Ecotopes, Natural Infection and Trophic Resources of *Triatoma brasiliensis* (Hemiptera, Reduviidae, Triatominae)

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Triatoma brasiliensis *is considered as one of the most important Chagas disease vectors in the northeastern Brazil. This species presents chromatic variations which led to descriptions of subspecies, synonymized by Lent and Wygodzinsky (1979). In order to broaden bionomic knowledge of these distinct colour patterns of* T. brasiliensis, *captures were performed at different sites, where the chromatic patterns were described: Caicó, Rio Grande do Norte (*T. brasiliensis brasiliensis *Neiva, 1911), it will be called the "brasiliensis population"; Espinosa, Minas Gerais (*T. brasiliensis melanica *Neiva & Lent 1941), the "melanica population" and Petrolina, Pernambuco (*T. brasiliensis macromelasoma, *Galvão 1956), the "macromelasoma population". A fourth chromatic pattern was collected in Juazeiro, Bahia the darker one in overall cuticle coloration, the "Juazeiro population". At the sites of Caicó, Petrolina and Juazeiro, specimens were captured in peridomiciliar ecotopes and in wilderness. In Espinosa the specimens were collected only in wilderness, even though several exhaustive captures have been performed in peridomicile at different sites of this municipality. A total of 298 specimens were captured. The average registered infection rate was 15% for "brasiliensis population" and of 6.6% for "melanica population". Specimens of "macromelasoma" and of "Juazeiro populations" did not present natural infection. Concerning trophic resources, evaluated by the precipitin test, feeding eclecticism for the different colour patterns studied was observed, with dominance of goat blood in household surroundings as well as in wilderness.*

Key words: *Triatoma brasiliensis* - ecotopes - trophic resources - natural infection - different colour patterns

Triatoma brasiliensis Neiva, 1911 has a wide geographical distribution (Alagoas, Bahia, Ceará, Goiás, Minas Gerais, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, Sergipe, Tocantins). It is regarded as one of the most important vectors of Chagas disease in the northeastern region (Silveira et al. 1984). The different melanic forms have been described as subspecies: *T. b. brasiliensis* Neiva, 1911; *T. b. melanica* Neiva & Lent, 1941 and *T. b. macromelasoma* Galvão, 1956. These subspecies were described on the basis of the pronotum, legs and hemelytron chromatic characters (Galvão 1956). Lent and Wygodzinsky (1979) have considered them as synonymous. A fourth chromatic pattern was collected by us in Juazeiro, Bahia, and will be referred to in this study as "Juazeiro population".

In order to broaden bionomic knowledge on *T. brasiliensis*, field captures were performed at different localities where the distinct chromatic variations were collected: Caicó, Rio Grande do Norte ("brasiliensis population"); Espinosa, Minas Gerais ("melanica population"); Petrolina, Pernambuco ("macromelasoma population") and in Juazeiro, Bahia ("Juazeiro population"). Comparative data among these different melanic forms were obtained regarding ecotopes, trophic resources and natural infection by *T. cruzi*.

The present study complements morphological (Costa et al. 1997a), biological (Costa & Marchon-Silva 1996, Costa et al. 1996a) and biochemical analysis which have detected phenotypical differences among the distinct chromatic patterns mentioned (Costa et al. 1997b). The aim was to clarify the taxonomic status at intra- or interspecific levels of different populations of *T. brasiliensis* utilizing multidisciplinary studies.

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MATERIALS AND METHODS

Field collections were carried out at different sites: Caicó, Espinosa, Petrolina and Juazeiro. Specimens were obtained by exhaustive and individual captures, utilizing a pyrethroid (pirisa, 5ml/500ml of water). The specimens were carefully picked up with tweezers. Collected insects were housed in flasks with filter paper. All captures were carried out during daytime. Domiciliar, peridomiciliar and silvatic ecotopes were examined.

