

## SHORT REPORT: VARIATION IN MITOCHONDRIAL 12S AND 16S RIBOSOMAL DNA SEQUENCES IN NATURAL POPULATIONS OF *TRITOMA INFESTANS* (HEMIPTERA: REDUVIIDAE)

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**Abstract.** Mitochondrial DNA sequences of the 12S and 16S ribosomal RNA genes were analyzed in five natural populations of the Chagas' disease vector *Triatoma infestans* from Argentina. DNA sequence comparisons of 878 basepairs (12S plus 16S) revealed 13 haplotypes. A total of 10 private haplotypes were found in four of the populations analyzed, suggesting low current levels of genetic exchange. The levels of genetic differentiation between the population of Chancaní (Córdoba) and other two of the populations analyzed indicated significant deviation from a pattern of unrestricted gene flow. The haplotypic diversity and the private haplotypes found in the geographically closest localities of Chancaní and El Jardín (La Rioja) suggest that the reduction in the population size by insecticide treatment did not avoid the recovery of the populations apparently from survivors of the same area.

*Triatoma infestans* is the main vector of *Trypanosoma cruzi*, the protozoan agent of Chagas' disease, in South America between latitudes 10°S and 46°S. The genetic analysis of vector populations may be useful in entomologic surveillance of Chagas' disease vector control programs because it may provide information on the source of the insects in recolonized areas after insecticide treatment. Previous analyses of 412-basepair<sup>5</sup> (bp) segments of the mitochondrial cytochrome B gene did not show variation within *T. infestans* populations.<sup>1</sup> Here, we analyzed mitochondrial DNA (mtDNA) fragments of the 12S and 16S ribosomal RNA genes in 40 specimens from natural populations of *T. infestans* in localities in four provinces of Argentina (Table 1). All sites were subjected to the same sampling strategy. The insects were collected in each place from different houses or peridomestic sites. Fragments of the 12S (371 bp) and 16S (507 bp) genes were sequenced from asymmetric polymerase chain reaction products by manual and automatic sequencing, respectively.<sup>2–4</sup> DNA sequences were aligned using the CLUSTAL method implemented in MegAlign, version 1.01 (LaserGene; DNASTAR, Madison, WI). Pairwise  $F_{ST}$  estimations, a measure of the variance of gene frequencies between populations, and the analysis of molecular variance were carried out using the software package ARLEQUIN 1.1.<sup>5–7</sup>

The DNA sequence comparisons of the 12S and 16S (878 bp) genes revealed 13 haplotypes determined by 17 variable sites (Table 2) (GenBank accession numbers AY226891–AY226906). Haplotype A is present in all the localities and only the geographically closest localities of El Jardín and Chancaní shared haplotypes C and A/C. The other 10 haplotypes were present exclusively in one of the populations. These private haplotypes, found in the majority of the populations, suggest limited current levels of genetic exchange. The haplotype network (Figure 1) obtained with the TCS software indicates that the different sequence types observed would have derived from a common ancestral haplotype (A), which persists in the current populations of *T. infestans* after the process of dispersion of this species.<sup>8</sup>

Intra-individual variation was observed in six of the insects analyzed (Table 2). Each heteroplasmic haplotype was entered into the programs and used in the calculations of variability and  $F_{ST}$  as two individual different sequences. The percentage of variation within populations was 94.4% and only 5.6% among populations. The levels of genetic differentiation between the populations of Chancaní and Santa María, and Chancaní and Las Lomitas were significant ( $F_{ST} = 0.165$ ,  $P < 0.01$  and  $0.173$ ,  $P < 0.05$ , respectively), suggesting that the magnitude of gene flow between these populations is not sufficiently large to mask differences eventually produced by genetic drift or local selective pressures.

El Jardín, with seven haplotypes, showed the highest haplotypic diversity, and Chancaní, with five haplotypes, showed the highest degree of nucleotide diversity (Table 2).<sup>9</sup> The effects of random genetic drift are more likely to cause losses in genetic variation when the insecticide treatments produce severe population reductions (bottlenecks). The haplotypic diversity and the private haplotypes found in the treated area of El Jardín and Chancaní indicate that the reduction in the population size did not avoid the recovery of the populations apparently from survivors of the same area. In Las Lomitas, where A is the most frequent

TABLE 1

Collection sites of *Triatoma infestans* and number of individuals studied in Argentina

Site of collection	Latitude	Longitude	Sample size
Santa María, Catamarca	26° 41' S	66° 02' W	8
Santa Rosa, Valle Viejo, Catamarca	28° 28' S	65° 47' W	3
El Jardín, General San Martín, La Rioja	31° 26' S	65° 58' W	9
Chancaní, Pocho, Córdoba	31° 26' S	65° 28' W	10
Las Lomitas, Patiño, Formosa	24° 42' S	60° 35' W	10

TABLE 2

Haplotypes, haplotype diversity, and nucleotide diversity found in the 12S and 16S ribosomal RNA genes in populations of *Triatoma infestans* in Argentina\*

