Isoenzymes Detect Variation in Populations of *Triatoma brasiliensis* (Hemiptera: Reduviidae: Triatominae)

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Triatoma brasiliensis *is one of the most important vectors of Chagas disease in the semiarid zone of the northeast of Brazil. Intraspecific morphological and behavioural variation has been reported for different populations. Results for four distinct populations using eight isoenzymes are reported here. The literature describes three subspecies:* T. brasiliensis brasiliensis *Neiva, 1911;* T. brasiliensis melanica *Neiva & Lent, 1941 and* T. brasiliensis macromelasoma *Galvão, 1956. These subspecies differ mainly in their cuticle colour pattern and were regarded as synonyms by Lent and Wygodzinsky (1979). In order to evaluate whether the chromatic pattern is a morphological variation of different melanic forms within* T. brasiliensis *or due to interspecific variation, field collections were performed in localities where these three subspecies have been described: Caicó (Rio Grande do Norte), the type-locality for* T. b. brasiliensis; *Petrolina (Pernambuco) for* T. b. macromelasoma *and Espinosa (Minas Gerais) for* T. b. melanica. *A fourth distinct chromatic pattern was found in Juazeiro (Bahia). A total of nine loci were studied. Values of Nei's genetic distance (D) were calculated.* T. b. brasiliensis *and* T. b. macromelasoma *are the closest populations with a D=0.295.* T. b. melanica *had a D ≥ 0.537 when compared to the others, a distance in the range of interspecific variation for other triatomine species.*

Key words: *Triatoma brasiliensis* - genetic distances - multilocus enzyme electrophoresis

In the semiarid region of northeast Brazil, *Triatoma brasiliensis* Neiva, 1911 is found colonizing domiciles and is regarded as one of the most important vectors of Chagas disease. Three subspecies of *T. brasiliensis* are reported in the literature: *T. brasiliensis brasiliensis* Neiva, 1911, *T. brasiliensis melanica* Neiva & Lent, 1941 and *T. brasiliensis macromelasoma* Galvão, 1956. Their descriptions are based on the different chromatic patterns of pronotum, hemelytron and legs. A taxonomic key for the different subspecies can be found in Galvão (1956). These subspecies were synonymized by Lent and Wygodzinsky (1979), who simply asserted that "inter-grading forms are frequent". Whether *T. brasiliensis* consists of different melanic populations or the chromatic patterns observed are due to interspecific variation is unknown.

In order to study this question, isoenzymatic, morphological and behavioural studies have been undertaken: morphological characters of the genital structures show considerable individual heterogeneity, thus making the differentiation of the chromatic patterns with these structures impossible. However, observations on the homogeneity and stability of colour patterns of colonies reared in laboratory and the allopatry registered during field captures, indicate that *T. brasiliensis* presents distinct geographic populations (Costa et al. 1996c). Studies on the biology, feeding sources and natural infection of the different chromatic variants have been carried out (Costa et al. 1995a, b). The results using multilocus enzyme electrophoresis for comparing these distinct populations of *T. brasiliensis* are reported here.

MATERIALS AND METHODS

The insects - One hundred and twenty specimens belonging to four chromatic populations of *T. brasiliensis* were tested: 30 F1 adults (15 females and 15 males) of each different chromatic patterns were used from colonies initiated with individuals collected from the field. The specimens captured were found in isolated colonies in different ecotopes. The localities where *T. brasiliensis* specimens were collected, the populations they represent and number of founder individuals are given in Table I. Voucher specimens are deposited in the Entomological Collection of Instituto Oswaldo Cruz-FIOCRUZ, Rio de Janeiro.

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TABLE I

Place of origin, ecotope and number of colony founder specimens in four populations of *Triatoma brasiliensis* used for isoenzyme electrophoresis analysis

Population	Locality	Location	Ecotope	Number of colony founders
brasiliensis	Caicó, Rio Grande do Norte	6º 27' 30" S 37º 05' 52" W	Peridomicile Sylvatic	47 73
macromelasoma	Petrolina, Pernambuco	9º 23' 35" S 40º 30' 27" W	Peridomicile Sylvatic	38 23
ssp.	Juazeiro, Bahia	9º 29' 49" S 40º 30' 11" W	Peridomicile Sylvatic	27 5
melanica	Espinosa, Minas Gerais	14º 55' 34" S 42º 49' 09" W	Peridomicile Sylvatic	- 75

Enzyme electrophoresis - The insects were cut between the prothorax and the mesothorax. Prothorax and head were ground in 200 µl of lysis buffer (500mMTris HCl, 26mM EDTA,10mM DTT, 10mM ε-amino-n caproic acid) and 4 µl of each homogenate was loaded for each track for gel electrophoresis.