For the precipitin test the standard protocols were carried out according to Weitz (1956) and Siqueira (1960). Rabbit serum immunized with total proteins of human blood and of domestic and silvatic animals was employed: bird (*Gallus gallus*), goat (*Capra aegagrus*), dog (*Canis familiaris*), horse (*Equus caballus*), pig (*Sus scrofa*), opossum (*Didelphis marsupialis*), rodent (*Rattus norvegicus*) and armadillo (*Dasypus novemcinctus*). Each triatomine captured was pressed dorsoventrally at the abdominal region and the faeces drops were deposited over strips of filter paper Klabin no. 80. This procedure allowed to keep the insects alive for further lab tests. After drying the samples, the chromatic pattern and the capture site were registered and all the material was stowed away at 2-8^{o}C. For the test, a 0.5 cm diameter cut was made in the papers containing the samples, and those were eluted by 500 ml of PBS (phosphate-buffer-solution) and left ovenight in the refrigerator. Each sample was tested with the nine antisera mentioned above. To begin with, 50 ml of antiserum were poured in a glass capillary. Next, the same volume of the sample was slowly poured so the two wouldn't mix, but rather formed a contact surface with each other. It is in this interface that the sample antigenes get into contact with the antiserum antibodies. When they have an adequate concentration, a precipitation ring is formed. The reading was performed every 30 min for 2 hr.

Each sample was read by two technicians and, whenever necessary the test were repeated to confirm the results. Positive controls (antiserum + homologous proteine) and negative controls (antiserum + PBS) were added in each test.

To determine the natural infection rate, the specimens captured were examined through abdominal compression, in order to obtain the faeces drops which were deposited on slides, diluted in PBS and observed by light microscopy.

Specimens that presented negative results were fed on normal mice and reexamined 10 days after the blood meal.

The positive specimens were bathed with White solution for 2 hr and later their digestive tubes were individually dissected, macerated and diluted in PBS. Part of this material was injected in mice and part of it was employed for isolation in culture (BHI) so that the strains could later be typed phenotypically and genotypically.

RESULTS

Caicó - Domiciliary and peridomiciliary collections were carried out in various sites: Sobradinho, Salgadinho and Fazendinha. In Sobradinho only one colony was found, with 34 specimens that lived on a stone wall, 5 m from a domicile and another colony, where 13 specimens were captured among rocks near another house in the same site.

In the wild, 73 specimens were taken in six sites (Bairro Nova Descoberta, Serraria, São Bernardo Elias, Salgadinho, Pedra do Sino and Seridó). The ecotopes where these specimens were found always presented the same characteristic features: hollow spaces between and under rocks where wild rodents find shelter and goats and other breeding animals eventually pass through.

Espinosa - Captures were performed in domiciliar and peridomiciliar ecotopes in various sites (Monte Azul, Charco, São Pedro, Faca 2, Teú and Urandi, the latter in Bahia). Only *T. sordida* specimens were found in abundance in a great variety of peridomiciliar ecotopes, i.e. among roof tiles, wood fences around stables or chicken coops, under tree barks, among leaves of palm trees that covered animal shelters of various species (dogs, chicken, pigs). The 95 *T. sordida* specimens examined were negative for *T. cruzi*.

In the wild, searches were conducted in rock cavy nests. In these ecotopes 75 specimens were captured from the following sites: Poço, Cana Brava, Sussuarana and Teú.

The specimens captured were found most of the time in deep rock fissures, that were inaccessible, rendering necessary the use of unhousing. Otherwise, whenever possible, rock fragments, under which the triatomines sheltered, were removed. However, some hungry specimens exposed themselves spontaneously, showing the rostrum in readiness to obtain food.

Petrolina and Juazeiro - In the peridomiciliar ecotopes of Petrolina (Catinguinha and Serrote do Urubu) 38 specimens were collected and 27 in Juazeiro (Junco and Aldeia). All the specimens were colonizing the most various ecotopes: under rocks, in wood fences around stables or animal shelters and in bundles of dry wood. In these sites, the household surrounding areas were more restricted than those of Espinosa or Caicó, and they presented many shelters for birds, pigs and goats near one another. The common presence of *T. sordida* and of *T. pseudomaculata* in the same habitat where specimens of *T. brasiliensis* were found was observed in Junco, Juazeiro.

