SHORT COMMUNICATION

Crossing Experiments Detect Genetic Incompatibility among Populations of *Triatoma brasiliensis* Neiva, 1911 (Heteroptera, Reduviidae, Triatominae)

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Triatoma brasiliensis *is composed of at least four geographic populations (brasiliensis, melanica, macromelasoma, and juazeiro) that have distinct chromatic, morphologic, biologic and ecologic patterns, and genetic composition. Reciprocal crosses between all pairwise combinations were carried out in order to evaluate the genetic and reproductive compatibility of these four populations. The F1 individuals developed normally and the resulting adults were crossed again to test the F2 and F3 viability. Genetic incompatibility was found between melanica and brasiliensis populations.*

Key words: *Triatoma brasiliensis* - populations - genetic incompatibility

Triatoma brasiliensis Neiva, 1911, the main Chagas disease vector in Brazil, (Silveira & Vinhaes 1999, Costa et al. 2003a) presents great chromatic variation, which has lead in the past to the description of two subspecies: *T. brasiliensis melanica* Neiva & Lent, 1941 and *T. brasiliensis macromelasoma* Galvão, 1956. These subspecies were synonymized as *T. brasiliensis* due to the allegation that intermediate forms could be found in the natural environment (Lent & Wygodzinsky 1979). The nominal subspecies and those of differentiated darker patterns (once described as subspecies) are here referred to as populations and called brasiliensis, melanica and macromelasoma. During the field collections carried out in several sites including the type localities, a fourth chromatic pattern was collected in Juazeiro (State of Bahia) and it will be referred to as the juazeiro population (Costa 1997, Costa et al. 1997a)

Recently, in an effort to clarify the evolutionary relationship of these distinct populations of *Triatoma brasiliensis sensu lato,* several approaches have been carried out: morphologic (Costa et al. 1997a), bionomic (Costa & Marchon-Silva 1998), ecologic (Costa et al. 1998, 2002), and molecular (Costa et al. 1997b, 2001). It was shown that *T. brasiliensis* is composed of at least four distinct geographic populations, and a great level of differentiation was observed among them. The genetic distances generated by isoenzymes and the mitochondrial DNA sequences revealed levels of divergence higher than those usually obtained in comparison between other closely related triatomine species (Costa et al. 1997b, 2001).

In order to evaluate the genetic and reproductive compatibility of these four *T. brasiliensis* populations, reciprocal crossing experiments among all possible combinations were conducted, and observed through the production of F3 eggs, according to recommendations to evaluate genetic compatibilities (Mayr 1963). The experimentation was conducted under laboratorial conditions of temperature ($X = 29.10^{o}$C, min. 22, and max. 31), and humidity ($X = 71.8\%$, min. 31, and max. 50). Fifth instar nymphs from colonies originated from individuals collected (n > 30) in the type localities (Caicó, Rio Grande do Norte - brasiliensis; Petrolina, Pernambuco - macromelasoma; Espinosa, Minas Gerais - melanica; and Juazeiro, Bahia - juazeiro) were isolated and kept individually until adulthood. Reciprocal crosses between all pairwise combinations were carried out, totaling 12 possible combinations. Three couples of each combination were formed and kept separately in plastic flasks ($10 \times 10 \times 16$ cm) containing folded filter paper. Intra population crosses were also performed for control, totaling 12 couples. All of these couples were maintained simultaneously and fed until repletion on mice once a month. All couples, experimental and control, produced viable eggs with variable percentages of

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eclosion (70-90%). Nymphs presented normal development to adulthood. No morphologic anomaly was observed in any of the nymphal instars (Table I). Subsequently, virgin F1 hybrid adults were crossed to test for the viability of the F2. Three couples generated by each of the previous combinations were formed. The same procedure was also utilized to assess the viability of the F3 eggs. All the hybrid couples were able to produce viable F2 and F3 eggs, with the exception of the combination of brasiliensis male × melanica female. In this combination a high mortality of the F2 fifth instar nymphs (80%) was observed. Only two females and two males reached adulthood (Table I). Of the other six nymphs, three died as fifth instars following ecdysis, two failed to reach adulthood and died after one year being fed monthly, and one presented incomplete ecdysis to adulthood and died. Two hundred and eleven F3 eggs were obtained from these two couples with 0% of eclosion.

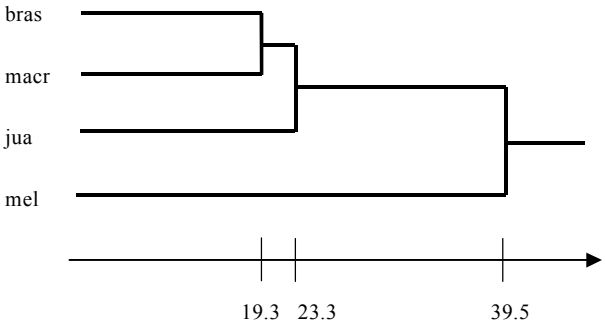
The heterogeneity among combinations shown in Table I was highly significant ($P_{X2} < 0.0001$), indicating unequal reproductive performance among the pairs. The average numbers of adults obtained from the six intergroup crossings (Table I) were used as an indicator of reproductive performance (Table II) to allow a characterization of populations based on the criterion of interfertility. This was illustrated by an UPGMA dendrogram (Sokal & Michener 1958) showing putative genetic compatibilities (Figure). Its topology suggested the brasiliensis-macromelasoma as the closest pair and melanica as the more external group, a pattern already obtained elsewhere from genetic data (Costa et al. 1997b, 2001).

