Isoenzyme electrophoresis of *Rhodnius* species: a phenetic approach to relationships within the genus

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Received 1 September 1998, Accepted 14 January 1999

Seventeen samples of *Rhodnius*, representing at least eight different species according to their morphological characteristics, were submitted to multilocus isoenzyme electrophoresis, revealing 17 different loci. A phenetic analysis of the enzyme data not only clustered the species in accordance with their geographical origin but also revealed interspecific relationships that differed from those expected from the morphology.

The genus *Rhodnius* Stal (Hemiptera: Reduviidae: Triatominae) currently comprises 13 species defined on the basis of morphological characters (Lent and Wygodzinsky, 1979; Schofield, 1994). All 13 are of Latin American distribution and several are of epidemiological significance as domestic vectors of *Trypanosoma cruzi* (Chagas), causative agent of Chagas disease (American trypanosomiasis). The species of greatest epidemiological significance are *R. prolixus* in Venezuela, Colombia and parts of Central America, and *R. palleceus* in Panama and southern Costa Rica. Several other species enter dwellings and peridomestic habitats and are of local importance in some areas, particularly *R. ecuadoriensis* in northern Peru and Ecuador, *R. stali* in Bolivia (previously cited as *R. pictipes*), and *R. neglectus* and *R. nasutus* in Brazil. *Rhodnius brethesi* is known to attack humans in parts of the Amazon region, although it is normally associated with piassaba palms. The other species seem to be entirely sylvatic, with little, if any, epidemiological importance.

The species of *Rhodnius* tend to be very similar in appearance, and their determination generally requires dissection of the male genitalia (Lent and Jurberg, 1969; Lent and Wygodzinsky, 1979). However, examination of large series often shows considerable overlap between key morphological characteristics, especially for the four species (*R. prolixus*, *R. robustus*, *R. neglectus*, and *R. nasutus*) sometimes known as the 'prolixus group' (Barrett, 1991; Harry, 1992, 1993), with some populations of the putative species being interfertile (Dujardin et al., 1991; Barrett, 1996). Because of this overlap, the distribution of many of the species cannot be clearly determined, either at the geographical level (Dujardin et al., 1991; WHO, 1991) or at the ecological level, in terms of the degree of domestic and sylvatic colonization. However, clarification of the distributions of each species is of considerable importance in assessing the species' relative
epidemiological significance and in designing effective control strategies when appropriate (WHO, 1991). The present report is of a genetic study based on isoenzyme electrophoresis of most of the available species of *Rhodnius*. The study confirms the usefulness of this technique for species determination (Solano et al., 1996) and allows a preliminary analysis of the geographical structuring of the genus.

**MATERIALS AND METHODS**

**The Insects**

Populations representing eight morphological species of *Rhodnius* were provided by different laboratories, or collected from natural habitats (Table 1). Together, these samples comprised 156 individuals, of which 23 were fifth-instar nymphs and 123 were adults. In all cases, the laboratory supplying the sample identified the bugs to species level on the basis of their morphological characters, following the keys of Lent and Wygodzinsky (1979).

**Isoenzyme Electrophoresis**

Cellulose-acetate electrophoresis was carried out according to Dujardin and Tibayrenc (1985) and Richardson et al. (1986). Twelve enzyme systems were assayed: ACON (aconitate hydratase or aconitase; EC 4.2.1.3); FDP (fructose bisphosphatase; EC 2.7.5.1); GPD (a-glycerophosphate dehydrogenase; EC 1.1.1.8); GPI (glucose phosphate isomerase; EC 5.3.1.90); 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); MPI (mannosephosphate isomerase; EC 5.3.1.8); 6PGD (phosphogluconate dehydrogenase; EC 1.1.1.44); PGM (phosphoglucomutase; EC 2.7.5.1); and PEP-B (aminopeptidase B, with L-leucyl-alanine as substrate; EC 3.4.13).

