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* Stal (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-Nuñez 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 *Triatoma infestans*, 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 el 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* Larrouse 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 Coyaima, department of Tolima (populations A and B, respectively); 36 sylvatic *R. pallescens* were collected around the Galeras, de-

This study received support from Universidad de Antioquia and Fundación Centro Internacional de Entrenamiento e Investigaciones Médicas (CIDEIM). German Lopez received training grant support from COLCIENCIAS (Grant No. 310-92).
Received 30 June 1994
Accepted 21 September 1994

partment of Sucre (population C) and 41 peridomiciliary *R. pallescens* in San Carlos, Antioquia (population D). Males and females were identified according to the key of Lent and Wygodzinsky (1979).

Fig. 1: map showing geographic distribution of the populations studied. Four distinct ecologic situations were represented: sylvatic and domiciliary *Rhodnius prolixus* were obteined from Coyaima, 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-aminocaproic 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 dehydrogenase (MDH) E.C. 1.1.1.37; isocitrate dehydrogenase (ICD) E.C. 1.1.1.42; pyruvate kinase (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 photographically.

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 frequency (Vq) standardized by the mean ($\bar{q}$) (Fst = $Vq/(\bar{q}(t-\bar{q}))$ (Futuyma 1986). Hierarchical cluster analysis by the complete linkage method was utilized to determine similarty between populations (Dunn & Everitt 1982). The coefficient of genetic distance between populations was defined as $D=(1-\cos\Theta)^{1/2}$ and $\cos\Theta=\Sigma(p_{iA}.p_{iB})^{1/2}$ where $\cos\Theta$ is a measusure of genetic distance between the two populations A and B, p_{iA} and p_{iB} 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. However, the enzymatic activity in the latter was too weak or bands too diffuse to allow reliable interpretation.

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 population of this same species presented only one polymorphic enzyme, PGM, having two phenotypes. 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 peridomiciliary individuals of this species displayed monomorphic phenotypes for all the enzymes examined. We found no evidence of any correlation between enzymatic patterns and sex.

The variability in the populations assayed was H=0.011 and P=9.09 for domiciliary *R. prolixus*; H=0.035 and P=72.7 for sylvatic *R. prolixus*; H=0.011 and P=18.2 for sylvatic *R. pallescens*.

