Genetic Variability and Differentiation between Populations of *Rhodnius prolixus* and *R. pallescens*, Vectors of Chagas’ Disease in Colombia

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*Enzyme polymorphism in Rhodnius prolixus and R. pallescens (Hemiptera, Reduviidae), principal vectors of Chagas’ disease in Colombia, was analyzed using starch gel electrophoresis. Three geographic locations were sampled in order to determine gene flow between populations and to characterize intra- and interspecific differences. Of 25 enzymes assayed 10 were successfully resolved and then used to score the genetic variation. The enzymes PEPD, GPI, PGM and ICD were useful to differentiate these species and PGD, PGM and MDH distinguished between sylvatic and domiciliary populations of *R. prolixus*. Both polymorphism and heterozygosity indicated greater genetic variability in sylvatic habitats (*H* = 0.021) compared to domiciliary habitats (*H* = 0.006) in both species. Gene flow between sylvatic and domiciliary populations in *R. prolixus* was found to be minimal. This fact and the genetic distance between them suggest a process of genetic isolation in the domiciliary population.

Key words: *Rhodnius prolixus* - *Rhodnius pallescens* - isoenzyme polymorphism - Chagas’ disease - Triatominae - Colombia

The blood-sucking bug *Rhodnius prolixus* Stål (Hemiptera, Reduviidae) is the main vector of *Trypanosoma cruzi*, the causative agent of Chagas’ disease, in Colombia, Venezuela and Central America (Lent & Wygodzinsky 1979). This disease is a public health problem throughout Latin America, where it has been estimated that 16-18 million people are infected (WHO 1990).

One of the main problems in the control of *T. cruzi* vectors is the recolonization of domiciliary habitats through the migration of triatomine bugs between palm trees and human dwellings (Gomez-Núñez 1969, Dujardin et al. 1991). To determine the mobility between sylvatic and domiciliary habitats it is informative to analyze the genetic structure of representative populations with respect to gene flow.

Enzyme systems provide useful genetic markers for population studies, allowing the genetic structure to be elucidated on the basis of polymorphic loci. Isoenzyme polymorphism has been used to characterize populations of Triatominae, the principal vector of *T. cruzi* in Bolivia. One study suggested a founder effect during the expansion of the populations (Dujardin & Tibayrenc 1985). In addition, no significant differences in enzyme phenotype were observed between sylvatic and domiciliary populations (Dujardin et al. 1987).

In Chile, Frias and Kattan (1989) reported that the domiciliary *T. infestans* show less enzymatic polymorphism than the sylvatic *T. spinolai* Porter. A similar approach was used to compare some species of the genus *Rhodnius* in Venezuela, where *R. prolixus* and *R. robustus* Larrousse were found to have identical isozyme patterns in spite of having evident morphological differences (Harry et al. 1992). The present study reports the genetic variability of 10 isoenzymes in sylvatic, domiciliary and peridomiciliary populations of *R. prolixus* and *R. pallescens* Barber. In addition, we analyze the rate of gene flow between sylvatic and domiciliary habitats.

**MATERIALS AND METHODS**

Insects - Three geographic locations were selected for the study (Fig. 1). The presence of triatomines in distinct habitats provided appropriate conditions to study gene flow and rendered feasible the characterization of intra- and interspecific genetic variability. Forty-one domiciliary and 43 sylvatic *R. prolixus* were collected around the village of Coyaíma, department of Tolima (populations A and B, respectively); 36 sylvatic *R. pallescens* were collected around the Galeras, de-
department of Sucre (population C) and 41 peri-
domiciliary R. pallescens in San Carlos, Antio-
quía (population D). Males and females were
identified according to the key of Lent and Wy-
godzinsky (1979).