**Data Analysis**

Estimation of the relative genetic variability was limited to calculation of the proportion of polymorphic genes (EP). Jaccard's distances were calculated as a conservative measure of genetic divergence, since these do not depend on gene frequencies nor on number of loci. The phenetic (UPGMA) analysis of relationships between *Rhodnius* species was then derived from the matrix of Jaccard's distances.

**RESULTS**

Seventeen loci for the 12 enzyme systems investigated were consistently seen. Nine of the enzyme systems (GPD, GPI, LAP, PGM, 6PGD, MPI, FDP, MDH and IDH) showed a single zone of activity for all specimens. Two zones of activity were seen for ACON (and named Acon1 and Acon2), two for ME (Me1 and Me2), and four for PEP-B (Pep1, Pep2, Pep3, and Pep4) (see Table 2). Four of these enzyme loci displayed heterozygotic patterns in some individuals: Idh in *R. pictipes*, *R. neglectus* and *R. stali*; Gpi in *R. pictipes* and *R. stali*; Fdp in *R. neglectus* and domestic *R. prolixus*; and Mpi in *R. ecuadoriensis* and sylvatic *R. prolixus*. Different alleles, without the corresponding heterozygote patterns, were found at the: Gpi locus in *R. neglectus*; Mdh locus in *R. stali*, *R. ecuadoriensis*, *R. neglectus* and *R. nasutus*; Pep1 locus in *R. pallescens* and *R. nasutus*; Pep3 and Pep4 loci in *R. ecuadoriensis*; Fdp locus in *R. pallescens*; Pep2 and Acon1 loci in *R. nasutus*, and 6pgd, Acon2, Gpi and Pgm loci in *R. pictipes*. None of the 17 loci varied in *R. brethesi*. Thus the average enzyme polymorphism for all species (EP) was 0.15. Amongst them, *R. brethesi* showed the lowest variability (EP = 0). Following domestic and sylvatic samples of *R. prolixus* (EP = 0.06), *R. pallescens* and *R. stali* (EP = 0.12 for each), *R. neglectus* (EP = 0.18), and *R. nasutus* and *R. ecuadoriensis* (EP = 0.24 for each). The highest variability was shown by *R. pictipes* (EP = 0.35).

From the 68 alleles revealed by this analysis, 29 (unique alleles) were limited to one species only (see Table 3). *Rhodnius pictipes* showed seven unique alleles, followed by *R. stali* (six unique alleles), *R. pallescens* (five), *R. ecuadoriensis* (three), domestic and sylvatic.
## TABLE 1

*Origins of the *Rhodnius* investigated*

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<th>No. of bugs</th>
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### TABLE 2

Allelic scores (1 = allele present and 0 = allele absent) and comparative levels of electrophoretic migration for 17 enzyme loci of the Rhodnius species studied*

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* Alleles are numbered from fastest to slowest migrating bands on the gels.


DISCUSSION

The absence of heterozygotic forms at several loci in *R. ecuadoriensis*, *R. nasutus*, *R. neglectus*, *R. pallescens*, *R. pictipes* and *R. stali* may be attributable to the small number of individuals of these species that could be examined in the present study. In the case of *R. ecuadoriensis*, this absence could be due to geographical differentiation, since the different alleles of *Mdh*, *Pep3* and *Pep4* were distributed according to the geographical origin of the specimens from Ecuador or Peru.

The small samples used for most of the species also mean that the present estimates of their genetic variability should be regarded with caution. It is notable, however, that the species of widest geographical distribution,
A complete lack of gene flow was revealed by isoenzyme comparison with domestic *R. prolixus* from houses in the same region. In the present analysis, the sylvatic 'R. prolixus' also appears very different from domestic *R. prolixus*, and may merit new status as a distinct taxonomic entity. Until further studies are complete, however, this population should be considered as the Tolima or Colombian form of *R. ecuadoriensis*, because of its relative proximity to other *R. ecuadoriensis* populations.

