Isoenzymes as a tool for the identification of Rhodnius species (Hemiptera:Reduviidae:Triatominae)

Article · January 1996

CITATIONS
22

READS
28

5 authors, including:

Philippe Solano
Institute of Research for Development
193 PUBLICATIONS **2,619** CITATIONS
SEE PROFILE

C. A. Romana
Environmental health Consultance
113 PUBLICATIONS **700** CITATIONS
SEE PROFILE

Michel Tibayrenc
Institute of Research for Development
463 PUBLICATIONS **11,557** CITATIONS
SEE PROFILE

Research and Reviews in Parasitology, 56 (1): 41-47 (1996)

Published by A.P.E.
Printed in Barcelona, Spain

ISOENZYMES AS A TOOL FOR THE IDENTIFICATION OF *RHODNIUS* SPECIES (HEMIPTERA: REDUVIIDAE: TRIATOMINAE)

P. SOLANO[1], J.P. DUJARDIN[1,2], C.J. SCHOFIELD[3], C. ROMANA[4] & M. TIBAYRENC[1]

[1]*Instituto Boliviano de Biologia de Altura (IBBA), C.P. 641, La Paz, Bolivia*

[2]*Laboratoire de Génétique Moléculaire des Parasites et des Vecteurs, UMR CNRS-ORSTOM 9926, ORSTOM, BP 5045, 911 Av. Agropolis, 34032 Montpellier Cedex 01, France*

[3]*Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London WC1 E7HT, UK*

[4]*Universidad de Antioquia, Facultad de Ciencias Exactas y Naturales, Laboratorio de Chagas, Ap. Aereo 1226, Medellin, Colombia*

Received 11 March 1996; accepted 16 April 1996

REFERENCE: SOLANO (P.), DUJARDIN (J.P.), SCHOFIELD (C.J.), ROMANA (C.) & TIBAYRENC (M.), 1996.– Isoenzymes as a tool for the identification of *Rhodnius* species (Hemiptera: Reduviidae: Triatominae). *Research and Reviews in Parasitology*, 56 (1): 41-47.

ABSTRACT: Some species of *Rhodnius* prove difficult to separate on overt morphological characters, leading to difficulties in determining the current distribution of several of the most epidemiologically significant species. Experiments of the present paper, as well as previous studies, indicate however an overall agreement between genetic distances computed from electrophoretic data and reproductive isolation (aborted eggs) between strains, whatever their morphological species attribution. The level of Nei's genetic distances between intersterile lines, even morphologically similar, is shown to be of the same order as for well established morphologically different species. This consistency of genetic distances with experimental reproductive isolation indicates the potential of isoenzyme electrophoresis to solve some of the taxonomic problems of the genus *Rhodnius*.

KEY WORDS: *Rhodnius, R. prolixus, R. robustus, R. neglectus, R. ecuadoriensis*, electrophoresis of isoenzymes, Chagas disease.

INTRODUCTION

The 13 described species of *Rhodnius* comprise one of the 14 genera of the subfamily Triatominae (Hemiptera: Reduviidae), many of which are important as vectors of *Trypanosoma cruzi*, causative agent of Chagas disease (South American trypanosomiasis). All species of *Rhodnius* seem to be of primarily arboreal habitats, often occupying ecotopes in palm tree crowns or epiphytic bromeliads. However, the genus includes some highly domesticated species such as *R. prolixus*, the major vector of Chagas disease in Venezuela, Colombia and parts of Central America, and *R. pallescens*, the major vector in Panama. Several other species enter dwellings and peridomestic habitats, and are of local importance in some areas. Some however, such as *R. paraensis* and *R. domesticus*, seem to be entirely silvatic, without epidemiological importance. Species of *Rhodnius* tend to be quite similar in appearence. Type material can be determined by overt morphological characteristics and, in some cases, dissection of the male genitalia (LENT & JURBERG, 1969; LENT & WYGODZINSKY, 1979). However, examination of large series often shows considerable overlap in many of the key morphological characteristics, especially for the four species sometimes known as the «prolixus group» (*prolixus, robustus, neglectus, nasutus*) (HARRY, 1994; HURTADO GUERRERO, 1992), with some populations of the putative species being interfertile (DUJARDIN *et al.*, 1991). Of these, *prolixus* is recognised as a domestic and peridomestic species, and most laboratory isolates derive from collections from houses and/or chicken coops. However, silvatic populations (mainly in palm tree crowns) have been recognised in parts of Venezuela and Colombia since the work of GAMBOA (1962). Palm tree crowns in these regions are also considered the main habitat of *robustus*, and these two species are distinguished only by the lighter-coloured tibia of the older nymphs and minor differences in the basal plate struts of the male genitalia (LENT & WYGODZINSKY, 1979). *R. neglectus* is also very similar to *prolixus* and *robustus*, but is generally distributed south of the Amazon region in the savanna-like areas of Central Brazil, where it is usually found in palm tree crowns but frequently forms small domestic and peridomestic colonies (DIOTAUITI & DIAS, 1984).

Because of this, the distribution of these species cannot at the present be clearly determined, either at the geographical level (DUJARDIN *et al.*, 1991; WHO, 1991), or at the ecological level in terms of relative degrees of domestic and silvatic colonisation. However, clarification of species limits and distribution is of considerable importance in assessing the relative epidemiological significance and control strategies against different species of *Rhodnius* (WHO, 1991).