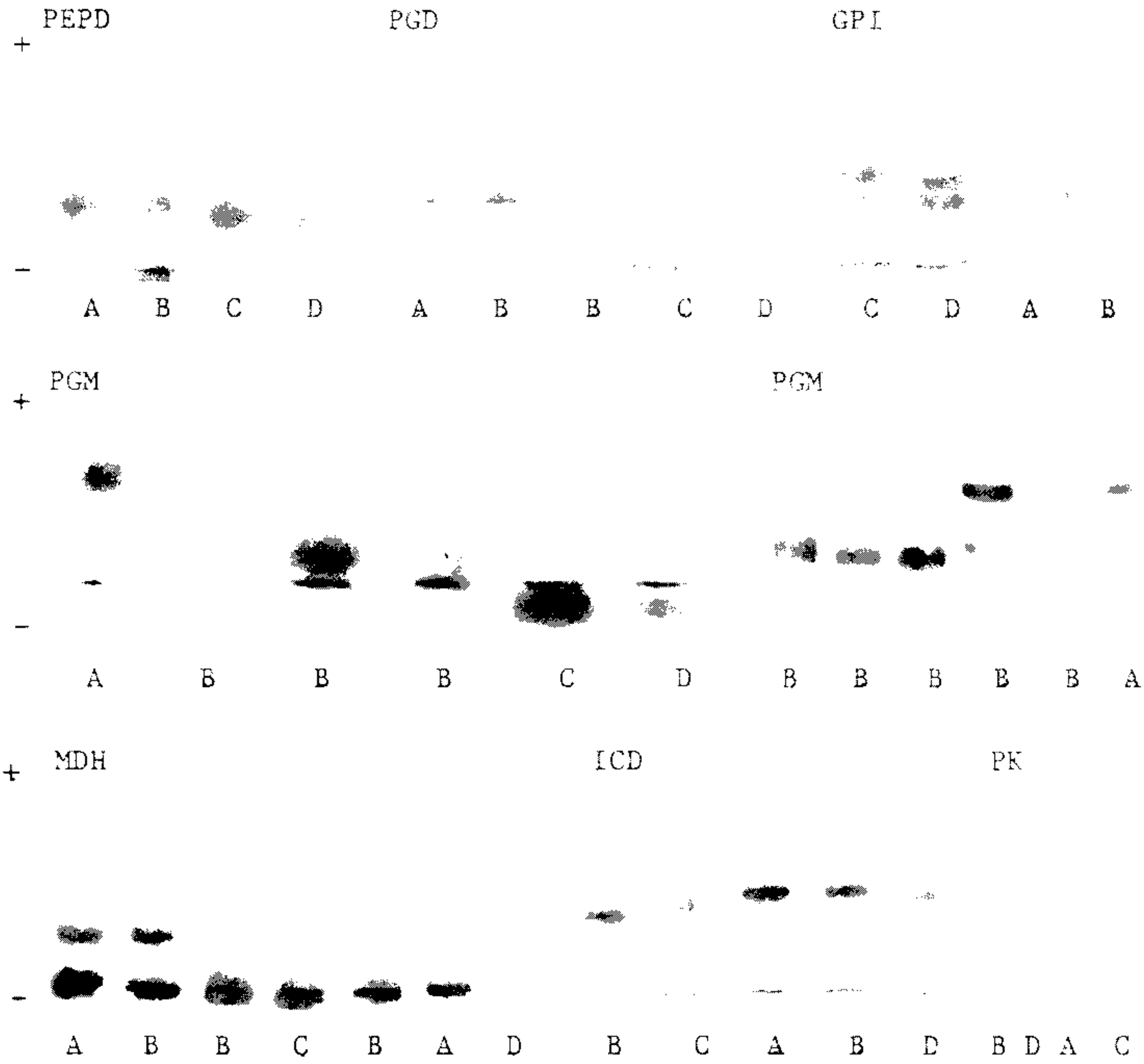

Fig. 2: photographic record showing variant isozyme profiles on starch gel electrophoresis of individuals of the four populations studied. A: domiciliary *Rhodnius prolixus*, B: sylvatic *R. prolixus*, C: sylvatic *R. pallescens* and D: peridomiciliary *R. pallescens*.

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

TABLE

Gene flow analysis between sylvatic and domiciliary populations of *Rhodnius prolixus* calculated with five polymorphic loci using genetic Wright's variance

Locus[a]	F_{ST}[b]
PGD	0.684
PGM	0.492
MDH	0.826
ICD	0.654
PK	0.323
Mean	0.598

[a]: only include loci that shown phenotypic differences between both populations
[b]: genetic Wright's variance

