Influence of blood meal and mating in reproduction patterns of *Triatoma brasiliensis* females (Hemiptera: Reduviidae) under laboratory conditions

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The influence of blood meal and mating on Triatoma brasiliensis *(Neiva) female fecundity, fertility, life-span and the preoviposition period were investigated under laboratory conditions. Nourishment increased fecundity, fertility and adult lifespan, whereas mating increased fecundity, fertility and decreased the preoviposition period. Females also required more than one mating to reach their full reproductive potential. Results indicate that both nourishment and mating are important in* T. brasiliensis *proliferation. Such information will help towards developing effective control strategies of this vector of Chagas disease.*

Key words: *Triatoma brasiliensis* - triatomine - fecundity - fertility

Triatoma brasiliensis is one of the most important species vectors of Chagas disease in Brazil, found mainly in the semi arid region of the Brazilian Northeast. Its large colonies in wild and domiciliary units, coupled with its high rate of *Trypanosoma cruzi* infection and ability to re-invade domiciliary environments shortly after insecticide application (Dias 1995) attest to its epidemiological importance. The purpose of this study was to investigate the influence of the blood meal and mating on the *T. brasiliensis* female to evaluate the role of these components in the fecundity, fertility, preoviposition period and life-span. Considering that reproductive success is intimately associated with the potential for colonizing or re-colonizing new ecotopes in endemic areas, this information will help in designing effective control strategies.

T. brasiliensis used in this study were third generation insects taken from an endemic area situated in the semi arid region of the state of Ceará, Brazil, and held in the laboratory at 28.6 ± 2.7ºC and 77.7 ± 10% humidity. Fifth instar nymphs from this colony were sex-screened (Espínola 1966) and male and female nymphs separated into different glass vials. They were fed fortnightly on live pigeons until the imaginal ecdysis (procedure approved by the Animal Use and Care Committee at Oswaldo Cruz Foundation, license L-0064/08).

The insects were divided into the following groups:

Animals fed fortnightly on pigeon blood

Multiple mated females - Twenty couples were placed into individual glass vials and any male that died before the female was immediately replaced with live males no older than 15 days post emergence. The number of matings by a female was determined by the number of Spermatophore casings found in the glass vials (Figure).

Single mated females - Thirty-six couples were placed into individual glass vials and after copulation the male was removed. Mating was confirmed by the presence of a Spermatophore casing in the glass vial and the female remained alone until death.

Unmated females - Thirty-five unmated females were collected immediately after the last ecdysis and placed into individual glass vials.

Animals receiving no food after the last ecdysis

Multiple mated females - Fourteen recently ecdysed females were separated into individual glass vials and each one stayed with a male all the time.

Unmated females - Forty-two virgin females were separated into individual glass vials immediately after the last ecdysis.

The groups were observed daily until the death of all females. The eggs laid by the mated female groups were kept for 30 days to ascertain the number that hatched into nymphs. Statistical analysis was carried out by the Mann-Whitney and Chi-square tests; a p-value less than 0.05 was considered statistically significant.

Feeding was a powerful stimulus for egg production. Mated and unmated fed animals produced approximately 15 fold the number of eggs compared to their unfed counterparts, a difference that was highly significant ($p < 0.001$). This finding is consistent with our knowledge of hematophagous species, such as triatomines, in which the ingestion of a blood meal initiates egg production by acting through the endocrine and nervous systems (Wigglesworth 1959, 1972). However, feeding alone did not increase egg production to its

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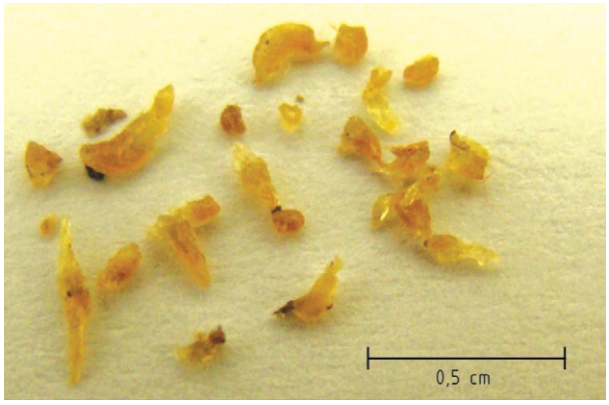

Spermatophore casings of *Triatoma brasiliensis*.

maximum level, as fed mated insects produced significantly more eggs than fed unmated ones. Thus mating also contributes to increased fecundity.