To address these problems, a series of additional approaches to vector identification are being applied to the Triatominae (DUJARDIN, 1988; DUJARDIN *et al.*, 1993). Cytogenetic techniques are currently being evaluated (PEREZ *et al.*, 1992), as are cuticular hydrocarbon analysis (PEACE, 1990; P. JUAREZ *et al.*, unpublished) and quantitative studies of metric characters (HURTADO GUERRERO, 1992; HARRY *et al.*, 1994) or sense organs (CATALA & SCHOFIELD, 1994). Isoenzyme analysis has also been used (DUJARDIN, 1990; DUJARDIN *et al.*, 1991; HARRY, 1992; HARRY *et al.*, 1992a, b; LOPEZ & MORENO, 1995). This genetic approach may provide the ad-

ditional advantage of helping in evaluate rates of gene flow between neighbouring domestic, peridomestic and silvatic populations (DUJARDIN *et al.*, 1988b; DUJARDIN, 1990; HARRY, 1992). We present here an evaluation of its taxonomic power with respect to the genus *Rhodnius*, using representatives of the *prolixus* goup (*prolixus, robustus, neglectus*) together with one species that is readily distinguishable from them (*R. ecuadoriensis*).

MATERIAL AND METHODS

The insects: Eight populations of *Rhodnius* (in this paper, the terms «population» or «strain» are considered equivalent) were provided from different laboratories (see below), together with a small collection of *R. ecuadoriensis* (RES) from palm trees dissected in 1993 near Porto Viejo, Ecuador. The determination (based on external morphology) and strain designations are shown in Table 1. Strains whose label ends with «J» (RPJ, RRJ, RNJ and REJ) are the FIOCRUZ reference strains (for *R. prolixus, R. robustus, R. neglectus* and *R. ecuadoriensis*, respectively), reared in the Department of Entomology at the Institute of Oswaldo Cruz (FIOCRUZ, J. Jurberg). The *ecuadoriensis* FIOCRUZ reference strain (REJ) originates from Peru (J.M. Barata, Department of Epidemiology, Faculty of Public Health, Lima, Peru - November 1979). The *neglectus* and *prolixus* FIOCRUZ reference strains (RNJ and RPJ) were shown to be completely intersterile by DUJARDIN *et al.* (1991). The *neglectus* FIOCRUZ reference strain (RNJ) was acquired in 1976 by FIOCRUZ, coming from the state of Goias, and transported alive in 1988 to the Institute of Tropical Medicine at Antwerp (ITMA) (Belgium). The *prolixus* FIOCRUZ reference strain (RPJ) was an old laboratory colony originating from Venezuela (locality unknow), while the *robustus* FIOCRUZ reference strain (RRJ) came from a sample collected in the Brazilian Amazons. In the present work, we also used the offspring of male RPJ and female RRJ (RRJ*RPJ) - the reverse cross also is fertile - which has been reared at the ITMA insectary since 1989 (DUJARDIN, unpublished data). The additional *prolixus* (RPH) and *neglectus* (RNB) strains were kindly provided by Dr. M. Harry (HARRY, 1992; HARRY *et al.*, 1992) and Dr. T. Barrett (Instituto Nacional de Pesquisas da Amazônia, Manaus), respectively. RNB is an old laboratory colony established at the Fundação Gonçalo Muniz (Salvador, Bahia, Brazil), received there in May 1984 from a sub-colony at Harvard University (USA), and sent in 1989 to the ITMA insectary. Together, these species comprised 107 individuals (25 of them were fifth instar nymphs).

Protein extraction: Individual insects were processed by dissecting out the alary muscles and immediately grinding them in 100 µl enzyme stabilizer solution (GODFREY & KILGOUR, 1976). This gave clearer final results than the previous method of DUJARDIN & TIBAYRENC (1985), in which the upper part of the insect (head and thorax) was crushed as a whole, mixing muscles and chitin.

Isoenzyme electrophoresis: Cellulose acetate electrophoresis was used. The electrophoretic conditions were according to DUJARDIN & TIBAYRENC (1985) with minor modifications. Fourteen enzyme systems were assayed, as follows: ACON (aconitate hydratase or aconitase, EC 4.2.1.3), DIA (diaphorase, EC 1.6.2.2), G6PD (glucose-6-phosphate dehydrogenase, EC 1.1.1.49), GAPD (glyceraldehyde-3-phosphate dehydrogenase, EC 1.2.1.12), aGPD (alpha-glycero-phosphate dehydrogenase, EC 1.1.1.8), GPI (glucose phosphate isomerase, EC 5.3.1.9), IDH (isocitrate dehydrogenase, EC 1.1.1.42), LAP (leucine aminopeptidase, EC 3.4.11), MDH (malate dehydrogenase, EC 1.1.1.37), ME (malic enzyme, EC 1.1.1.40), 6PGDH (6-phosphogluconate dehydrogenase, EC 1.1.1.44), PGM (phosphoglucomutase, EC 2.7.5.1), PEP-A (aminopeptidase A, EC 3.4.11, substrate l-leucyl-leucyl-leucine) and PEP-B (aminopeptidase B, EC 3.4.13, substrate l-leucyl-alanine).

