Enzymatic variability and phylogenetic relatedness among *Triatoma infestans*, *T. platensis*, *T. delpontei* and *T. rubrovaria*

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Four congeneric species of triatomine bugs, *Triatoma platensis*, *T. delpontei*, *T. rubrovaria* and *T. infestans,* were examined by multilocus enzyme electrophoresis. These four species are distributed sympatrically throughout Argentina, Paraguay and Uruguay, and were found to be closely related according to their known ethological, ecological and morphological traits. In order to evaluate previous hypotheses concerning the phylogenetic branching within this group, isoenzyme patterns were submitted to classical phenetic and genetic clustering analysis. Results are discussed in the light of occasional natural gene flow between *T. infestans* and *T. platensis*, and indicate that ecology is the main factor explaining both their present status and their morphological differentiation.

Keywords: Chagas disease, genetic distance, isoenzymes, Triatominae, Uruguay.

Introduction

In their major review of the biosystematics of Triatominae, Usinger *et al.* (1966) grouped together the four species considered in the present work, on the basis of their morphological, behavioural and ecological similarities. The four species share many common features, particularly *T. platensis* and *T. delpontei* (Lent & Wygodzinsky, 1979), although they are not difficult to distinguish by overt morphological characters such as connexival colour patterns and degree of pilosity (Lent & Wygodzinsky, 1979). *Triatoma infestans* is an important vector of American trypanosomiasis (Chagas disease) and is almost exclusively associated with humans and domestic animals. It is widely distributed in the southern part of Latin America (Argentina, Bolivia, Brazil, Paraguay, Peru and Uruguay) and typically found in the cracks and crevices of poor quality rural houses, emerging at night to feed on the people and domestic animals sleeping there. In contrast, *T. platensis* and *T. delpontei* are ornithophilic arboreal species, without relevance for the

transmission of human Chagas disease (Salvatella, 1986a,b, 1987); *T. platensis* commonly infests the woven stick nests of furnariid weaver birds (*Anumbius* spp.) in northern Argentina, Paraguay, Uruguay and southern Brazil, but has also been found in peridomestic habitats such as chicken coops. *Triatoma delpontei* seems more ecologically restricted, found almost exclusively in woven stick nests of colonial monk parrots (*Myiopsitta monarcha*), but occupying a similar geographical range to *T. platensis*.

The fourth member of the group, *T. rubrovaria*, is usually found amongst limestone outcrops in Uruguay and southern Brazil, often forming abundant colonies feeding on a wide range of rupicoline vertebrates (Salvatella, 1988). However, it is increasingly reported in domestic and peridomestic habitats in parts of Uruguay where *T. infestans* has been eliminated by Chagas vector control activities (Salvatella *et al.*, 1991b; Salvatella, 1993).

Considering a large number of morphological, physiological and behavioural characteristics, Usinger *et al.* (1966) concluded that *T. platensis* and *T. delpontei* were more closely related to each other than to *T. infestans*. They suggested that *T. platensis* and *T. delpontei* were 'most probably derived one from the other or from a direct common ancestor'.

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Nevertheless, epidemiological considerations have led other authors to suggest that *T. infestans* may have arisen as a domesticated form of *T. platensis* (Carcavallo *et al.*, 1986). Since then, no more data have been added to improve our knowledge about this closely linked species group, except for a recent study relating *T. infestans* with *T. platensis* on the basis of their chromosome C-banding (Panzera *et al.*, 1995). Isoenzyme markers, although they are considered to be more suitable for phylogenetic reconstruction than phenotypic traits (Richardson *et al.*, 1986), were not applied.

The goal of the present work was to clarify the relationships within this group of species by using multilocus enzyme electrophoresis, and to see if the enzyme patterns could be related to ecological associations of the four species.

Materials and methods

Insects

The origins of the 1044 insects used in this study are as follows.

Triatoma infestans One hundred and thirty-one specimens from Uruguay were collected from domestic and peridomestic habitats in the Departments of Soriano (south of Uruguay) and Rivera (North of Uruguay). The results obtained from these specimens were then plotted together with data previously obtained by Dujardin (1990) on 777 Bolivian *T. infestans* using similar techniques.