![Map showing geographic distribution of the populations studied.](image)

**Fig. 1:** map showing geographic distribution of the populations studied. Four distinct ecologic situations were represented: sylvatic and domiciliary _Rhodnius prolixus_ were obtained from Coyaina, department of Tolima (1); peridomiciliary _R. pallescens_ from San Carlos, department of Antioquia (2), and sylvatic _R. pallescens_ from Galeras, department of Sucre (3).

**Sample preparation** - The head, thorax and abdomen (posterior gut being removed from starved 30-40 day old adults) were ground separately in 250 μl of an enzyme stabilizer solution (dithiothreitol, E-aminoacapric acid and EDTA, each at 2 mM). The extracts were kept frozen at -70°C until they could be analyzed by electrophoresis.

**Electrophoresis and enzyme detection** - Standard horizontal starch gel electrophoresis procedures and enzyme development conditions described by Harris and Hopkinson (1976) and Miles et al. (1980) were employed, with the exception of sample loading (10 μl/well), voltage reduction to avoid overheating of gels and extension of running time to achieve resolution. The following 10 enzymes were assayed: aminopeptidase D (PEPD) E.C. 3.4.13.9; phosphogluconate

dehydrogenase (PGD) E.C. 1.1.1.44; glucose
phosphate isomerase (GPI) E.C. 5.3.1.9; phosphoglucomutase (PGM) E.C. 2.7.5.1; malate de-
hydrogenase (MDH) E.C. 1.1.1.37; isocitrate de-
hydrogenase (ICD) E.C. 1.1.1.42; pyruvate ki-
nase (PK) E.C. 2.7.1.40; esterase (ES) E.C.
3.1.1.1; alanine aminotransferase (ALAT) E.C.
2.6.1.2 and aspartate aminotransferase (ASAT) E.C. 2.6.1.1. The electrophoretic patterns were recorded graphically.

**Data analysis** - Genetic variability was calculated as the average of polymorphic loci (P) and mean heterozygosity (H). Gene flow was estimated by Fst, which is a measure of variation in allele frequencies among different populations. Specifically, Fst is the variance in allele fre-
quency (Vq) standardized by the mean (q) (Fst =
Vq/σ²(t-σ)). (Futuyma 1986). Hierarchical cluster analysis by the complete linkage method was utilized to determine similarity between popula-
tions (Dunn & Everitt 1982). The coefficient of genetic distance between populations was defined as

$$D = (1 - \cos \Theta) \frac{1}{\sqrt{2}}$$

where \( \cos \Theta \) is a measure of genetic distance between the two populations A and B, \( p_{AB} \) and \( p_{BA} \) being the gene frequencies for each allele at a
given locus in the two populations (Cavalli-
Sforza & Edwards 1967).

**RESULTS**

The electrophoretic patterns of the isoenzymes PEPD, GPI, PGM and ICD distinguished between _R. prolixus_ and _R. pallescens_ and PGD, PGM and MDH between sylvatic and domiciliary populations of _R. prolixus_ (Fig. 2). Phenotypes of PGD, MDH and PK were shared by sylvatic _R. prolixus_ and _R. pallescens_. The enzymes ES, ALAT and ASAT showed monomorphic patterns for all the samples assayed in both species. How-
ever, the enzymatic activity in the latter was too weak or bands too diffuse to allow reliable inter-
pretation.

The polymorphic isozyme profiles are shown in Fig. 2. The sylvatic population of _R. prolixus_ presented one phenotype for PEPD and GPI; two for PGD, MDH, ICD and PK and three phenotypes for PGM. In contrast, the domiciliary popu-
lation of this same species presented only one pol-
ymorphic enzyme, PGM, having two pheno-
types. Evidence for heterozygosity was found among sylvatic _R. prolixus_ for the enzymes PGD, PGM and PK. Only PK was polymorphic among the sylvatic _R. pallescens_ analyzed, while peri-
domiciliary individuals of this species displayed
monomorphic phenotypes for all the enzymes ex-
amined. We found no evidence of any correlation between enzymatic patterns and sex.