To investigate the influence of mating on reproduction in *T. brasiliensis*, we compared the mating status to the number of eggs laid (fecundity), % fertile eggs (fertility), preoviposition period and adult life-span of fed and unfed animals. In fed animals (Table I), multiple mating was more effective than a single mating as multiple mated females produced more eggs than single mated females and demonstrated a much higher % fertility ($p < 0.001$). Interestingly, fed unmated females laid as many eggs as the fed single mated females. However, both multiple mated and single mated fed animals showed a decrease in the preoviposition period compared to unmated fed females. Life-span means ranged from 89.8-115.2 days with no difference between mated and unmated animals.

In unfed animals (Table II), the mating status had an effect similar to that seen in fed animals. The multiple mated unfed insects produced significantly more eggs than their unmated counterpart and the mean value of the preoviposition period was shorter in the mated animals. This latter comparison was not significantly different, but any significance may have been masked by the very low oviposition frequency of the unfed females. The % fertility of multiple mated unfed females was also similar to that seen for multiple mated fed females although it has been reported recently that unfed females of *T. brasiliensis* reject the majority of attempts by males (Vitta & Lorenzo 2009). Since the males remained with the unfed females for the duration of the female's life span in our study, unfed *T. brasiliensis* will eventually mate given sufficient time. But without nourishment, the adult life-span of unfed animals was significantly reduced to approximately half the length for fed females.

According to several authors, egg production in triatomines is not solely dependent on ingestion of a large blood meal, for it can occur in the absence of feeding if unfed females have sufficient food stores in their digestive tract (Davey 1974, Garcia & Azambuja 1985, Davey & Singleton 1989). Further, the source of food may be a factor in fecundity. As a result of obtaining their nutrition from a single food source, the triatomine diet may lack certain vitamins and nutrients and the origin of the blood can influence their fecundity (Valle et al. 1987, Lima et al. 1990, Garneri et al. 2000). In our laboratory, the insects obtained their food from pigeons acquired in the market. Although it is possible that a shortage of hemoglobin, vitamins or proteins in a single food source may influence egg production, the high fecundity levels of our multiple mated fed females suggest that pigeon blood alone is sufficient for the maintaining a viable population of *T. brasiliensis*. The results of the present study are therefore considered to represent a similar response that would be recorded from insects in their native setting.

Besides ingestion of a blood meal, mating is a stimulus for egg production that appears to enhance, but is independent of the feeding stimulus. Lima et al. (1987b)

TABLE I

Mating versus fecundity, fertility, time of oviposition (days) or life span (days) of fed females

Fed females	Multiple mated (a)	Single mated (b)	Unmated (c)	p-value
n	20	36	35	-
Eggs laid[a]	53.2 ± 52.0	24.6 ± 40.0	15.6 ± 20.4	a x b (0.002) a x c (< 0.001) b x c (0.590)
Fertile eggs laid[a]	38.4 ± 42.7	11.9 ± 18.3	-	a x b (0.006)
% fertile eggs	72.1	38.9	-	a x b (< 0.001)[b]
Preoviposition period[a]	21.1 ± 18.5	23.4 ± 8.8	38.2 ± 23.6	a x b (0.022) a x c (0.001) b x c (0.015)
Adult life span[a]	115.2 ± 51.4	89.8 ± 59.8	109.5 ± 58.0	a x b (0.038) a x c (0.552) b x c (0.054)

Mann Whitney test. *a*: mean ± standard deviation; *b*: Chi-square test; n: number of specimens.

TABLE II

Mating versus fecundity, fertility, time of oviposition (days) or life span (days) of starved females

Unfed females	Multiple mated	Unmated	p-value
n	14	42	-
Eggs laid[a]	5.7 ± 4.4	1.1 ± 2.8	< 0.001
Fertile eggs laid[a]	3.64 ± 3.9	-	-
% fertile eggs	63.8	-	-
Preoviposition period[a]	23.7 ± 7.5	30.3 ± 17.9	0.645
Adult life span[a]	60.7 ± 17.8	53.5 ± 19.3	0.185

Mann Whitney test. *a*: mean ± standard deviation; n: number of specimens.

observed that unfed *Panstrongylus megistus* females which remained together with males after the last ecdysis were more fecund, laying more eggs than unfed unmated females. In our study, we observed the same response in *T. brasiliensis*. Mated unfed females produced on average five times more eggs than unmated unfed females.