Triatoma rubrovaria Thirty-one specimens were collected from peridomestic habitats in the Department of Artigas, Uruguay. Most were collected by the National Chagas Control Programme between 1989 and 1990.

Triatoma platensis Ninety-two specimens were studied: 82 were collected from nests of *Anumbius annumbi* in the Palmar de Quebracho region, Department of Paysandú, Uruguay); the remaining 10 were collected from similar nests in the Barra do Quaraí region, State of Río Grande do Sul, Brazil (Salvatella *et al.*, 1991a).

Triatoma delpontei Thirteen specimens were studied, of which 10 were laboratory-reared adults representing the fourth generation of material originally collected in Santiago del Estero, Argentina. Three further specimens were captured in a monk parrot nest in the Department of Artigas, Uruguay.

Enzyme electrophoresis

Analysis was performed by electrophoretic separation of isoenzymes on cellulose acetate plates (Helena Laboratories, Beaumont, TX). Seventeen enzyme systems were assayed, as follows: aconitase (aconitate hydratase, EC 4.2.1.3, ACON); aspartate aminotransferase (also known as glutamate-oxalo-acetate transaminase) (EC 2.6.1.1, GOT); diaphorase (EC 1.8.1.4, DIA); glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12, GAPD); glucose-6-phosphate dehydrogenase (EC 1.1.1.49, G6PDH); glycerol-3-phosphate dehydrogenase (EC 1.1.1.8, GPD); glucose-6-phosphate isomerase (EC 5.3.1.9, GPI); hexokinase (EC 2.7.1.1, HK); isocitrate dehydrogenase (EC 1.1.1.42, IDH); leucine aminopeptidase (cytosol aminopeptidase) (EC 3.4.11.1, LAP); malate dehydrogenase (EC 1.1.1.37, MDH); malate dehydrogenase (malic enzyme) (EC 1.1.1.40, ME); peptidase 1 (EC 3.4.11, PEP-1; leucyl-leucyl-leucine substrate); peptidase 2 (EC 3.4.11, PEP-2; leucyl-L-alanine substrate); peptidase 3 (EC 3.4.11, PEP-3; L-leucine-L-tyrosine substrate); phosphoglucomutase [EC 5.4.2.2 (formerly EC 2.7.5.1), PGM]; and 6-phosphogluconate dehydrogenase (EC 1.1.1.44, 6-PGDH).

Starved fifth instar nymphs and adults (both males and females) were used. Head and thorax samples were ground in liquid nitrogen with a glass mortar in an Eppendorf tube. Stabilizer was added (Godfrey & Kilgour, 1976) and the rest of the material was cut with entomological scissors until all the pieces has been crushed. Other conditions for sample preparation, electrophoresis and enzyme staining were as previously described by Dujardin & Tibayrenc (1985) except for GOT, HK, GAPD and DIA, which were processed following the techniques of Richardson *et al.* (1986).

Statistical methods

We characterized each specimen by its multilocus genotype (MLG), represented by the different allelic combinations (genotypes) found at all identified putative loci. For the whole sample, we could retain 53 different MLGs (Table 1) which were used for statistical analysis.

Our cladistic approach (Fig. 1) was based on the maximum parsimony method ('Wagner tree') of Farris (1970), and was computed using the software package PHYLIP 3.4 (Felsenstein, 1985). The 'independent alleles' coding of electrophoretic data (Agnese, 1989) was used, whereby each allele is considered as a character and the character-states

Table 1 The isoenzyme patterns of the 53 different multilocus genotypes (MLGs) found among the four *Triatoma* species studied