Data analysis: The estimation of genetic variability was limited to the calculation of the proportion of polymorphic genes (P). Allele frequencies at each locus were compared between strains using the following chi-square: $X2 = 2 \cdot nx \cdot ny \cdot \sum (xi - yi)^{2/(xi+nx+yi+ny)}$ where xi and yi are the frequencies of allele «i» for the populations x and y, nx and ny are the number of individuals of these populations (results not shown). Nei's standard unbiased genetic distances (Ds) were computed, as well as their variance (NEI, 1987, p. 226). From the Ds values, an UPGMA was constructed (Fig. 1).

RESULTS

Enzyme polymorphism

Eleven enzymes showed a single zone of activity (ACON, GPD, GAPD, GPI, G6PD, LAP, PGM, 6PGD, DIA, ME and IDH). For all specimens, two zones of activity were recorded for MDH and four zones for the two PEP systems (PEP-A and PEP-B): the corresponding loci were named *Mdh1, Mdh2, Pep1, Pep2, Pep3* and *Pep4*. For the 14 enzyme systems therefore, a total of 17 consistently scorable loci could be considered. Only three of them displayed heterozygote electrophoretic patterns

Strain*	(1)	(2)	(3)	N	(4)	(5)
RES	*R. ecuadoriensis*	Schofield	Ecuador	5	17	1
REJ	*R. ecuadoriensis*	Jurberg	Peru	1	17	1
RNB	*R. neglectus*	Barrett	Brazil	16	17	2
RNJ	*R. neglectus*	Jurberg	Goia, Brazil	16	17	1
RPH	*R. prolixus*	Harry	Venezuela	16	17	1
RRJ	*R. robustus*	Jurberg	Brazil	23	17	2
RPJ	*R. prolixus*	Jurberg	Venezuela	12	17	2
RPJ*RRJ	hybrids	Dujardin	Venezuela	18	17	2

Table 1.– Characteristics of the different strains analyzed. (1) = identification based on external morphology; (2) = responsible for this morphological identification; (3) = geographic origin of the strain; N = minimum number of specimens analyzed; (4) = number of enzymatic loci; (5) = number of variable loci. * = a three symbol code is used to identify the samples: the first symbol represents the genus (R for *Rhodnius*), the second one the species (P for *prolixus*, R for *robustus*, N for *neglectus*, and E for *ecuadoriensis*), and the last one the name of the scientist who made the morphological identification.

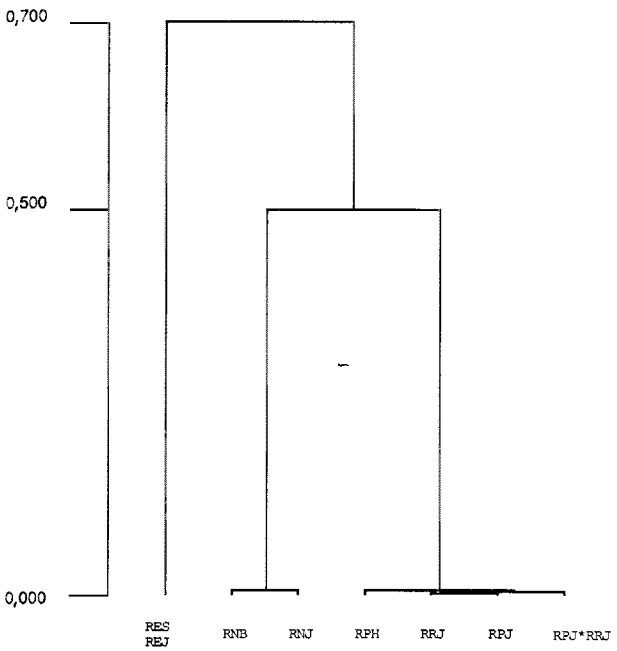

Fig. 1.– An UPGMA tree derived from Nei's standard unbiased genetic distances (Ds) between strains. Unless the rate of gene substitution varied greatly among strains, this tree could be regarded as a phylogenetic tree (NEI, 1987, p. 313).

