. Oswaldo Cruz 95 (Suppl. 1), 119–122.

Noireau, F., Cortez, M.G., Monteiro, F.A., Jansen, A.M., Torrico, F., 2005. Can wild *Triatoma infestans* foci in Bolivia jeopardize Chagas disease control efforts? Trends Parasitol. 21 (1), 7–10.

Nylander, J.A., Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24 (4), 581–583.

Nylander, J.A.A., 2004. MrModeltest v2. Program Distributed by the Author. Evolutionary Biology Centre, Uppsala University http://www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html (last accessed 31.08.10).

Paetkau, D., Slade, R., Burden, M., Estoup, A., 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Mol. Ecol. 13 (1), 55–65.

Panzera, F., Dujardin, J.P., Nicolini, P., Caraccio, M.N., Rose, V., Tellez, T., Bermudez, H., Bargues, M.D., Mas-Coma, S., O'Connor, J.E., Perez, R., 2004. Genomic changes of Chagas disease vector, South America. Emerg. Infect. Dis. 10 (3), 438–446.

Pérez, R., Hernández, M., Quintero, O., Scvortzoff, E., Canale, D., Méndez, L., Cohanoff, C., Martino, M., Panzera, F., 2005. Cytogenetic analysis of experimental hybrids in species of Triatominae (Hemiptera-Reduviidae). Genetica 125 (2), 261–270.

Piccinali, R.V., Canale, D.M., Sandoval, A.E., Cardinal, M.V., Jensen, O., Kitron, U., Gürtler, R.E., 2010. *Triatoma infestans* bugs in Southern Patagonia, Argentina. Emerg. Infect. Dis. 16 (5), 887–889.

Piccinali, R.V., Marcet, P.L., Noireau, F., Kitron, U., Gurtler, R.E., Dotson, E.M., 2009. Molecular population genetics and phylogeography of the Chagas disease vector *Triatoma infestans* in South America. J. Med. Entomol. 46 (4), 796–809.

Piry, S., Alapetite, A., Cornuet, J.M., Paetkau, D., Baudouin, L., Estoup, A., 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. J. Hered. 95 (6), 536–539.

Posada, D., Crandall, K.A., 2001. Intraspecific gene genealogies: trees grafting into networks. Trends Ecol. Evol. 16 (1), 37–45.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155 (2), 945–959.

Rambout, A., Drummond, A.J., 2009. Tracer v1.5. Program Distributed by the Authors. http://tree.bio.ed.ac.uk/software/tracer/ (last accessed 31.08.10).

Ramos-Onsins, S.E., Rozas, J., 2002. Statistical properties of new neutrality tests against population growth. Mol. Biol. Evol. 19 (12), 2092–2100.
Rannala, B., Mountain, J.L., 1997. Detecting immigration by using multilocus genotypes. Proc. Natl. Acad. Sci. U. S. A. 94 (17), 9197–9201.
Rice, W., 1989. Analyzing tables of statistical tests. Evolution 43 (1), 223–225.
Richer, W., Kengne, P., Cortez, M.R., Perrineau, M.M., Cohuet, A., Fontenille, D., Noireau, F., 2007. Active dispersal by wild *Triatoma infestans* in the Bolivian Andes. Trop. Med. Int. Health 12 (6), 759–764.
Rodriguero, M.S., 2009. Origen y consecuencias de la reproducción asexual en una especie de gorgojo de importancia agronómica. Doctoral Thesis. Departmento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina, p. 360.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19 (12), 1572–1574.
Rousset, F., 2008. Genepop'007: a complete re-implementation of the Genepop software for Windows and Linux. Mol. Ecol. Res. 8 (1), 103–106.
Schofield, C.J., 1988. Biosystematics of the Triatominae. In: Service, M.W. (Ed.), Biosystematics of Haematophagous Insects. Clarendon Press, Oxford, pp. 284–312.
Schofield, C.J., Lehane, M.J., McEwen, P., Catalá, S.S., Gorla, D.E., 1992. Dispersive flight by *Triatoma infestans* under natural climatic conditions in Argentina. Med. Vet. Entomol. 6 (1), 51–56.
Schlötterer, C., 2000. Evolutionary dynamics of microsatellite DNA. Chromosoma 109 (6), 365–371.
Schweigmann, N., Vallvé, S., Muscio, O., Guillini, M., Alberti, A., Wisnivesky-Colli, C., 1988. Dispersal flight by *Triatoma infestans* in an arid area of Argentina. Med. Vet. Entomol. 2 (4), 401–404.
Tajima, F., 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics 105, 437–460.
Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123 (3), 585–595.
Templeton, A.R., Crandall, K.A., Sing, C.F., 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132 (2), 619–633.
Torres-Pérez, F., Acuna-Retamar, M., Cook, J.A., Bacigalupo, A., García, A., Cattan, P.E., 2010. Statistical phylogeography of Chagas disease vector *Triatoma infestans*: testing biogeographic hypotheses of dispersal. Infect. Genet. Evol. 11 (1), 167–174.
True, J.R., 2003. Insect melanism: the molecules matter. Trends Ecol. Evol. 18 (12), 640–647.
Ward, J.P., Baker, P.S., 1982. The tethered flight performance of a laboratory population of *Triatoma infestans* Klug (Hemiptera: Reduviidae). Bull. Entomol. Res. 72 (1), 17–28.
Watterson, G.A., 1975. On the number of segregating sites in genetical models without recombination. Theor. Popul. Biol. 7, 256–276.
Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution 38 (6), 1358–1370.
World Health Organization, 2002. Control of Chagas Disease, Second Report of the WHO Expert Committee, WHO Technical Report Series 905. Geneva, Switzerland, 120 pp.
Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4 (3), 535–538.
Yeo, M., Acosta, N., Llewellyn, M., Sánchez, H., Adamson, S., Miles, G., López, E., González, N., Patterson, J., Gaunt, M., Rojas de Arias, A., Miles, M., 2005. Origins of Chagas disease: *Didelphis* species are natural hosts of *Trypanosoma cruzi* I and armadillos hosts of *Trypanosoma cruzi* II, including hybrids. Int. J. Parasitol. 35 (2), 225–233.
Zuccon, A., Zuccon, D., 2010. MrEnt v.2.2. Program Distributed by the Authors. http://www.mrent.org (last accessed 31.08.10).