MLG	1	2	3	5	6	7	9	10	11	12	13	14	16	18	19	21	22	23	24	n
1*	11	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	22	11	65
2*	33	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	22	11	107
3	33	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	23	12	53
4	22	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	23	12	1
5	33	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	23	11	18
6	11	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	23	11	3
7	33	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	23	12	1
8	33	33	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	23	22	1
9	33	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	23	22	108
10	33	12	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	23	22	1
11	33	23	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	23	22	1
12	33	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	23	22	6
13	33	23	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	22	11	1
14	33	11	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	22	12	1
15	33	12	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	22	12	10
16	33	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	22	12	104
17	11	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	22	12	3
18	33	23	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	22	12	1
19	33	23	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	22	22	5
20	33	12	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	22	22	9
21	33	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	22	22	156
22	11	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	22	22	5
23	33	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	33	11	29
24	11	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	33	11	2
25	33	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	33	12	52
26	11	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	33	12	5
27	33	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	33	22	123
28	11	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	33	22	19
29	33	23	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	33	22	1
30	33	12	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	12	11	1
31	33	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	12	22	1
32	33	22	22	22	00	11	11	11	11	33	11	22	11	22	22	22	22	12	12	1
33	11	22	22	22	00	11	22	11	00	33	22	22	22	22	22	11	22	22	11	2
34	22	22	22	22	00	11	22	11	00	33	22	22	22	22	22	11	22	22	11	56
35	22	22	22	22	00	11	22	11	00	33	22	22	12	22	22	11	22	22	11	5
36	22	22	22	22	00	11	22	11	00	13	22	22	22	22	22	11	22	22	11	7
37	12	22	22	22	00	11	22	11	00	33	22	22	22	22	22	11	22	22	11	9
38	12	22	22	22	00	11	22	11	00	13	22	22	22	22	22	11	22	22	11	3
39	12	22	22	22	00	11	22	11	00	33	22	22	12	22	22	11	22	22	11	1
40	22	22	22	22	00	11	22	11	00	33	22	22	22	22	22	11	22	23	11	1
41	22	22	22	22	00	11	22	11	00	11	22	22	22	22	22	11	22	23	11	2
42	22	22	22	22	00	11	22	11	00	13	22	22	12	22	22	11	22	22	11	1
43	22	22	22	22	00	11	22	11	00	11	22	22	22	22	22	11	22	22	11	1
44	11	22	11	22	11	22	22	11	00	22	22	22	22	22	22	11	11	22	22	1
45	11	22	11	22	11	22	22	11	00	23	22	22	22	22	22	11	11	22	22	5
46	11	22	11	22	11	22	22	11	00	33	22	22	22	22	22	11	11	22	22	9
47	44	44	33	11	00	22	22	22	00	33	22	11	22	11	11	22	22	22	33	1
48	44	44	33	11	00	22	22	22	00	33	22	11	22	11	11	22	22	12	33	1
49	44	44	33	11	00	22	22	22	00	33	22	11	22	12	11	22	22	22	33	5
50	44	44	33	11	00	22	22	22	00	33	22	11	22	22	11	22	22	22	33	17
51	44	44	33	11	00	22	22	22	00	33	22	11	22	22	11	22	22	23	33	1
52	45	44	33	11	00	22	22	22	00	33	22	11	22	22	11	22	22	22	33	1
53	34	44	33	11	00	22	22	22	00	33	22	11	22	11	11	22	22	22	33	1
																			Total:	1024