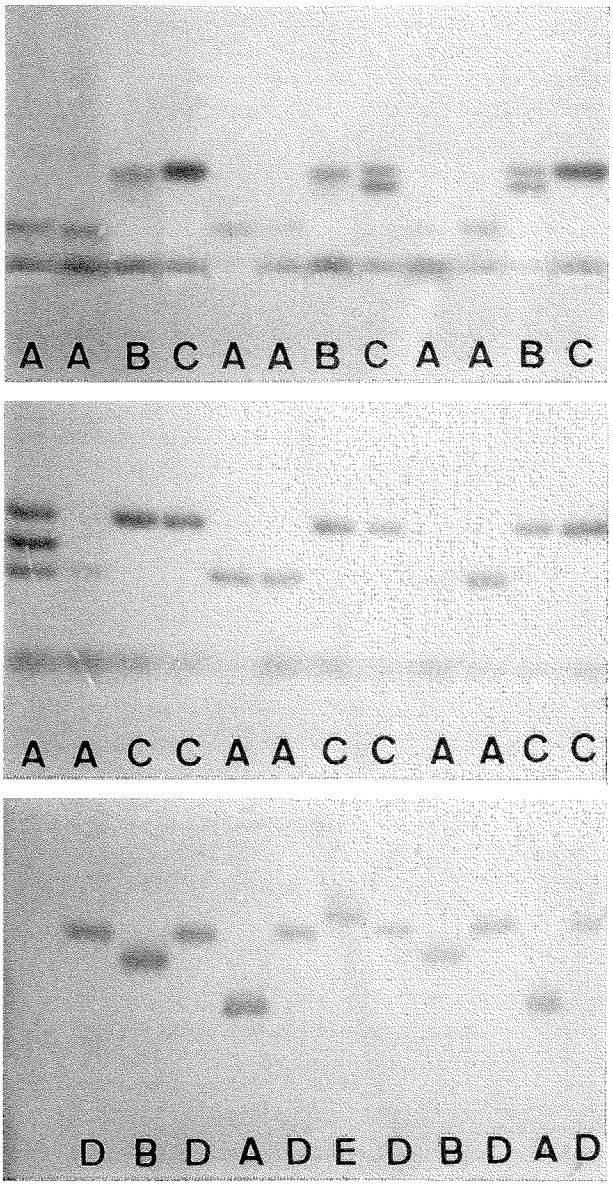

Fig. 2.– Photographic record showing variant isozyme profiles on acetate gel electrophoresis of individuals of PGM (top), GPD (mid) and GPI (bottom). A = RNJ; B = RPJ; C = RRJ; D = RES; E = *Triatoma infestans*.

for some individuals: *Gpd* (Fig. 2), *Pgm* (Fig. 2) and *Mdh1* (see Table 2, also explaining the corresponding alele nomenclature).

The *Gpd* locus was polymorphic in only one of the two *neglectus* strains (RNB), with three-banded heterozygous patterns suggesting a dimeric quaternary structure for this enzyme (Fig. 2). The *Pgm* locus was monomorphic in the *prolixus* strain of Harry (RPH), while it was polymorphic in the *prolixus* (RPJ) and the *robustus* (RRJ) reference strains, as well as in their offspring (RPJ*RRJ), with a monomeric structure inferred from the typical two-banded heterozygous patterns (Fig. 2). In all strains, the anodal zone of activity of MDH (*Mdh1*) showed for some individuals typical heterozygous patterns suggesting a dimeric structure. Four alleles were scored at this locus, two of them (*Mdh1-1* and *Mdh1-2*) found in all the strains except the *R. ecuadoriensis* strains, which showed two other, unique alleles (*Mdh1-3* and *Mdh1-4*). The wild and laboratory *R. ecuadoriensis* (RES and REJ, respectively) showed no variable loci except at *Mdh1*. Across the strains, the proportion of polymorphic loci ranged from 5,9% (1/17) to 11,8% (2/17).

Between-strain analysis

According to NEI (1987, p. 227), if the X2 used for allele frequencies comparisons (see Material and Methods) is significant for one locus, any estimate of genetic distance is different from zero. Our results (not shwn) indicate that all distances were significantly different fro zero, except the ones between RPH and either RPJ or RPJ*RRJ, between RRJ and RPJ and between RPJ and RPJ*RRJ. NEI's significant genetic distances ranged from 0,028 (between *R. robustus* and the hybrid RPJ*RRJ strain) to 0,850 (between *R. ecuadoriensis* and *R. neglectus*). Interfertile lines (RPJ and RRJ) showed no diagnostic locus (Ds=0,039), nor did different strains of the same species (RNJ and RNB, RES and REJ). Between established intersterile lines (RNJ and RPJ), genetic distances (from 0,498 to 0,539 + –0,020 SD) were of the same order as between *R. ecuadoriensis* and

Alleles	RES REJ	RNB	RNJ	RRJ	RPJ	RPH	RPJ*RRJ
Gpd-1	+	+		+	+	+	+
Gpd-2		+	+				
Gapd-1	+	+	+				
Gapd-2				+	+	+	+
Gpi-1	+						
Gpi-2				+	+	+	+
Gpi-3		+	+				
Idh-1				+	+	+	+
Idh-2		+	+				
Idh-3	+						
Mdh1-1		+	+	+	+	+	+
Mdh1-2		+	+	+	+	+	+
Mdh1-3	+						
Mdh1-4	+						
Mdh2-1		+	+	+	+	+	+
Mdh2-2	+						
Me-1		+	+				
Me-2				+	+	+	+
Me-3	+						
Pep3-1		+	+	+	+	+	+
Pep3-2	+						
Pep4-1		+	+				
Pep4-2	+			+	+	+	+
Pgm-1				+	+	+	+
Pgm-2				+	+		
Pgm-3	+						
Pgm-4		+	+				
6Pgdh-1	+						
		+	+	+	+	+	+

Table 2.– Comparative levels of electrophoretic migration among strains at 11 loci. Different positions on the gel were attributed to distinct alleles, which were numbered 1, 2, etc., starting from the faster migrating allele. For instance, *Mdh1-1* (i.e. allele 1 of the locus *Mdh1*) was the fastest migrating band (or zone of activity) detected among the whole set of strains, out of a total of four different levels (*Mdh1-1* to *Mdh1-4*). When two levels are shown for the same strain, for instance *Gpd-1* and *Gpd-2* in RNB, it means that heterozygote forms did exist at this locus for some specimens. Out of the 17 loci scored, this table shows only eleven loci, those exhibiting differences among srains or some of them (eleven loci); the remaining loci not shown (*Lap, Dia, Pep1,, Pep2, Acon* and *G6pd*) never varied among strains. «+» = presence of a band, or zone of enzymatic activity.