Fifteen enzymatic systems were tested by agarose gel electrophoresis as described by Rosa-Freitas et al. (1992): HBDH - β-hydroxybutyrate dehydrogenase (E.C. 1.1.1.30), MDH - Malate dehydrogenase (E.C. 1.1.1.37), ME - Malic enzyme (E.C. 1.1.1.40), IDH - Isocitrate dehydrogenase (E.C. 1.1.1.42), 6PGD - 6-phosphogluconate dehydrogenase (E.C. 1.1.1.43), LEDH - Leucine dehydrogenase (E.C. 1.4.1.9), NP - Purine nucleoside phosphorylase (E.C. 2.4.2.1.), HK - Hexokinase (E.C. 2.7.1.1), PGM - Phosphoglucomutase (E.C. 2.7.5.1), PEP 2 - Aminopeptidase (E.C. 3.4.11), PEP3 - Aminopeptidase (E.C. 3.4.11), PEPD - Proline dipeptidase (E.C. 3.4.13.9), FUM - Fumarase (E.C. 4.2.1.2), ACON - Aconitase (E.C. 4.2.1.3), MPI - Manose-6-phosphate isomerase (E.C. 5.3.1.8). Single bands were interpreted as belonging to homozygotes, while double bands were interpreted as heterozygotes.

In Table II the number 100 is the most common allele displayed by the different enzymes. The other numbers refer to the relative mobility of the other alleles.

Data from the allelic frequency of the homozygotes and heterozygotes were used to calculate Nei's standard genetic distance between populations (Nei 1987), using the Nei software program (R Cibulskis, Liverpool School of Tropical Medicine). The genetic distance matrix produced by this program was transformed into a dendrogram by using the NTSys software package and UPGMA for clustering (Rohlf 1992).

RESULTS

Eight out of the 15 systems tested gave readable results for four populations of *T. brasiliensis*. The enzymes NP, LEDH, HBDH, showed no activity. Four enzymes, PEP3, PEPD, MPI and 6PGD, produced only weak bands and were discarded from the analysis. Thus the enzymes used were: MDH, ME, IDH, HK, PGM, FUM, PEP2 and ACON (Fig. 1). MDH and PEP2 each showed two loci. PEP2 produced one electromorph as a faint and undefined zone which was not scored. Thus a total of nine loci were considered: *Mdh-1*, *Mdh-2*, *Me*, *Idh*, *Hk*, *Pgm*, *Pep2-2*, *Fum* and *Acon*. Different electromorph mobilities were scored as distinct allelic expressions (Table II).

Allelic polymorphism was observed in all loci, except for *Acon*, which was monomorphic within and between populations. Fixed allelic expression within *T. brasiliensis* populations was seen for *Mdh-2*, *Me*, and *Pep2-2*. Different populations could be separated by distinct biochemical profiles: *Me*-116 and *Pgm*-89 have distinguished *T. b. melanica* from the other populations. High *Idh*-108 allelic frequency characterized *T. brasiliensis* ssp. *Mdh-1-83* could separate *T. b. brasiliensis*. *Mdh-2-150* grouped *T. b. melanica* and *T. brasiliensis* ssp., whereas the *Mdh-2-100* grouped *T. b. brasiliensis* and *T. b. macromelasoma*. *Pep2-2*-400 distinguished *T. b. brasiliensis* and *Pep2-2-300* identified the undetermined form.

Males and females showed no difference in their allelic expression.

The proportion of polymorphic loci for the four populations of *T. brasiliensis* was P(0.99)= 0.361 (Table II).