1, *Gpd-1*; 2, *Gpd-2*; 3, *Acon*; 5, *Dia-2*; 6, *Dia-3*; 7, *Dia-4*; 9, *Hk*; 10, *Idh-1*; 11, *Idh-2*; 12, *Lap-1*; 13, *Lap-2*; 14, *Mdh-1*; 16, *Me-1*; 18, *Pep-1*; 19, *Pep-2*; 21, *Pep-4*; 22, *Gpi*; 23, *Pgm*; 24, *6-Pgdh*. Loci numbered 4, 8, 15, 17 and 20 (*Dia-1, G-6-pdh, Mdh-2, Me-2* and *Pep-3*, respectively) are not shown as they did not vary among species. Numbers 11, 12, 22, 23, 33, etc. represent the symbols of each allele (1, 2, 3, etc.) constituting different genotypes. 00 denotes that no bands were detected.
MLGs 1–32, *T. infestans*; MLGs 33–43, *T. platensis*; MLGs 44–46, *T. delpontei*; MLGs 47–53, *T. rubrovaria*.
n = total number of individuals recorded for a given MLG.
*The two genotypes are present both in Uruguay and Bolivia, i.e. there is no specific genotype of *T. infestans* in Uruguay.
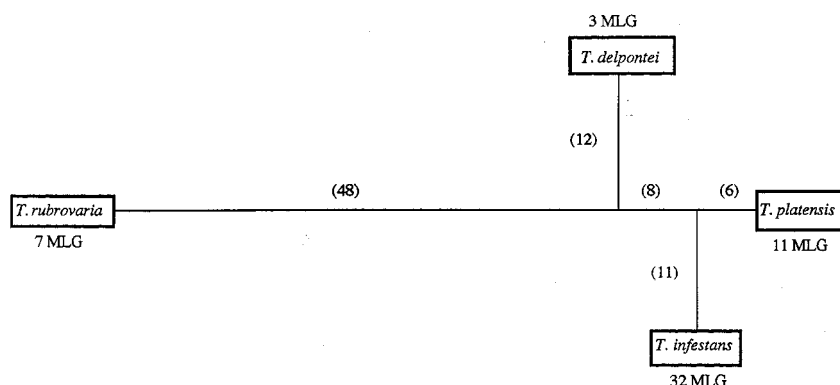
3 MLG

| T. delpontei |

(12)

(48)

| T. rubrovaria | (8)   (6) | T. platensis |
7 MLG   11 MLG

(11)

| T. infestans |
32 MLG

Fig. 1 An unrooted Wagner tree depicting the phylogenetic relationships among the four *Triatoma* species under study (Uruguayan and Bolivian specimens of *T. infestans* share common genotypes, so they are included within the same *T. infestans* box). The numbers in brackets refer to the patristic (or evolutionary) distances among the species. The patristic distance between any two species is given by the sum of the patristic distances quoted along the branches that join these two species. For example, the patristic distance between *T. delpontei* and *T. platensis* is $12 + 8 + 6 = 26$. It appears that the latter two species are not particularly closely related to each other, compared with the *T. infestans* and *T. platensis* pair, separated by patristic distance $11 + 6 = 17$. The number of different multilocus genotypes (MLGs) recorded in each species is given by the species name. *Triatoma infestans* from Bolivia and from Uruguay were not separated on this figure because of their common MLGs, MLG 1 and MLG 2.

are 'present' or 'absent'. To evaluate the robustness of the distance-Wagner trees, we used the bootstrap procedure of Felsenstein (1985).

Phenetic analysis (Fig. 2) was visualized by constructing a UPGMA dendrogram (Sokal & Michener, 1958) derived from the matrix of standard genetic distances (Nei, 1972) computed from the array of 53 MLG. This analysis gave similar results, either taking account of sample size, or not.

Results

Technical results

Eleven enzyme systems (GPD, ACON, G6PDH, IDH, LAP, MDH, ME, PEP-1, PEP-2, PEP-3, GPI, PGM and 6-PGDH) were revealed as in Dujardin & Tibayrenc (1985), except for ACON where only one band could be scored. PEP-1, PEP-2 and PEP-3 revealed the same bands with different intensities. Two additional enzyme systems also gave reproducible patterns (HK and DIA). GAPD and GOT activity was too weak or sporadic to allow reliable interpretation. GAPD and GOT activity was too weak or sporadic to allow reliable interpretation.