	RES REJ	RNB	RNJ	RPH	RRJ	RPJ
RNB	811 (27)					
RNJ	850 (28)	34 (nc)				
RPH	723 (25)	513 (20)	539 (20)			
RRJ	720 (25)	498 (20)	514 (20)	31 (nc)		
RPJ	737 (25)	514 (20)	529 (20)	40 (nc)	31 (nc)	
RPJ*RRJ	713 (25)	503 (20)	530 (20)	30 (nc)		

Table 3.– Nei's genetic unbiased distance (Ds) and its standard deviation. Ds values are multiplied by 1000. Values between brackets are standard deviations, computed after $[(1-I)/(Ir)]^{1/2)}$ with I = the Nei's standardized identity (corrected for sample sizes) and r the number of loci. Since the variance estimation is not allowed when I is higher than 0,85, the standard deviation was not computed in each case (see

the remaining species (Ds from 0,713 + –0,025 SD to 0,850 + –0,028 SD). There were six loci (35%) able to distinguish the *neglectus* strains from the *prolixus* and *robustus* ones (*Gapd, Gpi, Idh, Me, Pep-4, Pgm*), and a minimum of nine loci (53%) distinguishing the strains of ecuadoriensis from any of the other populations.

DISCUSSION

Within-population study

The within-population polymorphism found here (P ranging from 0,06 to 0,12) may be regarded as very low for invertebrates (FERGUSON, 1980; NEI & GRAUR, 1984). However, sample size was very small in some strains. Furthermore, laboratory colonies can be affected by strong genetic drift and/or selection that may greatly lower their genetic variability although, unexpectedly, the reverse may be true (LEBERG, 1992). Nevertheless, low values of P were similarly reported in larger samples of wild caught *R. pictipes* (P = 0,10 to 0,19: DUJARDIN *et al.*, 1988a; HARRY *et al.*, 1992b) and *R. pallescens* (P = 0,18: LOPEZ & MORENO, 1995), in laboratory strains of *R. neglectus* (P = 0,07: DUJARDIN *et al.*, 1991), *R. prolixus* (P = 0,07 to 0,23: DUJARDIN *et al.*, 1988a; HARRY *et al.*, 1992a, b, c) [but see LOPEZ & MORENO (1995) for wild caught *R. prolixus* in Colombia] and *R. robustus* (P = 0,00 to 0,16: HARRY, 1992; HARRY *et al.*, 1992b), suggesting that our results could reflect a general trend to low polymorphism in the genus *Rhodnius*. In this regard, two comments could be added: 1) *R. ecuadoriensis* from sylvatic environment in Ecuador (RES) showed only one variable locus out of 17, the same one found as the unique variable locus for the insectary specimen (REJ), originating from Peru; 2) in a genetic drift process occurring in laboratory, there is no reason to expect the same alleles to be fixed at the same loci in different strains. Nevertheless, different old populations separated for many generations were almost identical in the present set of data (RNJ versus RNB, RPH versus RPJ).

Between-populations study

The first comparison of different *Rhodnius* species based on isoenzyme analysis failed to separate *prolixus* from the *neglectus* of Garcia-Zapata (RNGZ1 and RNGZ2: DUJARDIN *et al.*, 1988; DUJARDIN *et al.*, 1991), although these were clearly different from *R. pictipes* (DUJARDIN *et al.*, 1988). Moreover, the RNGZ populations were found to be completely interfertile with the various *prolixus* isolates (DUJARDIN, 1990; DUJARDIN *et al.*, 1991) (Table Reprod Isolat). Subsequently however, various *prolixus* strains were found to differ at 4-5 loci from the FIOCRUZ reference strain of *neglectus* (RNJ) (DUJARDIN, 1990; DUJARDIN *et al.*, 1991). Also, marked isoenzymatic differentiation was found by HARRY (1993) between reproductively isolated strains of prolixus and neglectus (HARRY, 1992).

Other isoenzymatic studies of the *prolixus* group have failed to reveal differences between interfertile lines of *prolixus* and *robustus* collected from palm trees in the state of Trujillo (Venezuela), an area where silvatic populations of *prolixus* have never been reported (HARRY, 1992; HARRY *et al.*, 1992a, b), although again, both were clearly different from *pictipes* (HARRY *et al.*, 1992a).

For the present work, the clustering of four different presumed species (*ecuadoriensis, neglectus, prolixus* and *robustus*) into three distinct groups (Fig. 1) may be discussed taxonomically using the results of both crossing experiments and genetic analysis.

According to MAYR (1969), reproductive isolation due to biological mechanisms is the main criterion for species attribution. Interfertility between *prolixus* and *robustus*, giving viable offspring since 1989 (RPJ*RRJ), does not allow us to confirm the morphologically based species distinction. However, it does not rule out the possibility of natural, recent mating barriers separating these taxa in field conditions. On the other hand, intersterility (aborted eggs) between *neglectus* (RNJ) and *prolixus* (RPJ) is a strong argument for separating these populations into distinct species (DUJARDIN *et al.*, 1991). Similar results were reported earlier by CARVALHEIRO & BARRETO (1976). However, this criterion alone has been a matter of controversies (TEMPLETON, 1989) and the arguments leading to interpret reproductive isolation prudently are numerous (HARRY, 1994): for instance, the presence of bacterial symbiotes in *Drosophila* may produce an «infectious sterility» (WILLIAMSON *et al.*, 1971).