Our study also made an unexpected observation regarding mating in fed females. From the first studies of the triatomine reproduction, it has been stated that only one mating is enough for the triatomine female to maintain fertile eggs for her entire life-span (Neiva 1910, Lima et al. 1987a, Pires et al. 2004). However, we observed a much higher fertility rate and fecundity in multiple mated fed females compared to single mated fed females. This finding suggests that a female is not able to maintain adequate amounts of spermatozoids in her spermathecae when mated only once. This inability could result from the lone male's failure to deliver to the female enough spermatozoids or other male secretions in a single copulation, or that the female empties much of the contents of her spermathecae as a single egg passes through the genital tract to the substrate. In the latter case, a female would require more than one copulation to continue to produce fertilized eggs. Other factors may also be involved, such as the reproductive competence of a single male or the duration of copulation, a factor in successful inseminations with Lygaeidae bugs, cousins to the triatomine (Micholitsche et al. 2000, Wang et al. 2008). Our finding warrants a further investigation of reproductive physiology of *T. brasiliensis* especially the organs and processes associated with copulation, insemination and egg laying.

Regarding the time spent as an adult until the first oviposition occurred, we observed that regularly fed unmated females took almost twice as long to initiate ovipositioning than regularly fed multiple or single mated females. This preoviposition period appears to be related more to mating than to feeding as the initiation of egg laying in unmated insects lags behind mated insects whether fed or not. Due to the nature of this study, insects were not sacrificed to determine the progression of egg maturation in the ovaries. Therefore, it is possible that the unmated animals retained their unfertilized eggs whereas the mated animals laid their fertilized eggs as they were ovulated. This observation has been made for *Rhodnius prolixus* in which the fed unmated female will begin egg laying several days after the fed mated female (Chiang 1998) although egg production continues at the same rate in each during this time (Davey et al. 1986). Asin and Crocco (1992) also observed that in *Triatoma infestans*, mating stimulated ovipositioning, since mated females started to lay eggs from the 13th day onwards after imaginal ecdysis, whereas unmated females did not lay eggs during the first gonadotrophic cycle. These authors also suggest that unmated females could keep their eggs in the genital tract, because a big number of ovulated eggs were found in them compared to the mated ones.

Brasileiro (1982) observed that unmated females of *T. brasiliensis* had a higher longevity than that of multiple mated females. In our study, we did not observe any significant difference in life-span between the mated and unmated animals whether they were fed or not. We did observe that feeding greatly increased the life-span such that regularly fed insects lived approximately twice as long as unfed insects.

Compared to the life-spans reported for other fed triatomines, the life-span we observed for *T. brasiliensis* females (90-120 days) is similar to *Triatoma gerstaeckeri* and *Triatoma sanguisuaga* (Wood 1964) but considerably shorter compared to adult life-spans of over 200 days recently reported for some triatomines (Martinez-Ibarra & Katthain-Duchateau 1999, Cabello & Lizano 2001, Wolff et al. 2004). Conversely, the life-spans we observed for unfed animals are similar to those reported for unfed *Triatoma lecticularia* (Jurberg & Costa 1989) and unfed *T. brasiliensis* (Costa & Perondini 1973). Perhaps, the food source used for our colony may be adequate for maximal fecundity, but may lack some essential substances required for improving longevity. Guarneri et al. (2000) observed that *T. brasiliensis* fed on pigeons had a shorter life than those fed on mice, although there was no difference in fecundity between the blood sources.

Studies on all the biological aspects of secondary triatomine species are important as they may contribute to the design of control programs. The Brazilian Northeast is the endemic region of Chagas disease with more problems, because it is the dispersion center of *T. brasiliensis*, actually considered the most difficult triatomine to control (Dias 2007). In these regions, this species is always found in large densities in its natural ecotope, the Caatinga, where the ecological characteristics facilitate its development. Deforestation together with other human interferences in this environment has caused the displacement of this species to peridomiciles and from these to intradomiciles. In our study, *T. brasiliensis* females displayed good reproductive performance, even in starvation or absence of multiple copulations providing a physiological basis for its reproductive success in the wild. These results indicate the need for constant vigilance in maintaining an effective control program for this species.

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