

Biological aspects of crosses between *Triatoma maculata* (Erichson, 1848) and *Triatoma pseudomaculata* Corrêa & Espínola, 1964 (Hemiptera: Reduviidae)

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Due to morphological similarities between Triatoma maculata and T. pseudomaculata, which comprise the "maculate complex", both had been regarded as the same species until 1964. Considering that the studies on triatomine hybridization permit hypotheses formulation concerning origin and divergence of species, enabling a quantitative analysis of taxonomic relationships between species, the present investigation was aimed at broadening further understanding related to the capacity of hybrid production by determining the degree of reproductive isolation between T. maculata and T. pseudomaculata. Our results have demonstrated that T. maculata and T. pseudomaculata showed no differences regarding reproduction patterns and they are able to cross, generating infertile hybrids.

Key words: *Triatoma maculata* - *Triatoma pseudomaculata* - hybrids - Triatominae

Triatomines are hematophagous insects belonging to the order Hemiptera, family Reduviidae, and subfamily Triatominae, comprising a group of 138 species (Galvão et al. 2003, Forero et al. 2004, Costa et al. 2006, Costa & Felix 2007). The epidemiological importance of such group lies in their potential capacity of transmitting the etiological agent of Chagas disease, the flagellate protozoan *Trypanosoma cruzi* (Chagas 1909).

In epidemiological terms, *T. maculata* is considered a species of secondary importance (Lent & Wygodzinsky 1979). It can occur in domestic, peridomestic, and silvatic habitats, frequently found in hen houses, corals, nests of birds (Lent & Wygodzinsky 1979, Torrealba et al. 1985, Carcavallo et al. 1997), nests of small mammals (Torrealba et al. 1985), pig pens (Lent & Wygodzinsky 1979), and pigeon coops, although its natural ecotope seems to be palm tree crowns and dry trees (Carcavallo et al. 1997). *T. pseudomaculata* is also found in hollows and under the bark of dry trees (Noireau et al. 2005). It is also a species of secondary epidemiological importance, often found in peridomestic habitats associated with domestic fowl (Silveira & Rezende 1994, Borges et al. 1999, Sarquis et al. 2006).

Due to morphological similarities, most triatomine species can be grouped into specific complexes (Carcavallo et al. 2000), which have been much discussed in order to reassess species distinction and establish precise terms for their identification. The Lent and Wygodzinsky (1979) revision included *T. maculata* and *T. pseudomaculata* in the 'infestans complex' together

with 19 other species, although Carcavallo et al. (2000) grouped the two together as the 'maculata complex'.

T. maculata and *T. pseudomaculata* are allopatric species (Figure). According to Schofield (1988), *T. maculata* could be a derivative of *T. pseudomaculata*, geographically separated by association with migratory birds, although Santos (2003) and Hypša et al. (2002) rejected the hypothesis that *T. maculata* and *T. pseudomaculata* had the same origin.

In their original description of *T. pseudomaculata*, Corrêa and Espínola (1964) noted that hybrids could be obtained from crosses between *T. maculata* from Roraima, Brazil, and *T. pseudomaculata* from Paraíba, Brazil, but with a high number of non-viable eggs – particularly when *T. pseudomaculata* males were crossed with *T. maculata* females. Later, exploring further crosses, including *T. pseudomaculata* from Paraíba and Minas Gerais, Brazil, Espínola (1974) reasserted the validity of *T. pseudomaculata* as a separate species and, by observing differences between specimens from the two localities, suggested recognition of *T. pseudomaculata pseudomaculata* from Northeast Brasil, and *T. pseudomaculata minasensis* from Minas Gerais, as distinct subspecific entities. Perlowagora-Szumlewicz and Correia (1972), Perlowagora-Szumlewicz et al. (1974), Perlowagora-Szumlewicz (1976), in a series of studies of hybridization of Triatominae, concluded that viable reciprocal crosses (male of one species with female of another, and vice versa) between *T. pseudomaculata* and *T. sordida* demonstrated genetic proximity between these two species, whereas unilateral crosses (successful in only one direction, male of one species and female of the other, or vice versa) demonstrated more distant relationships, as between *T. pseudomaculata* and *T. infestans*.

Considering that triatomine hybridization (a) allows formulation of hypotheses concerning origin and divergence of species, (b) may help understand the systemat-

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Received 13 December 2006

Accepted 7 March 2007



Map of Brazil showing the geographical distribution of *Triatoma maculata* (black) and *T. pseudomaculata* (gray) in Brazilian territory (Carcavallo et al. 1999).

ics of the group (Pérez et al. 2005), and (c) experimental hybridizations have enabled quantitative analyses of taxonomic relationships correlated with degrees of morphological similarities between species (Usinger et al. 1966), the present investigation was aimed at broadening our understanding of the degree of reproductive isolation between *T. maculata* and *T. pseudomaculata*.