In the evolutionary concept, two populations are different species if they descend from distinct lineages and keep their own identity (WILEY, 1989). This concept could be used indirectly to attribute populations or laboratory strains to distinct species when genetically quite different. In practice, we could do so only when the genetic distance is higher than that commonly reported between conspecific populations. Although we are obliged to accept an arbitrary threshold, studies on various arthropod groups indicate that NEI's genetic distance between conspecific populations hardly exceeds 0,10 (DUJARDIN, 1990; KREUTZER *et al.*, 1990; LANZARO *et al.*, 1993; ESTRADA-FRANCO *et al.*, 1993; DUJARDIN *et al.*, 1996). In this study, the magnitude of the genetic distances found between the intersterile lines of *prolixus* RPJ (or *robustus* RRJ) and *neglectus* RNJ was more than one hundred times the distances found between fertile strains, indicating probable evolutionary divergence. Slightly higher distances were scored between morphologically well-recognized species (CATALA & SCHOFIELD, 1993; LENT & JURBERG, 1969; LENT & WYGODZINSKY, 1979) such as between the strains of *ecuadoriensis* on the one hand (RES, REJ), and the other populations. Similarly, DUJARDIN *et al.* (1988a) found 9 diagnostic loci and HARRY *et al.* (1992) found 12 diagnostic loci between *R. pictipes* and either *R. prolixus* or *R. robustus*. These results give confidence that a high

genetic distance between *neglectus* and *prolixus* reflects evolutionary divergence. In contrast, neither weak genetic distance, nor laboratory interfertility, between two populations is definitive evidence of lack of speciation between them (DUJARDIN *et al.*, 1987). Insignificant genetic distance and interfertility between *robustus* and *prolixus* (HARRY *et al.*, 1992a, b, 1993; this paper) remain compatible with a recent speciation event favoured by an ecological separation. As stated by HARRY *et al.* (1992b, 1993), further studies are needed to elucidate this problem. Indeed, if there are different species, a lack of gene flow should be apparent between *robustus* and *prolixus* collected in sympatry.

We would propose that high genetic distances (Ds), combined (or not) with reproductive isolation, confirmed the status of *neglectus* (RNJ or RNB) as a species distinct from the other two (*prolixus* and *robustus*). Unfortunately, formal attribution of the RNB and RNJ strains to the type species «*neglectus*» remains somewhat uncertain, because their geographic origin cannot be confirmed as representing the type locality. If they were confirmed as *R. neglectus*, then the clear electrophoretic differences observed here with *R. prolixus*, also reported by DUJARDIN *et al.* (1991) and HARRY (1993), would allow an easy solution to the question of identifying the specimens invading houses in Brazil (DIOTAIUTI *et al.*, 1984; GARCIA-ZAPATA *et al.*, 1985; DUJARDIN *et al.*, 1991) and help improve our knowledge about the geographic distribution of these morphologically similar species (WHO, 1991).

ACKNOWLEDGEMENTS

We are grateful to the following persons for kindly providing some of the insects used in this study: Dr. J. Jurberg (Instituto Oswaldo Cruz, Rio de Janeiro, Brazil), Dr. T. Barrett (UNPq, Belem), Dr. M. Harry (Université de Paris, France), Dr. J. Pereira (Universidad de la Republica, Montevideo, Uruguay), Dr. N. Schweigmann (Argentina). We thank Mme P. Roelants (IMTA, Antwerp, Belgium) for carrying out the experimental crosses referred to in this work, and Dr. J. Stevens (University of Bristol, UK) for reviewing the manuscript. Collections of *Rhodnius ecuadoriensis* in Ecuador were supported by the Association Emile Brumpt, and this work was supported by the STD Programme of the Commission of the European Communities (Grants No. TS3-CT91-0029 and No. TS3-CT92-0092).

REFERENCES

ALENCAR (J.E. DE), 1987.– *Historia Natural da Doenca de Chagas no Estado do Ceara*. Imprensa Universitaria da UFC, Fortaleza. 341 pp.

AVISE (J.C.), AQUADRO (C.F.) & PATTON (J.C.), 1982.– Evolutionnary genetics of birds. V. Genetic distances within Mimidae (mimic trushes) and Vireonidae (vireos). *Biochemical Genetics*, 20: 95-104.

BARRETT (T.V.) & HURTADO GUERRERO (J.C.), 1992.– Compatibilidade reprodutiva entre 14 colonias de *Rhodnius prolixus* e especies afims. In: *XXVIII Congreso da Sociedade Brasileira de Medicina Tropical*, Belem: 30.

CATALA (S.) & SCHOFIELD (C.J.), 1994.– Antennal sensilla of *Rhodnius*. *Journal of Morphology*, 219: 193-203

CARVALHEIRO (J.R.) & BARRETO (M.P.), 1976.– Estudos sôbre reservatórios e vectores silvestres do *Trypanosoma cruzi*. LX - Tentativas de cruzamento de *Rhodnius prolixus* Stal, 1859, com *Rhodnius neglectus* Lent, 1954 (Hemiptera, Reduviidae). *Revista do Instituto de Medicina Tropical de São Paulo*, 18: 17-23.