Nei's genetic distance (Nei 1987) among the four chromatic populations of *T. brasiliensis* were calculated (Table III). The distances encountered

TABLE II

Allelic frequencies at nine enzymes loci in four populations of *Triatoma brasiliensis*

Enzymes	Alleles	*brasiliensis*	*melanica*	*macromelasoma*	ssp.
Mdh-1	100	0.0000	1.0000	1.0000	0.9667
	83	1.0000	0.0000	0.0000	0.0333
	n	60	60	60	60
Mdh-2[a]	-150	0.0000	1.0000	0.0000	1.0000
	100	1.0000	0.0000	1.0000	0.0000
	n	60	60	60	60
Me	116	0.000	1.0000	0.000	0.0000
	100	1.0000	0.0000	1.0000	1.0000
	n	60	60	60	60
Idh	108	0.1666	0.0333	0.0667	0.8667
	100	0.8333	0.9667	0.9333	0.1333
	n	60	60	60	60
Hk	100	0.2885	1.0000	0.3462	0.5385
	33	0.7115	0.0000	0.6538	0.4615
	n	52	52	52	52
Pgm	100	1.0000	0.0000	1.0000	1.0000
	89	0.0000	0.9667	0.0000	0.0000
	56	0.0000	0.0333	0.0000	0.0000
	n	60	60	60	60
Pep2-2 [a]	300	0.0000	0.0000	0.0000	1.0000
	100	0.0000	1.0000	1.0000	0.0000
	-400	1.0000	0.0000	0.0000	0.0000
	n	60	60	60	60
Fum	100	0.6333	0.6000	0.6000	0.7000
	96	0.3667	0.4000	0.4000	0.3000
	n	60	60	60	60
Acon	100	1.0000	1.0000	1.0000	1.0000
	n	32	32	32	32
P(0.99)		0.333	0.333	0.333	0.444

Acon was monomorphic in all samples; n = number of alleles sampled; *a* : *Mdh-2* (-150) and *Pep-2* (-400) displayed a cathodic mobility

for the four chromatic populations of *T. brasiliensis* ranged from 0.295 to 1.128. The smallest genetic distance observed was between *T. b. brasiliensis* and *T. b. macromelasoma* (D= 0.295). The next D=0.418 was obtained for *T. b. macromelasoma* and the undetermined form. A D=0.537 separated *T. b. melanica* and *T. b. macromelasoma.* The distances obtained for *T. b. brasiliensis* and the undetermined chromatic form was 0.599. *T. b. melanica* and the undetermined chromatic form had a D=0.654. The greatest distance obtained was 1.128, between *T. b. brasiliensis* and *T. b. melanica*.

DISCUSSION

It has long been argued whether the different chromatic patterns presented by different populations of *T. brasiliensis* represent intraspecific variation or are due to interspecific isolation. During field collections at type localities, only one chromatic form was found in each site. These colour patterns are maintained in laboratory reared colonies. They have been stable and homogenous for three generations (Costa et al. 1996c). The four *T. brasiliensis* populations have been kept in the laboratory under the same environmental conditions of temperature, feeding sources and feeding intervals. This suggests that these colour variations are not modified by environmental conditions, and are due to genetic factors.

Males and females of *T. brasiliensis* populations showed no isoenzymatic differences, as has been previously observed for *T. infestans* (Dujardin & Tibayrenc 1985) and three *Rhodnius* species (Harry et al. 1992).

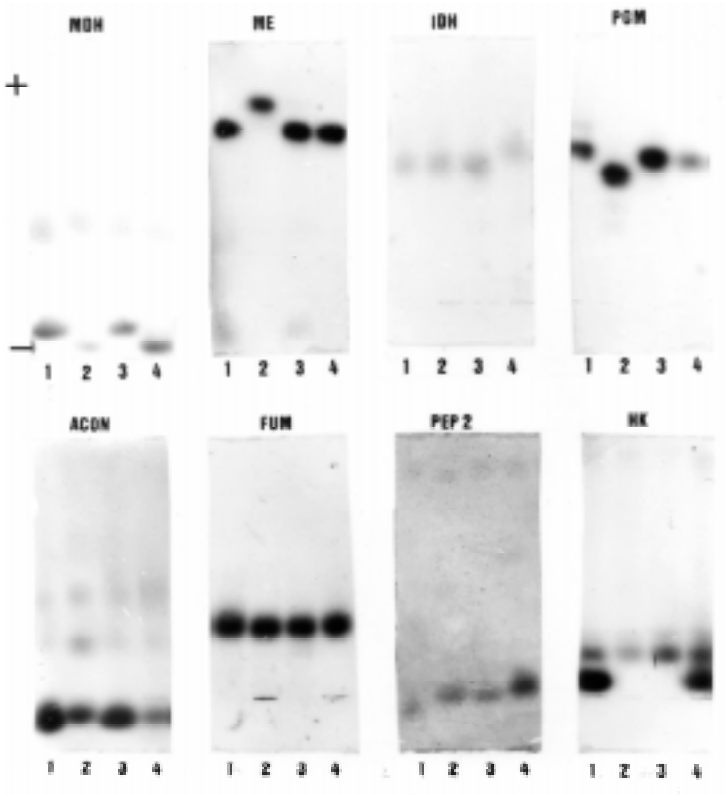

Fig.1: agarose gel eletrophoretic patterns of eight enzymes belonging to four populations of *Triatoma brasiliensis*. 1: *T. b. brasiliensis*; 2: *T. b. melanica*; 3: *T. b. macromelasoma*; 4: *T. brasiliensis* ssp.