MATERIALS AND METHODS

Parental specimens of the *T. maculata* colony were obtained in November 2002 in palm trees locally known as “inajá” (*Attalea maripa*), in the municipality of Mucajá, Roraima (2°26'16,3" N and 61°0,1'14,8" W). *T. pseudomaculata* specimens were captured in peridomestic habitats in September 2003 in the municipality of Sobral, state of Ceará (3°47'22" S and 40°24'08" W) Brazil, in collaboration with Fundação Nacional de Saúde – Funasa (*The National Health Foundation*). The colonies were maintained in the insectary of the Laboratory of Triatomine and Epidemiology of Chagas Disease at Centro de Pesquisa René Rachou-Fiocruz, Belo Horizonte, Brazil, in an incubator (at $27 \pm 1^\circ\text{C}$, relative

humidity $65 \pm 5\%$) and offered weekly feeds on Swiss mice anesthetized with Thiopental. To perform the experiments, either field collected insects or up to the F1 generation, obtained under laboratory conditions, were used.

For inter and intraspecific (control) crosses, 10 couples for each group were used as follows: (1) *T. pseudomaculata* females \times *T. maculata* males; (2) *T. maculata* females \times *T. pseudomaculata* males; (3) *T. pseudomaculata* males \times females; and (4) *T. maculata* males \times females.

The insects were sexed at the fifth instar and males and females were kept separately with weekly blood-feeding. The couples were introduced no more than three days after the adult molt, and each couple was then kept in a separate container. Each female was checked daily for eggs whose viability was then observed for at least 15 days. Viable offspring were then maintained to the adult stage.

Data obtained from a daily follow up of hybrid offspring were submitted to a parametric statistical analysis (ANOVA) concerning average of laid eggs and hatch time, and a non-parametric analysis (Kruskal Wallis) regarding percentage of hatched eggs. Analyses were carried out through the software Graph Pad Prism version 3.00. Fertility of the hybrids was evaluated from a pool of insects (Table I). Hybrid males and females were kept together and observed weekly for one month to verify the presence of eggs and spermatophore elimination.

RESULTS

None of the parameters under study showed significant differences between specific crosses (Table II).

Crosses involving *T. maculata* females showed a lower number of eggs laid (Table II) and a longer hatching period (Table II) when compared with the *T. pseudomaculata* control crosses; whereas, crosses involving *T. maculata* males showed a shorter hatching period in relation to the *T. pseudomaculata* control crosses (Table II). The percentage of hatched eggs was significantly different only for crosses between *T. maculata* male and *T. pseudomaculata* female (Table II) when compared to the *T. maculata* control crosses. A couple from this group was observed not to have their eggs hatched. Spermatophore elimination by females was noted in order to detect mating between hybrid specimens and backcross hybrids. This provided evidence that hybrids are able to mate, even if they produce unviable eggs.

TABLE I

Backcross groups formed from a pool of hybrids descendants of the cross between *Triatoma maculata* and *T. pseudomaculata* and their parental species. The crosses had been kept at $27 \pm 1^\circ\text{C}$ and relative humidity $65 \pm 5\%$ and observed during one month

Male	Female
Hybrids of the cross between <i>T. maculata</i> male and <i>T. pseudomaculata</i> female	<i>T. maculata</i> <i>T. pseudomaculata</i>
<i>T. maculata</i> <i>T. pseudomaculata</i>	Hybrids of the cross between <i>T. maculata</i> male and <i>T. pseudomaculata</i> female
<i>T. maculata</i>	Hybrids of the cross between <i>T. pseudomaculata</i> male and <i>T. maculata</i> female

TABLE II
Average of daily observation of the crosses between *Triatoma maculata* (Tm) and *T. pseudomaculata* (Tp) during 15 days since the first laid (10 couples for each cross)

Parameters observed	Cross			
	Tm ♂ x Tm ♀	Tp ♂ x Tp ♀	Tm ♂ x Tp ♀	Tp ♂ x Tm ♀
Laid eggs	22,5 ± 8,1	30,5 ± 8,2 ^a	21,5 ± 7,8	21 ± 5,9 ^a
Hatching time	18,1 ± 0,3	17,2 ± 0,4 ^a	16,6 ± 0,5 ^a	19,9 ± 0,4
Success (percentage of hatched eggs)	96 ± 6,6 ^a	89 ± 11,4	72 ± 28 ^a	90 ± 10,9

a: different values for each parameter observed ($p < 0.05$).