Isoenzymatic polymorphism

Locus numbering was according to Dujardin & Tibayrenc (1985), except for GPD which clearly revealed two loci, and ACON which revealed only one. Because the DIA and the PEP systems each gave four loci, and GPD, IDH, LAP, MDH and ME each gave two, the studied set of enzymes represented a total of 24 genetic loci (Dujardin & Tibayrenc, 1985; Frias & Kattan, 1989). Over the whole set of four species, seven loci appeared to be variable, namely *Gpd-1*, *Gpd-2*, *Lap-1*, *Me-1*, *6-Pgdh*, *Pgm* and *Pep-1* (0.29 of the total loci). Only one locus (*Gpd-1*) was variable in the Uruguayan populations of *T. infestans* (0.04 of the total loci), compared with three in the Bolivian specimens (*Gpd-2*, *6-Pgdh* and *Pgm*, i.e. 0.13 of the total loci) (Dujardin & Tibayrenc, 1985). Four loci (*Gpd-1*, *Lap-1*, *Me-1* and *Pgm*) varied in the sample of *T. platensis* (0.17 of the total loci), and three (*Gpd-1*, *Pep-1* and *Pgm*) in *T. rubrovaria* (0.13 of the total loci). One polymorphic locus (*Lap-1*) (0.04 of the total loci) was detected in *T. delpontei* (see Table 1). The unbiased estimate of gene diversity (or expected heterozygosity, *H*) (Nei, 1987) and its standard error were computed for the
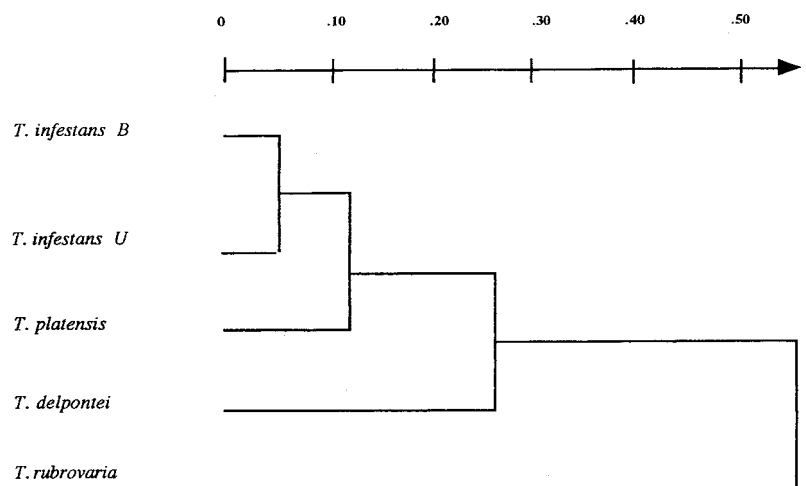
Fig. 2 A UPGMA dendrogram based on Nei's unbiased genetic distance, illustrating the genetic relationships among the four *Triatoma* species under study. B, Bolivia; U, Uruguay.

full sample of four different species ($H = 0.076 \pm 0.031$) and for each species (mean = 0.028 ± 0.019).

Allelic distribution

Considering just the variable loci, the patterns of allelic distribution among the four species suggested a basic phylogenetic framework. *Triatoma rubrovaria* exhibited 13 unique alleles (0.27), whereas five unique alleles (0.10) were found in the Bolivian *T. infestans*, four (0.08) in the Uruguayan *T. infestans* and *T. delpontei*, and only two (0.04) in *T. platensis*. This latter result implies some gene communality between *T. platensis* and another species: 17 alleles (0.35) are shared by the *T. infestans/T. platensis* pair, whereas 14 (0.29) are shared by the *T. platensis/T. delpontei* pair. Also supporting the external phylogenetic position of *T. rubrovaria*, we found no more than three to six shared alleles in any three-species group in which *T. rubrovaria* was included. Only three alleles were common to the four species, whereas 10 common alleles (0.21) were found between *T. infestans*, *T. platensis* and *T. delpontei*.

Phylogenetic clustering

Triatoma delpontei was not grouped with *T. platensis*, as illustrated by the cladogram depicting the phylogenetic relationships (Fig. 1) and the phenogram based on Nei's distance (Fig. 2). In contrast, these statistics suggest that *T. infestans* and *T. platensis* are more closely related to each other than to either *T. delpontei* or *T. rubrovaria*, which is undoubtedly the most external species of the group. The close relationship between *T. infestans* and *T. platensis* is

supported by the bootstrap analysis where these two species appeared as the most closely related pair 87 times out of 100 replicates (Fig. 1).