DIOTAIUTI (L.), SILVEIRA (A.C.) & ELIAS (M.), 1984.– Sobre o encontro de *Rhodnius prolixus* Stal, 1859, em Macauberias. *Revista Brasileira de Malariologia e Doenças Tropicais*, 36: 11-14.

DOREA (R.C.C.), POVOA (M.M.), MILES (M.A.), DE SOUZA (A.A.A.) & BARATA (J.M.), 1982.– Electroforese de enzimas para estudos de Triatomineos com referencia especial a subpopulaçoes de *Panstrongylus megistus*. *Revista Brasileira de Biologia*, 42: 521-526.

DUJARDIN (J.P.), 1988.– Potential of genetic methods for systematics of Triatominae. In: *V Reunião sobre esquisa aplicada em doença de Chagas* (Araxa, 3-5 Nov. 1988), Abstracts of Papers: 26.

DUJARDIN (J.P.), 1990.– *Intérêt de la génétique des populations dans l'étude des vecteurs de la trypanosomiase américaine*. Thèse de Doctorat en Sciences Biomédicales Expérimentales, Université de Liège, Liège.

DUJARDIN (J.P.) & TIBAYRENC (M.), 1985.– Etude de 11 enzymes et données de génétique formelle pour 19 loci enzymatiques chez *Triatoma infestans*. *Annales de la Société Belge de Médicine Tropicale*, 65: 271-280.

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. *Journal of Medical Entomology*, 24: 40-45.

DUJARDIN (J.P.), LE PONT (F.), GARCIA-ZAPATA (M.T.), CARDOZO (L.), BERMUDEZ (H.), TIBAYRENC (M.) & SCHOFIELD (C.J.), 1988a.– *Rhodnius prolixus, R. neglectus, R. pictipes* and *Triatoma infestans*: an electrophoretic comparison. In: *V Reunião sobre esquisa aplicada em doença de Chagas* (Araxa, 3-5 Nov. 1988), Abstracts of Papers: 83.

DUJARDIN (J.P.), LA FUENTE (C.), CARDOZO (L.) & TIBAYRENC (M.), 1988b.– Dispersing behaviour of *T. infestans*: evidence from a genetical study of field populations in Bolivia. *Memórias do Instituto Oswaldo Cruz*, 88: 235-240.

DUJARDIN (J.P.), GARCIA-ZAPATA (M.T.), JURBERG (J.), ROELANTS (P.), CARDOZO (L.), PANZERA (F.), DIAS (J.C.P.) & SCHOFIELD (C.J.), 1991.– Which species of Rhodnius is invading houses in Brazil? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 85: 679-680.

DUJARDIN (J.P.), PEREIRA (J.), SOLANO (P.) & TIBAYRENC (M.), 1994.– Les réponses de la génétique des populations aux problèmes soulevés par la lutte contre les vecteurs de la maladie de Chagas. In: *Pour une épidémiologie totale, Compte-rendus du Colloque et des Ateliers «Hommes-Santé-Tropiques» 1990-1993* (J.L. Jacquemin edit.), Labratoire de Parasitologie et Epidémiologie Tropicale, Unité Alphonse Laveran, La Milétrie, Poitiers: 121-125.

DUJARDIN (J.P.), LE PONT (F.), CRUZ (M.), LEON (R.), TARRIEU (F.), GUDERIAN (R.), ECHEVERRIA (R.) & TIBAYRENC (M.), 1996.– Cryptic speciation in *Lutzomyia* (*Nyssomyia*) *trapidoi* (Fairchild & Hertog) (Diptera: Psychodidae) detected by multilocus enzyme electrophoresis. *American Journal of Tropical Medicine and Hygiene*, 54: 42-45.

ESTRADA-FRANCO (J.G.), LANZARO (G.C.), MA (M.C.), WALKER-ABBEY (A.), ROMANS (P.), GALVAN-SANCHES (C.), CESPEDES (J.L.), VARGAS SAGARNAGA (R.), LAUGHINGHOUSE (A.), COLUMBUS (I.) & GWADZ (RW.), 1993.– Characterization of *Anopheles pseudopunctipennis sensu lato* from three countries of Ne-

otropical America from variation in allozymes and ribosomal DNA. *American Journal of Tropical Medicine and Hygiene*, 49: 735-745

FERGUSON (F.), 1980.– *Biochemical Systematics and Evolution.* Blackie, Glasgow & London.

GAMBOA (C.J.), 1962.– Dispersión de *Rhodnius prolixus* en Venezuela. *Boletin de la Dirección de Malariología y Saneamiento Ambiental*, 3: 262-272.

GARCIA-ZAPATA (M.T.), VIRGENS (D.), SOARES (V.A.), BOSWORTH (A.) & MARSDEN (P.D.), 1985.– House invasion by secondary triatomine species in Mambai, Goias-Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 18: 199-201.

GODFREY (D.G.) & KILGOUR (V.), 1976.– Enzyme electrophoresis in characterizing the causative agent of Gambian Trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 71: 217-225.

HARRY (M.), 1992.– *Variabilité génétiõque de populations vénézuéliennes de Rhodnius spp., vectrices de Trypanosoma cruzi, parasite responsable de la maladie de Chagas.* Thesis, Paris VI.

HARRY (M.), 1993.– Isozymic data question the specific status of some blood-sucking bugs of the genus *Rhodnius*, vectors of Chagas disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 87: 492.