In this study, the F1 specimens were utilized and during the period in which they were being reared there was no high mortality that could promote a bottle neck effect which could explain the lack of heterozygosity observed. Pasteur et al. (1988) asserted that deviations from Hardy-Weinberg equilibrium, among other factors, can be caused by sub-structuring in the population of the sample being studied, between which mating is not random (Wahlund effect). The F1 specimens utilized in this study reflect the inbreeding conditions of the isolated colonies forming non-panmitic subgroups. Comparison among isoenzymatic results of these different chromatic forms showed a high genetic distance, that could be interpreted as an indication of the existence of different well defined taxa (Table III).

Many studies have calculated the genetic distance for well defined taxa such as: *T. infestans* and *T. delpontei* (D= 0.290 - 0.292, by Pereira et al. 1996) and for *T. infestans* and *T. sordida* (D= 1.333, by García et al. 1995b). There are also two published comparisons of *T. infestans* and *T. platensis*: Pereira et al. (1996) analyzed 24 loci,

TABLE III

Nei's genetic distance among four population of *Triatoma brasiliensis* from Brazil

Population	*brasiliensis*	*macromelasoma*	ssp.	*melanica*
brasiliensis	0.000			
macromelasoma	0.295	0.000		
ssp.	0.599	0.418	0.000	
melanica	1.128	0.537	0.654	0.000

registering D= 0.094 - 0.124 and García et al. (1995b) which analyzed 14 loci, with a D=0.45. These data demonstrate the wide range of genetic distances measurements between species, although biochemical analysis carried out at intraspecific level have shown a low genetic distance. Dujardin et al. (1987) analyzed wild and domestic populations of *T. infestans* from Cochabamba (Bolívia) concluding they are virtually identical comparing the 19 enzymatic gene loci indicating lack of speciation between these two populations (D= 0.001 - 0.004). García et al. (1995a) studied nine colonies of *T. infestans* established with individuals collected at different localities in South America. They observed uniformity of allele frequencies among populations, explaining the result in terms of the recent and rapid dispersal of the species from the site of origin, Cochabamba Valley in Bolivia (D= 0.001 - 0.011).

The present study has allowed us to distinguish the four populations of *T. brasiliensis* demonstrating that they are genetically distinct from one another (Table III). All the genetic distances have situated them at the interspecific level, when compared to the results above mentioned (Fig. 2). *T. brasiliensis melanica* has a higher genetic distance (> 0.537) from the other *T. brasiliensis* populations. Although specimens of *T. b. macromelasoma* and the *T. brasiliensis* ssp. had been collected in nearby localities (Petrolina and Juazeiro, separated by the San Francisco River) they presented a significant genetic distance (D= 0.418).

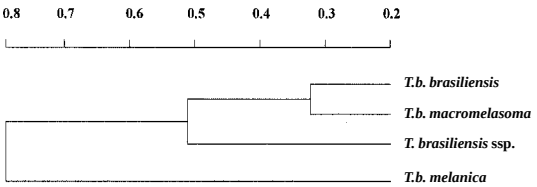

Fig. 2: dendrogram showing the genetic distances among populations of *Triatoma brasiliensis.*

In order to elucidate the taxonomic status of these different chromatic populations, it would be necessary: define the geographic distribution of each population, analyze the colour patterns and the genitalia (Costa & Marchon-Silva 1993), morphological studies of eggs using scanning electron microscopy (Costa et al. 1996a), apply other genetic analysis such as chromosome and DNA markers and determine the existence or non existence of reproductive isolation mecanisms via directed crosses (Costa et al. 1996b).

The validation of distinct taxonomic *status* for these different chromatic patterns will be important for the basic knowledge of this group of insects. This information will improve the understanding of the role of these "distinct melanic forms" in the transmission and possibly in the control of Chagas disease.

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