	Haplotype																Populations					
	12S								16S								1	2	3	4	5	
	1	1	2	3	1	1	1	1	1	1	2	3	3	4	4	4	7	3	2	2	7	
	9	2	2	4	6	1	1	1	4	5	8	7	0	2	0	0	9					
	2	2	8	4	3	0	5	8	5	7	9	4	7	2	4	5	6					
A	c	t	t	a	c	—	—	t	t	a	a	g	a	t	t	c	a	7	3	2	2	7
B	.	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	1	0	0	0	0
C	.	.	.	.	.	.	.	.	.	.	.	.	g	.	.	.	.	0	0	2	4	0
A/C	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	0	0	1	2	0
D	.	.	.	.	.	g	.	.	.	.	.	.	.	.	.	.	.	0	0	1	0	0
E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	t	.	0	0	1	0	0
F	.	.	.	.	.	.	t	.	.	.	.	.	.	.	.	.	y	0	0	1	0	0
G	.	.	.	.	.	.	.	y	.	r	.	.	.	.	.	.	.	0	0	1	0	0
H	t	.	.	t	.	.	.	.	g	.	a	.	.	c	.	.	.	0	0	0	1	0
I	t	.	.	.	.	.	.	.	g	.	a	.	.	c	.	.	.	0	0	0	1	0
J	.	.	.	g	.	.	.	.	.	.	.	.	.	.	.	.	.	0	0	0	0	1
K	.	c	c	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	0	0	0	1
L	.	.	.	.	.	.	.	.	.	.	.	.	.	w	.	.	.	0	0	0	0	1
Number of haplotypes																	2	1	7	5	4	
Sample size																	8	3	9	10	10	
Haplotype diversity ( $h = 1 - \sum f_i^2$ )																	0.219	0.000	0.840	0.740	0.480	
Nucleotide diversity ( $\pi$ , percent)																	0.029	0.000	0.123	0.219	0.083	

\* 1 = Santa María, Catamarca; 2 = Santa Rosa, Valle Viejo, Catamarca; 3 = El Jardín, General San Martín, La Rioja; 4 = Chancaní, Pocho, Córdoba; 5 = Las Lomitas, Patiño, Formosa. Variable nucleotide positions are indicated in sequential order in both genes. The sequence of the haplotype A is used as a reference. Deletions relative to other sequences are denoted by a dash. Nucleotides identical to the reference are indicated by a dot. Intra-individual variation is indicated as r (a/g), y (t/c), and w (a/t).

haplotype, the presence of three private haplotypes indicates an intermediate reduction of genetic variation. Conversely, although the small sample size for Santa Rosa may have limited the finding of variability, the absence of variation in this locality, as well as the low variability observed in Santa Maria, suggest severe bottlenecks derived from the insecticide treatments.

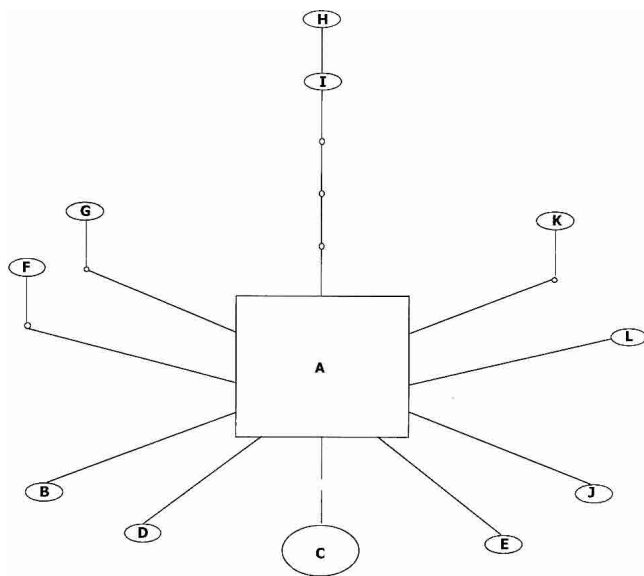


FIGURE 1. Haplotype network. The maximum number of steps connecting parsimoniously two haplotypes is indicated; one step is indicated by lines between two haplotypes and each additional base substitution by a small circle. The haplotype with the highest ancestral probability is displayed as a square, while other haplotypes are displayed as ovals. The size of the square or oval corresponds to the haplotype frequency.

Received November 7, 2002. Accepted for publication March 12, 2003.

Acknowledgment: We thank Octavio Fusco (Servicio de Secuenciación del Instituto Nacional de Parasitología Dr. Mario Fatala Chabén) for sequencing the 16S ribosomal DNA sequences.

Financial support: This research was partially supported by the grants from the Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba and Banco de la Nación Argentina, the Secretaría de Ciencia y Tecnología de la Universidad Nacional de La Rioja, and Mundo Sano. Beatriz A. García, Laura Fichera, and Elsa L. Segura are Career Investigators of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina.

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