HARRY (M.), 1994.– Morphometric variability in the Chagas disease vector *Rhodnius prolixus. Japanese Journal of Genetics*, 69: 223-250.

HARRY (M.), GALINDEZ (I.) & CARIOU (M.L.), 1992a.– Isozyme variability and differenciation between *Rhodnius prolixus, R. robustus*, and *R. pictipes*, vectors of Chagas disease in Venezuela. *Medical and Veterinary Entomology*, 6: 37-43.

HARRY (M.), MORENO (G.) & GOYFFON (M.), 1992b.– Genetic variability in populations of *Rhodnius prolixus* vector of Chagas' disease in Venezuela. *Evolución Biológica*, 6: 175-194.

HURTADO GUERRERO (J.C.), 1992.– *Analise morfometrica de 14 colonias de Rhodnius prolixus e especies afims (Hemiptera: Reduviidae: Triatominae).* Thesis, Universidade do Amazonas, Manaus, 326 pp.

KREUTZER (R.D.), PALAU (M.T.), MORALES (A.), FERRO (C.), FELICIANCELI (D.) & YOUNG (D.G.), 1990.– Genetic relationships among phlebotomine sandflies (Diptera: Psychodidae) in the verrucarum species group. *Jounal of Medical Entomology*, 27: 1-8.

LANZARO (G.C.), OSTROVSKA (K.), HERRERO (M.V.), LAWYER (P.G.) & WARBURG (A.), 1993.– *Lutzomyia longipalpis is* a species complex: genetic divergence and interspecific hybrid sterility among three populations. *American Journal of Tropical Medicine and Hygiene*, 48: 839-847.

LEBERG (P.L.), 1992.– Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. *Evolution*, 46: 477-494.

LENT (H.) & JURBERG (J.), 1969.– O genero *Rhodnius* Stal, 1859, com um estudo sobre a genitalia das especies (Hemiptera, Reduviidae, Triatominae). *Revista Brasileira da Biologia*, 30: 297-312.

LENT (H.) & WYGODZINSKY (P.), 1979.– Revision of the Triatominae (Hemiptera: Reduvidae), and their significance as vectors of Chagas disease. *Bulletin of the American Museum of Natural History*, 163: 123-520.

LOPEZ (G.) & MORENO (J.), 1995.– Genetic variability and differentiation between populations of *Rhodnius prolixus* and *R. pallescens*, vectors of Chagas disease in Colombia. *Memórias do Instituto Oswaldo Cruz*, 90: 353-357.

MAYR (E.), 1969.– *Principles of Systematic Zoology.* McGraw-Hill, New York.

NEI (M.), 1987.– *Molecular Evolutionnary Genetics.* Columbia University Press, 512 pp.

NEI (M.) & GRAUR (D.), 1984.– Extent of protein polymorphism and the neutral mutation theory. *Evolutionnary Biology*, 17: 73-118.

PEACE (C.), 1990.– *Cuticular hydrocarbon analysis of Rhodnius spp.* Thesis, University of Salford, Salford, 31 pp.

PEREZ (R.), PANZERA (Y.), SCIAFEZZO (S.), MAZZELLA (M.C.), PANZERA (F.), DUJARDIN (J.P.) & SCVORTZOFF (E.), 1992.– Cytogenetics as a tool for triatomine species distinction (Hemiptera-Reduviidae). *Memórias do Instituto Oswaldo Cruz*, 87: 353-361.

RIBEIRO (A.J.), 1982.– Polymorphism of an alpha-esterase of *Rhodnius prolixus* hemolymph (Hemiptera, Reduviidae, Triatominae). *Revista Brasileira de Parasitologia*, 42: 653-654.

SCHOFIELD (C.J.) & KILGOUR (V.), 1978.– Isoenzyme characterization of triatomine bug flight muscle. *Revista do Instituto da Medicina Tropical, Sao Paulo*, 20: 254-255.

SHAW (C.R.) & PRASAD (R.) 1970.– Starch gel electrophoresis of enzymes. A compilation of recipes. *Biochemical Genetics*, 4: 297-320.

SOLANO (P.), DUJARDIN (J.P.), PEREIRA (J.) & TIBAYRENC (M.), 1993.– Isozymic versus morphological differences among species of the genus *Rhodnius*. In: *Proceedings of the IV Congress of Evolutionnary Biology* (Montpellier, August 1994).

TEMPLETON (A.R.), 1989.– The meaning of species and speciation: a genetic perspective. In: *Speciation and its consequences* (D. Otte & J.A. Endler edit.), Sinauer, Sunderland, Massachusetts: 3-27.

THIOULOUSE (J.), 1990.– MacMul and GraphMu: two Macintosh programs for the display and analysis of multivariate data. *Computers and Geoscience*, 16: 1235-1240.

WHO, 1991.– Control of Chagas disease. *WHO Technical Report Series* 811, World Health Organization, Geneva, 95 pp.

WILEY (E.O.), 1989.– *Phylogenetics: the theory and practice of phylogenetic systematics.* Ed. Wiley-Interscience, 439 pp.

WILLIAMSON (D.L.), EHRMAN (L.) & KERNAGAN (R.P.), 1971.– Induction of sterility in *Drosophila paulistorum*: effect of cytoplasmic factors. *Proceedings of the National Academy of Sciences*, USA, 68: 2158-2160.