Life Cycle and Reproductive Patterns of *Triatoma* rubrofasciata (De Geer, 1773) (Hemiptera: Reduviidae), under Laboratory Conditions

Marina Vianna Braga/+, Zeneida Teixeira Pinto*, Marli Maria Lima

Laboratório de Biologia e Controle de Insetos Vetores *Laboratório de Educação Ambiental e em Saúde, Departamento de Biologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

The life cycle and reproductive patterns of Triatoma rubrofasciata were studied along with laboratory conditions for the establishment of a prolific colony. The insects were divided into four groups: two of them were maintained at room temperature $(20.5^{\circ}\text{C to }33^{\circ}\text{C and }85\% \pm 5\%$ of relative humidity), the other two in a climatic chamber (CC) (temperature: 29°C , humidity: $80\% \pm 5\%$). The groups were fed weekly or fortnightly on Swiss mice. The females from the group kept in the CC and fed weekly had longer life span, as well as a higher number of eggs, fertile eggs and hatchings; the group kept in the CC and fed fortnightly had a shorter life span for the 1st, 2nd and 3rd instars and a lower mortality rate for all instars. It was concluded that a constant high temperature (CC at 29°C) is the most suitable condition for the maintenance of a colony of T. rubrofasciata regardless of the interval between repasts.

Key words: Triatoma rubrofasciata - temperature - feeding - colony - reproductive pattern

Triatoma rubrofasciata (De Geer) is the only triatomine species reported from port cities througout the tropical and subtropical regions of the World (Lent & Wygodzinsky 1979). According to those authors, this species is not an important vector of Chagas disease. However, it is occasionally found naturally infected by Trypanosoma cruzi Chagas, inside human dwellings (Lucena & Magalhães Netto 1939, Dias & Neves 1943). It is commonly the vector of Trypanosoma conorhini (Donovan) which infects Rattus rattus, and this insect is in close association with the rat. Sherlock and Serafim (1972) collected T. rubrofasciata in the State of Bahia, which presented a high level of natural infection by T. conorhini, never by T. cruzi.

The establishment of a longterm colony of this triatomine species in the laboratory is believed to be difficult, since most of them have been shortlived, probably due to unfavorable environmental and feeding conditions. This investigation is aimed at providing more information regarding a better maintenance of this species in confinement.

MATERIALS AND METHODS

A total of 261 eggs was collected from the second generation of a colony from Evandro Chagas Institute, Belém, PA, Brazil, kindly provided by

Dr Adelson AA de Souza, and kept in the Biology and Control of Vector Insects Laboratory, at Oswaldo Cruz Institute, Fiocruz, Brazil. As soon as the hatching occurred, the 1st instar nymphs were divided into four groups: two of them were kept in a climatic chamber (CC) (temperature: 29°C; humidity: 80% approximately) and the other were kept at room temperature (RT) (temperature: 20.5°C to 33°C; relative humidity (R.H).: 91.3 \pm 4.3%). Both nymphs and adults were fed on Swiss mice. The groups were studied as follows: (a) group kept at RT and fed weekly (34 first instar nymphs); (b) group kept at RT and fed fortnightly (55 first instar nymphs); (c) group kept in CC and fed weekly (55 first instar nymphs); (d) group kept in CC and fed fortnightly (35 first instar nymphs).

The insects were kept in glass containers, wrapped in black cardboard and covered with nylon netting bound by an elastic band. A filter-paper was placed on the bottom of the containers so as to absorb the insects' excreta and another was folded and placed vertically inside the container to be used as substrate. Each instar was put in separate containers. The adults were individually identified (Mac Cord et al. 1983) and were separated in couples after the completion of the nymphal cycle. In the four groups all females were kept permanently with males, except in the case of death of the males. The matings were confirmed by the presence of a spermatophore capsule inside the glass containers where couples were kept.