In wilderness, the specimens were collected in ecotopes with similar features as those already described. In Petrolina, the captures were carried out in Catinguinha and in Serrote do Urubu, where 10 and 23 specimens respectively were captured. In Juazeiro captures were carried out in Sacrabetó, Junco, Tapera and Aldeia and only at the first site five specimens were collected.

Regarding the trophic resources, 46 specimens of "brasiliensis population" from peridomiciliar ecotopes and 57 from wilderness were examined. The feeding sources most often observed were goats (56% and 54%, respectively), followed by dogs (37%) in the peridomicile and armadillos (51%) in wilderness (Table I).

In Espinosa, 47 specimens of "melanica population" were examined, all of them captured in wilderness where the most explored feeding resource was goat (57%), followed by armadillo (36%) and opossum (28%) (Table II).

In Petrolina, for the 58 specimens examined in household surroundings and in wilderness, goat blood was the most reacting feeding resource (59 and 61%, respectively), followed by human blood in peridomicile (44%) and bird and armadillo (35%) in wilderness (Table III).

In all studied sites, each triatomine presented a positive reaction for more than one feeding source.

TABLE I

Blood meal sources of *Triatoma brasiliensis* Neiva, 1911. The "brasiliensis population", in the locality of Caicó, Rio Grande do Norte

Locality	No. of specimens examined	No. of positive reactions for anti-serum[a]								
		Bird	Goat	Dog	Horse	Opossum	Human	Pig	Rodent	Armadillo
In the peridomicile										
Sobradinho	46	15	26	17	2	5	14	2	6	13
%		32	56	37	4	11	30	4	13	28
In the wild										
Bairro Nova Descoberta	20	6	3	7	9	0	6	4	2	12
Pedra do Sino	5	2	3	1	0	0	0	0	0	2
Serraria	13	4	11	5	0	5	0	0	1	6
São Bernardo Elias	16	4	11	8	1	0	3	0	1	6
Seridó	3	0	3	1	1	0	0	0	1	3
Total	57	16	31	22	11	5	9	4	5	29
%		28	54	39	19	9	16	7	9	51

a: 100 of 103 examined specimens reacted for more than one blood meal; 13 specimens presented no reaction to the anti-serum used.

TABLE II

Blood meal sources of *Triatoma brasiliensis* Neiva, 1911. The "melanica population", in the locality of Espinosa, Minas Gerais

Locality	No. of specimens examined	No. of positive reactions for anti-serum[a]								
		Bird	Goat	Dog	Horse	Opossum	Human	Pig	Rodent	Armadillo
In the wild										
Poço	13	4	6	4	1	7	5	1	1	6
Sussuarana	17	2	12	1	0	2	4	4	1	7
Cana Brava	14	1	9	1	0	1	3	2	1	3
Teú	3	3	0	1	0	3	0	0	1	1
Total	47	10	27	7	1	13	12	7	4	17
%		21	57	15	2	28	26	15	9	36

a: 32 of 47 specimens examined reacted for more than one blood meal; 1 specimen not reactive.

In Juazeiro, the 27 specimens from the peridomicile ecotopes examined also presented a higher percentage for goat blood (59%), followed by human blood (48%). In wilderness, the five triatomine examined were positive for armadillo blood (Table IV).

Even in wilderness, we usually found traces of human activities related to the breeding of animals, especially caprines.

The average natural infection rate for a total of 298 specimens varied for each chromatic pattern among different sites. In the peridomicile, only the "brasiliensis population" have presented natural infection for *T. cruzi*. Forty-seven specimens from Sobradinho site were examined and seven were positive, with an average of 14.9%. In wilderness, the "brasiliensis population" showed an average of natural infection rate of 15.1%. The highest infection rate was observed in the site of Nova Descoberta (36%). For "melanica population", which was only found in the silvatic environment, the rate was 6.6% (Table V). All specimens collected in Petrolina and Juazeiro presented a negative rate for *T. cruzi*.