The variability in the populations assayed was

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<th>Population</th>
<th>H</th>
<th>P</th>
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<tr>
<td>Dcorr</td>
<td>0.011</td>
<td>9.09</td>
</tr>
<tr>
<td>C</td>
<td>0.035</td>
<td>72.7</td>
</tr>
<tr>
<td>A</td>
<td>0.011</td>
<td>18.2</td>
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for domiciliary _R. prolixus_;

for sylvatic _R. prolixus_;

for sylvatic _R. pallescens_.
The mean variability for *R. prolixus* was $H=0.019$ and $P=81.8$ and for *R. pallescens* $H=0.005$ and $P=18.2$. At the ecologic habitat level, the variability for both species was $H=0.021$ and $P=81.8$ for sylvatic populations and $H=0.006$ and $P=9.0$ for domiciliary/peridomiciliary populations.

The gene flow between sylvatic and domiciliary populations of *R. prolixus* was minimal with $F_{ST}$ scores above 0.025 for five polymorphic loci (Table). The genetic distance between *R. pallescens* populations using the Cavalli-Sforza coefficient was 0.236. A greater genetic distance of 0.636 was observed between sylvatic and domiciliary populations of *R. prolixus*. The genetic distance between both species was estimated to

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<td><strong>Gene flow analysis between sylvatic and domiciliary populations of <em>Rhodnius prolixus</em> calculated with five polymorphic loci using genetic Wright's variance</strong></td>
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<tr>
<td>Locus</td>
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<tr>
<td>PGD</td>
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<td>PGM</td>
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<td>MDH</td>
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<td>PK</td>
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<tr>
<td>Mean</td>
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\(^a\) only include loci that shown phenotypic differences between both populations  
\(^b\) genetic Wright's variance
Comparison of the variability between sylvatic and domiciliary or peri domiciliary vectors indicates greater variability in sylvatic than domiciliary populations. Hence the polymorphism in natural populations of *R. prolixus* could be used to indicate local and geographical variations of this species. The variety of biological niches available to vectors in sylvatic habitats may influence the maintenance of genetic variability, similar to the heterogeneity occurring in sylvatic stocks of *T. cruzi* studied by Saravia et al. (1987).

The remarkable monomorphism in the domiciliary populations of *R. prolixus* suggests a genetic drift process. If this is true, the domiciliary populations of vectors could have been established by a small number of individuals with minimal gene flow. This possibility is consistent with the results of the analysis by genetic Wright's variance. The high values of *Fst* indicate genetic isolation of domiciliary *R. prolixus*. Consequently, it is not surprising that the strains of *T. cruzi* of domiciliary origin show characteristic genetic profiles quite different to those of sylvatic origin. This hypothesis is supported by results from Widmer et al. (1985) who showed that domiciliary and peridomiciliary *T. cruzi* stocks from geographically dispersed foci were phenotypically uniform. In addition, enzyme polymorphism in 54 stocks of *T. cruzi* from vectors, mammalian reservoirs and infected humans, showed that the variability in foci of sylvatic transmission was greater than in foci of domiciliary transmission and the patterns of heterogeneity correlated with the type of transmission cycle, domiciliary or sylvatic (Saravia et al. 1987).

Our findings are in accord with the results of radioisotope labelling of bugs in Venezuela, where migration between palms and houses was not evident (Gomez-Nunez 1969). On the other hand, the average rate of gene flow among established populations of a species is often quite low (Futuyma 1986). This is especially likely in triatomine bugs because the immigrating individuals must compete with residents to survive and reproduce. In addition, it appears that the sylvatic and domiciliary foci of *R. prolixus* were stable. Blood meal availability may be a determinant of the observed genetic isolation of these colombian populations.

These findings suggest a founder effect in the domiciliary vectors and provide diagnostic loci for species and populations of the same species of *Rhodnius*. In practical terms the results support the feasibility of the prevention of recolonization using materials and construction designs that discourage the establishment of sylvatic populations in the domiciliary habitat.
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