DISCUSSION

Observations of the biology of *T. maculata* and *T. pseudomaculata* are available from several sources. Espínola et al. (1981) assessed *T. maculata* crosses under laboratory conditions at temperatures ranging from 26.3 to 32.2°C, average relative humidity of 60.4% and with the bugs blood fed on chickens. The number of eggs laid varied from one to 17 eggs a day, with an average daily oviposition of three eggs; 8.1% of the eggs were infertile. In our study, *T. maculata* showed an average daily oviposition of 12.6, producing 4% of infertile eggs. Feliciangeli and Rabinovich (1985) observed an average of 19.1 days of incubation for *T. maculata* eggs, which is similar to our results, in which average incubation time was 18.1 days. Silva et al. (1995), by studying the influence of temperature on the development of *T. maculata* blood fed on chicken, verified a difference in the egg incubation period: 29 and 16 days at 25 and 30°C, respectively. Luitgards-Moura et al. (2005) have also shown that higher temperatures diminish the incubation period; with temperatures ranging from 21.5 to 32°C (average 27.6°C) and weekly blood feeding on mice, these authors reported a variation in the period of egg incubation between 14 and 42 days, with a significant negative correlation between temperature and incubation time (-0.98).

Previous investigations on the biology of *T. pseudomaculata* have shown that the average incubation period is 18 days, at temperatures $28 \pm 1^\circ\text{C}$ and 80% relative humidity, blood fed weekly on pigeons (Gonçalves et al. 1997). These results are in accordance with ours, where temperature and relative humidity were kept at $27 \pm 1^\circ\text{C}$ and $65 \pm 5\%$, respectively, with an average egg hatching time of 17.2 days.

Our results show no differences between *T. maculata* and *T. pseudomaculata*, concerning the number, hatching time, and viability of eggs laid. Besides maintenance conditions, slight differences in the biology of *T. maculata* and *T. pseudomaculata* shown here, in relation to previous studies (Gonçalves et al. 1997), might be due to genetic variations among the populations used, and to the length of time that colonies had been kept under laboratory conditions.

At the time of description of *T. pseudomaculata*, Espínola carried out crosses between *T. maculata* and *T. pseudomaculata* and verified that they produced unvi-

able eggs, mainly when crosses were between *T. pseudomaculata* males and *T. maculata* females (Corrêa & Espínola 1964). In the present study, the eggs hatching period showed no statistical difference in the crosses between *T. maculata* females and *T. pseudomaculata* males, or between *T. maculata* males and *T. pseudomaculata* females; 72% of the eggs laid by *T. pseudomaculata* females in the cross with *T. maculata* hatched, and 90% of the eggs laid by *T. maculata* females crossed with *T. pseudomaculata* were successful. Such results demonstrate that the direction of the crosses did not significantly interfere with embryonic development. A significant decrease in the percentage of eggs hatched in the cross between *T. maculata* males and *T. pseudomaculata* females (72%) was observed in relation to the control cross of *T. maculata* (96%). However, one must consider that, in this interspecific cross, there was a couple whose eggs did not hatch, a fact that may also occur in intraspecific crosses, probably due to either the male or the female infertility. The interspecific cross with *T. maculata* female showed the worst results when compared with the control cross with *T. pseudomaculata*; when *T. maculata* males were included in the interspecific crosses, eggs showed a shorter development length than that with *T. pseudomaculata*. Such results show the influence of female *T. maculata* specimens in relation to the control cross with *T. pseudomaculata*.

In accordance with the species concept proposed by Dobzhansky (1937): "a species is a group of fully fertile individuals, but reproductively isolated from other similar groups due to their physiological features (producing any parental incompatibility, hybrid sterility, or both)". The ease with which *T. maculata* and *T. pseudomaculata* intercross in the laboratory, despite not producing fertile hybrids in nature, may be explained by the fact that they are allopatric species (Figure). Because they do not co-exist, there would be no selection of features (either morphological or behavioral) that would lead to reproductive isolation between them. Several other triatomine species, morphologically similar or not, genetically close or not, are known to produce hybrids under either natural or experimental conditions (Mazzotti & Osório 1941, 1942, Abalos 1948, Ryckman 1962, Espínola 1971, Carvalheiro & Barreto 1976, Franca-Rodríguez et al. 1979, Zárate & Zárate 1985, Galíndez et al. 1994, Pérez et al 2005).

ACKNOWLEDGMENTS

To Dr CJ Schofield for English review of this paper.

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