Discussion

Intraspecific analysis

With more than 20 loci, at least 20–30 individuals should be examined at each locus (Nei, 1987) in order to get reliable conclusions about the isoenzyme variability. In our study only the *T. delpontei* sample did not fit this condition. Thus, the apparently low polymorphism in this species (calculated heterozygosity $H = 0.019 \pm 0.019$) might be attributed to our small sample size of 13 specimens) although, as pointed out by Nei (1987), the most important factor in minimizing the variance of genetic distance estimates is the number of loci examined rather than the number of individuals. In the case of *T. delpontei*, however, the apparently low polymorphism could also be attributed to the origins of the specimens, most of which were from a fourth generation laboratory colony formed from an unknown number of founders. However, it is worth noting that among the same set of 24 loci, the same unique variable locus (*Lap-1*) was found in the laboratory-reared specimens from Argentina, originally collected from Santiago del Estero, and in the silvatic specimens from Uruguay (nearly 1000 km distant). It seems possible, therefore, that the low rate of polymorphism recorded here may be a general feature of *T. delpontei*.

The calculated heterozygosity was also low for the three other species studied here, ranging from 0.020 ± 0.011 for *T. platensis* to 0.023 ± 0.017 for *T.*

rubrovaria and 0.050 ± 0.029 for *T. infestans*. This is in accordance with studies on other species of Triatominae indicating a trend towards low isozymic polymorphism amongst Triatominae (Dujardin & Tibayrenc, 1985; Harry, 1992; Harry *et al.*, 1992; Solano *et al.* 1996).

Another notable result is the pattern of genetic variation observed in three of the four species for the GPD system. For insects this enzyme is generally involved in metabolic functions associated with flight muscle activity, and tends to be monomorphic or poorly polymorphic in most Diptera (Pasteur & De Stordeur, 1976; Coyne *et al.*, 1979; Mery *et al.*, 1979; Lakovaara & Keranen, 1980). The relatively high variability at these loci in *T. infestans* has been tentatively explained by the low level of flight activity in this species (Dujardin *et al.*, 1988).

The present data confirm this tendency to polymorphism at these loci in two additional triatomine species, *T. platensis* and *T. rubrovaria*, which exhibited complex variable isozyme patterns similar to those previously described in *T. infestans* (Dujardin, 1990). The flight behaviour of these two species is not known, but we suspect it may be similar to that of *T. infestans* because all three species tend to live in close association with their vertebrate hosts.

Interspecific comparisons

According to Richardson *et al.* (1986, p. 55), no more than two or three individuals need to be sampled to represent a population in a phylogenetic study, and additional samples need only be taken where there is evidence of considerable genetic variation within a species. Indeed, dendrograms from subsamples are most likely to resemble the full data set when heterozygosity levels within taxa are low, allele frequencies are near zero or one, and there are clear patterns of fixed or nearly fixed alleles unique to certain groups and subgroups of taxa (Archie *et al.*, 1989). This description appears to fit our material, so that our within-species sample sizes (from 13 to 1044 specimens) could be considered as representative.

The genetic distance between *T. infestans* and *T. platensis* was very low compared to that between *T. platensis* and *T. delpontei* (see Figs 1 and 2) even though there is no doubt that these are definitely separate species (Lent & Wygodzinsky, 1979).

Two hypotheses, which are not mutually exclusive, can be advanced to explain the low genetic distance between *T. infestans* and *T. platensis*. The two species may have diverged recently from each other or from a common ancestor, or their actual phylogenetic divergence may be minimized by persistent gene flow in the field. In support of the first hypothesis are the conclusions of a recent cytogenetical study showing more similarity between *T. platensis* and *T. infestans* than between *T. platensis* and *T. delpontei* (Panzera *et al.*, 1995). Another indication of recent common ancestry between *T. infestans* and *T. platensis* is their interfertility. In this respect, our data are fully consistent with previous studies which recorded complete interfertility between *T. platensis* and *T. infestans*, whereas partial sterility was found between *T. platensis* and *T. delpontei* (Abalos, 1948; Franca, 1985) and production of sterile hybrids was observed between *T. infestans* and *T. rubrovaria* (Usinger *et al.*, 1966).