From positive specimens, 15 strains were isolated, 14 of which were typed and biochemically characterized, confirming natural infection by *T. cruzi* (Costa et al. 1996b).

TABLE III

Blood meal sources of *Triatoma brasiliensis* Neiva, 1911. The "macromelasoma population", in the locality of Petrolina, Pernambuco

Locality	No. of specimens examined	No. of positive reactions for anti-serum[a]								
		Bird	Goat	Dog	Horse	Opossum	Human	Pig	Rodent	Armadillo
In the peridomicile										
Catinguinha	18	2	7	4	0	0	8	0	2	7
Serrote do Urubu	9	4	9	1	0	2	4	0	3	2
Total	27	6	16	5	0	2	12	0	5	9
%		22	59	18	0	7	44	0	18	33
In the wild										
Catinguinha	10	2	6	3	0	0	0	0	2	3
Serrote do Urubu	21	9	13	7	2	3	1	0	4	8
Total	31	11	19	10	2	3	1	0	6	11
%		35	61	32	6	10	3	0	19	35

- 5 specimens presented no reaction to the anti-serum used; *a*: 44 of 58 specimens examined reacted for more than one blood meal.

TABLE IV

Blood meal sources of *Triatoma brasiliensis* Neiva, 1911. The "Juazeiro population", in the locality of Juazeiro, Bahia

Locality	No. of specimens examined	No. of positive reactions for anti-serum[a]								
		Bird	Goat	Dog	Horse	Opossum	Human	Pig	Rodent	Armadillo
In the peridomicile										
Junco	6	3	5	1	0	3	1	0	0	1
Aldeia	21	5	11	6	0	6	12	0	0	8
Total	27	8	16	7	0	9	13	0	0	9
%		30	59	26	0	33	48	0	0	33
In the wild										
Sacrabetó	5	2	4	4	1	3	1	0	1	5
%		40	80	80	20	60	20	0	20	100

a: all 32 examined specimens reacted for more than one blood meal.

TABLE V

Rates of natural infection with *Trypanosoma cruzi* of *Triatoma brasiliensis* Neiva, 1911 according to distinct colour patterns in wild environment at different sites

Site		"brasiliensis population"				"melanica population"					
		Caicó, RN				Espinosa, MG					
		Nova Descoberta	Serraria	São Bern. Elias	Sobradinho	P. do Sino	Seridó	Teú	Poço	Cana	Sussuarana
No. of samples collected		22	14	20	9	5	3	15[a]	17	26	17
Total		73				75					
Total of positive samples in each instar	♂	2		1	1				1	1	
	♀	2					1		2		
	N5	1									
	N4	3									
Total of positive samples		8	0	1	1	0	1	0	3	1	0
Total		11				4					
% of positiveness		36.4	0	5	11.11	0	33.33		17.65	3.85	0
% $\overline{X}$ of positiveness		15.07				6.6					

a: specimens have died few minutes after capture, in response to the concentration of the pyrethroid utilized as unhousing, and for this reason they were not scorable.

TABLE VI

Natural infection rate with *Trypanosoma cruzi* of the different populations of *Triatoma brasiliensis* Neiva, 1911 in the peridomicile and wild environment

No. of specimens	"brasiliensis population"	"melanica population"	"macromelasoma population"	"Juazeiro population"
Collected	120	75	71	32
Examined	120	60	56	30
Infected with *T. cruzi*	18	4	0	0
% of infection	15	6,66	0	0

DISCUSSION AND CONCLUSIONS

The "brasiliensis population" appears to be the most important chromatic pattern from the epidemiological point of view. Comparing specimens from the Herman Lent collection this pattern presents the most wide geographical distribution: Ceará, Paraíba, Piauí e Rio Grande do Norte; it was not observed simpatry among the different colour patterns (Costa et al. 1997b); the "brasiliensis population" have been captured naturally infected in wilderness and in peridomicile ecotopes; this pattern can also occurs indoors in Caicó (Fundação Nacional de Saúde - FNS pers. com.).