The present results also reveal a clear distinction between *R. stali* and *R. pictipes*. *Rhodnius stali* is a recently described but poorly known species from the states of Acre and Mato Grosso (Brazil), and from the department of Chaparé (Bolivia), where it has been found invading human dwellings (H. Bermudez, unpubl. obs.). It has long been confused with *R. pictipes*, from which it was recently separated on the basis of the characters of the male genitalia (Lent et al., 1993); the present results support the validity of this separation, revealing considerable enzymatic divergence between the two species.

The results of the present study clearly distinguish domestic *R. prolixus* from *R. neglectus* and *R. nasutus* but, in agreement with their morphological similarity, these three species were closely clustered in the phenetic analysis (Fig.). The small distance between *R. neglectus* and *R. nasutus* may indicate that their speciation was very recent and/or the possibility of gene exchange in parapatric zones. In fact, these two species of *Rhodnius* showed fewer isoenzymatic differences than seen between populations of *T. infestans* and

### TABLE 3

Jaccard's distances computed from the allelic frequencies

<table>
<thead>
<tr>
<th></th>
<th>STA</th>
<th>NAS</th>
<th>NEG</th>
<th>DomPr</th>
<th>ECU</th>
<th>SyHoPr</th>
<th>PAL</th>
<th>BRE</th>
<th>PIC</th>
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<td>NEG</td>
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*STA, Rhodnius stali; NAS, R. nasutus; NEG, R. neglectus; DomPr, domestic *R. prolixus*; ECU, *R. ecuadoriensis*; SylvPr, sylvatic 'R. prolixus'; PAL, R. pallescens; BRE, R. brethesi; PIC, R. pictipes.*
RELATIONSHIPS WITHIN THE GENUS *Rhodnius*

![UPGMA tree](image)

Fig. An UPGMA tree derived from Jaccard's distances between *Rhodnius* species, with the positions of *Rhodnius stali* (STA), *R. brethesi* (BRE), *R. pictipes* (PIC), *R. nasutus* (NAS), *R. neglectus* (NEG), domestic *R. prolixus* (DomPr), *R. ecuadoriensis* (ECU), sylvatic *R. prolixus* (SylvPr) and *R. pallescens* (PAL) indicated.

*T. platensis* examined using identical techniques (Pereira *et al.*, 1996), and these two species of *Triatoma* are known to be interfertile (Usinger *et al.*, 1966). The clustering of domestic *R. prolixus* with *R. nasutus* and *R. neglectus* into one group may indicate that they had a common ancestral form—a hypothesis which should be verified by other, phylogenetic approaches.

With the exception of domestic *R. prolixus*, the present isoenzyme analysis grouped the *Rhodnius* species in accordance with their broad geographical origin; the members of Group 1 (*R. pictipes*, *R. brethesi* and *R. stali*) are all species of the Amazon–Orinoco forest, whereas the members of Group 2 (*R. neglectus* and *R. nasutus*) are from the drier, cerrado and caatinga regions of central and north-eastern Brazil. In contrast, the Group-3 species (*R. pallescens*, *R. ecuadoriensis* and the sylvatic *R. prolixus* or Tolima form) are all from valleys to the west of the Andean mountains. Only domestic *R. prolixus* would appear to distort this pattern, since they can be found in regions both east and west of the Andes, and in parts of Central America. Nevertheless, the present isoenzyme data indicate that domestic *R. prolixus* originate in...
the eastern part of South America, together with the other species of Groups 1 and 2; its occurrence in Central America is probably the result of human intervention (Dujardin et al., 1998) and its occurrence in Colombia west of the Andes may also the result of its passive transport in association with humans.

ACKNOWLEDGEMENTS. We are grateful to the Drs T. V. Barrett, C. B. Beard, H. Bermudez, C. Ponce, J. Jurberg and C. J. Schofield and Professor J. Moreno for kindly providing some of the specimens used in this study. This work was supported by the STD Programme of the Commission of the European Communities (contract numbers TS3*CT91.0029 and TS3*CT92.0092) and benefited from international co-operation through the ECLAT network.

REFERENCES