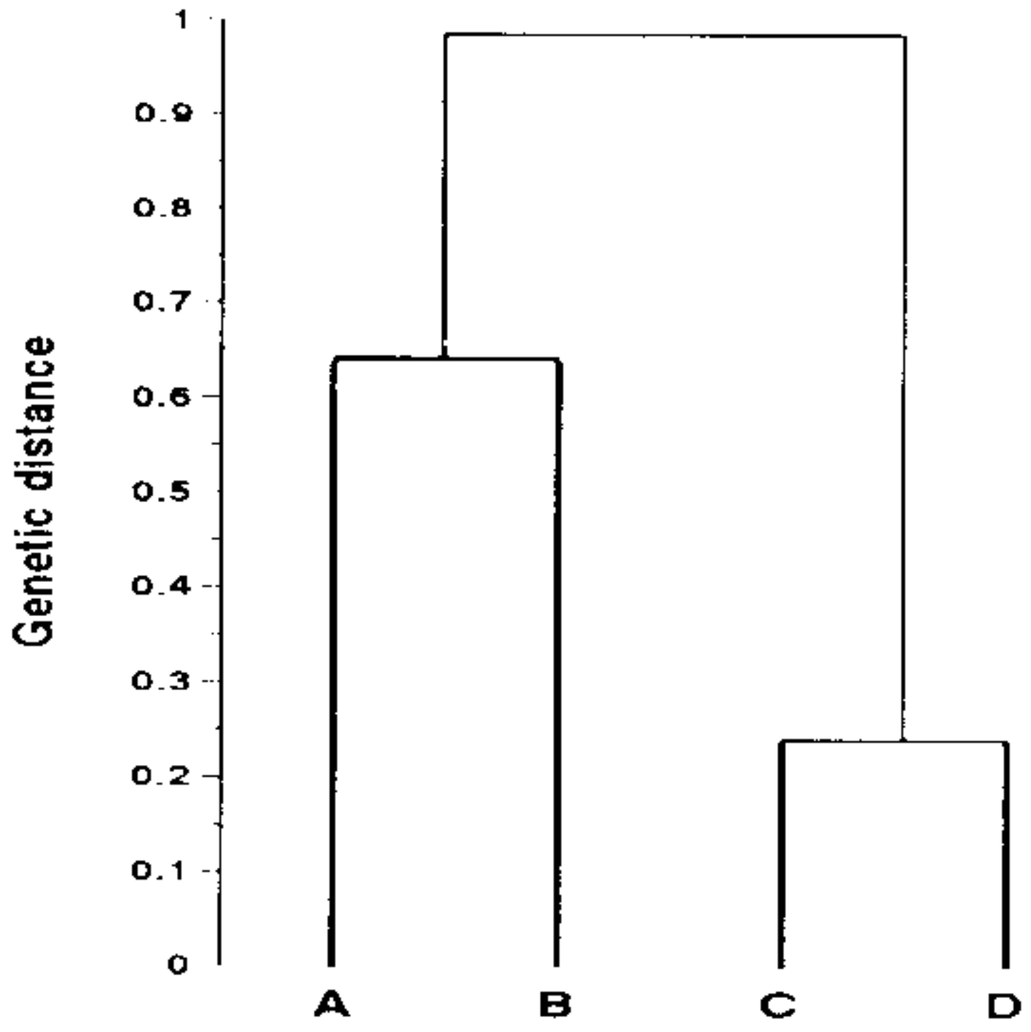

Fig. 3: dendogram summarizing the genetic distance of four populations of *Rhodnius* using the Cavalli-Sforza coefficient. A: domiciliary *R. prolixus*, B: sylvatic *R. prolixus*, C: sylvatic *R. pallescens* and D: peridomiciliary *R pallescens*.

be 0.982. The genetic distance relationships are summarized in Fig. 3.

DISCUSSION

The enzymes PEPD, PGD, GPI, PGM, MDH, ICD and PK had the most readily detectable activity and therefore were most suitable for genetic analysis of triatomine vectors. The PGM locus was routinely scorable for differentiation between *R. prolixus* populations in addition to comparisons between *Rhodnius* species. On the other hand, the enzymes PEPD, PGD, GPI, MDH and ICD appeared to be more useful in differentiating between species. The occurrence of three bands in phenotypes of GPI indicates a dimeric structure. This is consistent with the known quaternary structure for this enzyme (Harry et al. 1992).

Our results indicate that the total genetic variability was low for the populations of both species analyzed at H=0.019 for *R. prolixus* and H=0.005 for *R. pallescens*. Heterozygosity values of approximately 0.1 are usually recorded for invertebrates (Nevo 1983, Ayala and Kiger 1984). Nevertheless, in the present study the heterozygosity was almost four times greater in *R. prolixus* than *R. pallescens*. This is comparable with the observed genetic variability reported by Harry et al. (1992) for *R. prolixus* from Venezuela where the heterozygosity ranged between 0.04 to 0.10. *T. infestans* from Bolivia showed heterozygosity values of 0.04 (Dujardin & Tibayrenc 1985). Such low genetic variability appears to be a common feature of specialist insects (Nevo 1983).

Comparison of the variability between sylvatic and domiciliary or peridomiciliary 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 F_{ST} indicate genetic isolation of domiciliary *R. prolixus*. Consequently, 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-Nuñez 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.

ACKNOWLEDGEMENTS

To Dr Nancy Gore Saravia of the Fundación Centro Internacional de Entrenamiento e Invetigaciones Médicas (CIDEIM), who provided helpful suggestions in the development of these studies. To Mrs Berta Velez for technical asistance. To Dr Juan Carlos Ortíz, Unidad de Bioquímica (CIDEIM) and Dr Bruce Alexander, Unidad de Entomología (CIDEIM) for critical reading of early drafts of this manuscript. This research was developed and presented in partial fulfillment of the requirements for the Master of Science degree, Departamento de Biología, Universidad de Antioquia, Medellín, Colombia.

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