The close kinship revealed in the present work by isozyme analysis between *T. infestans* and *T. platensis*, and the results of our phylogenetic analysis, do not support the hypothesis of a 'common ancestor' for *T. delpontei* and *T. platensis*, despite the fact that these two species are similar both morphologically (Lent & Wygodzinsky, 1979) and ecologically (Usinger *et al.*, 1966). Moreover, the present data also suggest that *T. infestans* and *T. platensis* are more closely related to each other than to either of the two other species. Nevertheless, the phylogenetic reconstruction proposed here should be corroborated by further studies involving both larger samples and additional genetic markers.

The second hypothesis is that apparent phylogenetic clustering could be drastically modified by unequal gene flow that could exist even between different species. The geographical range of *T. delpontei* represents a relatively small zone within the range of *T. platensis*. Both species are sympatric, even being found in different nests on the same tree. In spite of this sympatry and their partial fertility, no hybrids seem to occur in nature, or if they do, they must be very rare. Gene flow would in any case be reduced by 50 per cent owing to the sterility of a mating between *T. platensis* females and *T. delpontei* males. Contrasting with this partial reproductive isolation and the probable absence or scarcity of natural hybrids, complete laboratory fertility and the occurrence of natural hybrids have been found between *T. platensis* and *T. infestans* (Usinger *et al.*, 1966; Franca, 1985). Indeed, *T. platensis* occasionally enters peridomestic habitats such as chicken coops, which in Uruguay are frequently invaded by *T. infestans*, and natural hybrids may occur (Usinger *et al.*, 1966). Two arguments lending support to the hypothesis that genetic distances reflect gene flow more than ancestral relationships would be the following: (i) when considering *T. infestans* from

Bolivia (a country where *T. platensis* has not been recorded), the genetic distance between *T. infestans* and *T. platensis* is slightly larger (unbiased $D_s = 0.12$) than when considering Uruguayan *T. infestans* (unbiased $D_s = 0.094$); and (ii) when comparing genetic distances on the one hand, and biological traits such as morphology, ecology and interfertility on the other, more apparent concordance is observed between genetic distances and interfertility (genetic distances increase while interfertility declines). Nevertheless, these arguments remain speculative, and from the present data it is not possible to decide between these two hypotheses (recent ancestry between *T. infestans* and *T. platensis*, or actual ancestry masked by persistent gene flow between them).

Speciation and ecology

The fact that genetic barriers to gene exchange have not developed between *T. platensis* and *T. infestans* implies that their status as distinct species is almost entirely based upon their ecological separation. Although *T. platensis* is clearly able to invade human dwellings and peridomestic habitats by feeding on poultry, it maintains its association with wild birds as does *T. delpontei*. It thus appears that *T. platensis,* which has developed all the morphological features typical of arboreal triatomines like *T. delpontei*, has acquired a highly specialized adaptation to bird nests: this, and the competition with domiciliary *T. infestans*, might handicap its adaptation to the human environment. According to this point of view, the morphological proximity of *T. platensis* and *T. delpontei* would be mainly the consequence of convergence and not of common ancestry. On the other hand, the same ecological adaptations could be regarded as isolating mechanisms playing a role in speciation.

These ecological specificities and the non-negligible Nei's genetic distance are good arguments for maintaining *T. platensis* and *T. infestans* as distinct species. In this regard, their interfertility and the probable natural gene flow between them could modify this situation in some cases of ecological perturbations, lowering their present separation. Another indication that ecology could play an important role in triatomine speciation processes are the observations that (i) no natural hybrids are observed between *T. delpontei* and *T. platensis*, which are never found in the nests of the same bird species, whereas (ii) natural hybrids are occasionally observed between *T. infestans* and *T. rubrovaria*

(Lent & Wygodzinsky, 1979) which sometimes do coexist in human habitats.

We conclude therefore, in agreement with Usinger *et al.* (1966), that ecological factors may be the main force acting in the speciation of Triatominae. However, in disagreement with the same authors, our results are compatible with the hypothesis that morphological similarities in Triatominae are largely the consequence of convergence, reflecting the ecological niche more than common ancestry. If this conclusion is true, morphology should be regarded with caution as the main criterion for phylogenetic studies in Triatominae.

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