The "macromelasoma" and the "Juazeiro populations" were also found in wilderness and in peridomiciliar environments. However, the natural infection rate for *T. cruzi* was negative for both. Even though, Petrolina and Juazeiro are located in different states (Pernambuco and Bahia, respectively). These two populations belong to neighboring sites which are separated by the São Francisco river, thus, sharing a parapatric condiction with the same environmental support capacity.

The "melanica population" was found only in wilderness, showing a different behavior from the other patterns examined. This pattern is clearly involved in the maintenance of the wild cycle of *T. cruzi*.

Regarding trophic resources, feeding eclecticism was observed for all different populations of *T. brasiliensis.* However, goat blood stood out in wilderness as well as in peridomiciliar areas for the four population samples analyzed. These data agreed with observations of Lent and Wygodzinsky (1979) and also with observations in field captures related to caprine herds, that roam from household surrounding areas to wilderness. This feature has shown the interconnection between these two ecotopes and perhaps the arbitrariness in discriminating their boundaries.

Several authors registered the occurrence of triatomine that presented positivity to more than one trophic resouce (Siqueira 1960, Pinto Dias et al. 1989, Salvatella et al. 1994). Romoser et al. (1989) emphasized the value to the knowdledge of the local fauna and the detection of any evidence of a host presence to obtain a better evaluation of the positive results for the different trophic resourses, mainly for phylogenetically related animals.

The fact that in Caicó, Petrolina and Juazeiro the different chromatic patterns have been found both in household and wild environments, indicates the possibility that in these sites the natural environment (rock cavy nests) could be suffering impact pressure and the feeding sources may not be fulfilling the basic needs, which would stimulate the invasion of new environments, as emphasized by Aragão (1975) concerning feeding opportunism. The wilderness is much poorer in vegetation in Caicó, Petrolina and Juazeiro than in Espinosa. Furthermore, in the three former sites, the high population density of *T. sordida* observed in Espinosa was not found. Thus the household surrounding areas at those sites offer less competitive conditions to allow colonization by *T. brasiliensis*.

According to information received from FNS, the low population density of triatomines found the peridomicile areas in Caicó results from the fact that it had recently been sprayed and is under permanent control, due to the high level of triatomine infestation found in the mentioned area. No other species were found during the searches. FNS records show that this species is frequent in household surroundings.

It was in the sites of Petrolina and Juazeiro that the species was found most remote from its natural environment. Indeed, it was collected in various human ecotopes, such as stables, chicken coops and other human artifacts. In Caicó, the specimens were collected in household surroundings, in environments that resemble natural ecotopes, i.e., stone walls, that somehow reproduced the rock cavy nests where they are found in wilderness. These walls are characteristic and frequently found in this region. They abundantly reproduce the features of the natural shelter of "brasiliensis population", because all housing units are bounded by this type of construction.

The "melanica population" seldom invades households in Espinosa (inhabitants and FNS, pers. com.). When the population density in the natural shelters at rock cavy nests increases rapidly at a certain time of the year, it becomes necessary to promote a burning of the nests, because the insects voraciously attack people who approach them, often to work with the rocks or in connection with agricultural activities.

Studies carried out by Gomes (1993) in two sites at São Raimundo Nonato, in Piauí, concerning the feeding profile and the mobility of *T. brasiliensis* and other triatomine species, have demonstrated that this species presents ample mobility and is found in household surroundings as well as indoors (bedrooms). Through the precipitin test, they demonstrated a high positivity for the armadillo blood (72.1%). Subsequently these specimens were examined concerning the chromatic patterns that define the *T. brasiliensis* populations. They were characterized as "brasiliensis population".

The broad ecological valence disclosed for different analyzed chromatic patterns of *T. brasiliensis*, through different colonized ecotopes, through variety of trophic resources and through natural infection rates stresses the need to increase environmental knowledge on the vectorial potential of *T. brasiliensis* in different areas not yet analyzed.

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