The present study showed that in spite of the morphologic, biologic, ecologic, and molecular differences previously demonstrated among these four *T. brasiliensis*

TABLE II

Average numbers of obtained adults from crosses between four populations of *Triatoma brasiliensis*: brasiliensis (bras), melanica (mel), macromelasoma (macr) and juazeiro (juaz). Each cell contains an average from twelve crosses: the three F1 and three F2 crosses between groups plus the corresponding reciprocal crosses (see Table I). Values in diagonal are average numbers of adults obtained from within population crosses, six ones instead of twelve (see Table I)

		Population			
		bras	macr	juaz	mel
Population	bras	58.67			
	macr	56.00	65.67		
	juaz	57.17	41.00	42.67	
	mel	33.25	29.67	35.25	41.67



A tree illustration of relative reproductive compatibility among populations of *Triatoma brasiliensis*: brasiliensis (bras), melanica (mel), macromelasoma (macr), and juazeiro (juaz) using the average distance method (UPGMA). The values of Table II were used as parameters describing each population; the UPGMA algorithm used the Euclidian formula to compute differences.

TABLE I

Number of adults obtained from each of the three couples (parentals and hybrids F1) formed for the crossing experiments carried out between four populations of *Triatoma brasiliensis*: brasiliensis (bras), melanica (mel), macromelasoma (macr) and juazeiro (juaz)

Parentals	F1	F1	F1	Subtotals	F2	F2	F2	Subtotals
Inter-group crossings								
bras ♂ × mel ♀	67	65	0	132	2	2	0	4
bras ♀ × mel ♂	51	55	61	167	25	32	39	96
bras ♂ × macr ♀	126	55	0	181	87	80	10	177
bras ♀ × macr ♂	54	74	0	128	107	0	79	186
bras ♂ × juaz ♀	14	62	119	195	48	68	60	176
bras ♀ × juaz ♂	66	86	76	228	57	7	23	87
mel ♂ × macr ♀	20	105	0	125	21	26	32	79
mel ♀ × macr ♂	42	52	0	94	30	8	20	58
mel ♂ × juaz ♀	98	32	60	190	0	15	31	46
mel ♀ × juaz ♂	13	68	0	81	55	36	15	106
macr ♂ × juaz ♀	22	20	18	60	92	0	79	171
macr ♀ × juaz ♂	4	55	149	208	42	0	11	53
Control crossings								
bras × bras	27	83	74	184	45	69	54	168
mel × mel	51	69	0	120	42	43	45	130
macr × macr	72	55	80	207	60	70	57	187
juaz × juaz	63	60	40	163	57	36	0	93

populations, they were able to freely interbreed and to produce viable progeny in the laboratory. Interestingly, the two populations (melanica and brasiliensis) known to present the lowest genetic identity values, based on either alloenzyme data or mitochondrial DNA sequences (Costa et al. 1997b, 2001), or the highest ecologic distinctiveness (Costa et al. 2002), also revealed the lowest reproductive compatibility, disclosing sterility in F2 crosses.

According to Ryckman (1962) the reproductive isolation is the best criteria to assess the taxonomic status of a determined "population". In this regard, the present data suggest that brasiliensis and melanica populations are in the beginning of a true speciation process. Our data also indicate that despite their high genetic heterogeneity, the four differentiated populations of *T. brasiliensis* are still reproductively compatible. However, reproductive compatibility is not proof of conspecificity. Actually, many examples exist of possible hybridization among well established species of Triatominae (Usinger et al. 1966, Perlowagora-Szumlewics & Correia 1972). Due to the apparent "gradient" in the likely speciation process among the *T. brasiliensis* populations, and the well-known possibility of hybridization among many species of Triatominae (Usinger et al. 1966), the taxonomic status of these four distinct *T. brasiliensis* "populations", at least of one of them (melanica), should be reconsidered at specific level. We are preparing this revision in the lights of the present and previous studies on this complex (Costa et al. 2003b).

Under the perspective of vector control, the present results, as well as the previous ecologic and molecular approaches, showed that the distinct allopatric and parapatric "populations" of *T. brasiliensis lato sensu* could be treated as individual targets. From an epidemiologic point-of-view, it was also important to stress that these "populations" are not simply morphologic variants of the same biologic entity, which could explain why they have different epidemiologic importance (Costa et al. 1998, 2003a), and why they are not expected to behave as a single species in response to any environmental change.

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