The following parameters were observed: number of ecdyses, mortality rate of the nymphs as

Accepted 23 March 1998

⁺Corresponding author. Fax: +55-21-521.6913. E-mail: mvbraga@gene.dbbm.fiocruz.br Received 11 August 1997

well as the life span of each instar and adults, number of days between the first repast after female emergence until first oviposition (preoviposition period); egg production (fecundity), number of fertile eggs (fertility), number of eggs hatching and number of matings per couple. The data were statistically analyzed by the Kruskal-Wallis one-way test and the Chi-square test (Siegel 1956).

RESULTS

The period of maturation of the eggs was of 17.3 ± 1.3 days and 80.5% hatched. Table I shows the life cycle of all nymphal stages. First, 2nd and 3rd instars from the group kept in CC and fed fortnightly had a shorter life cycle. When kept in CC, this species spent 83 to 376 days from egg hatching to imaginal ecdysis, and when kept at RT it spent 115 to 677 days. Regardless of the feeding interval, the CC was favorable for the total development of the triatomines.

The group kept in a CC and fed fortnightly had the lowest nymphal mortality among the four groups ($c^2 = 11.1$; P<0.05) (Table II).

On Table III are the results of maximum and minimum life span for males and females of all groups. In three out of the four groups the males had a longer life span than the females, and both sexes lived longer when fed weekly.

Table IV shows the reproductive patterns of each group tested. The CC condition was favorable for the couples, since the preoviposition period was shorter for both groups, the shortest period being for the group fed fortnightly. The females fed weekly laid more eggs, had higher fertility and number of hatchings, and the group fed fortnightly presented a higher number of matings.

By the results of Kruskal-Wallis' statistical test, 11 out of the 12 parameters observed were found to be significantly different between the four experimental groups. The ecdyses from 1st to 2nd instar occurred in greater number among the nymphs kept in the CC and fed fortnightly (H=58.6. P<0.05). In the next stages (3rd, 4th and 5th), the highest number of ecdyses occurred in the group kept in a CC and fed weekly (H=39.9, P<0.05, H=27.4, P<0.05 and H=45.6, P<0.05, respectively). The preoviposition period did not present differences at any temperature or feeding conditions (H=3.7, P>0.05), showing a minimum of two days for the females fed weekly and kept at controlled temperature and a maximum of 42 days for the females that were kept under the same conditions of temperature and fed fortnightly. The life span of females (H = 19.7; P<0.05), fecundity (H = 35.6, P<0.05), fertility (H = 32.3; P<0.05) and number of hatching (H = 31.9, P<0.05) were greater in the group kept in a CC and fed weekly, whereas the number of matings (H = 19.9, P<0.05) was higher for the group kept in CC and fed fortnightly.

DISCUSSION

T. rubrofasciata is an important species of triatomine since it has been found infected by *T. cruzi* by several authors (Lucena & Magalhães Netto 1939, Dias & Neves 1943, Leal et al. 1965, Sherlock & Serafim 1974, Sherlock 1979, Brazil

TABLE I

Life cycle (minimum and maximum of days) of *Triatoma rubrofasciata* for each group tested

| Instars | Groups | | | | | | | | |
|-----------------------|---|--------------------------------------|----------------------------------|--------------------------------------|--|--|--|--|--|
| | Room temperature/ fed weekly | Room temperature/ fed fortnightly | Climatic chamber/ fed weekly | Climatic chamber/ fed fortnightly | | | | | |
| | Min Max n | Min Max n | Min Max n | Min Max n | | | | | |
| 1st | $ \begin{array}{ccc} 16 & 57 & 33 \\ (26.5 \pm 7.4)^a \end{array} $ | 32 100 52 (37.9 ± 11.3) | $11 32 54 \\ (19.1 \pm 4.0)$ | $09 	25 	34 	(15.2 \pm 5.8)$ | | | | | |
| 2nd | $\begin{array}{ccc} 22 & 100 & 31 \\ (37.4 \pm 14.1) \end{array}$ | 14 136 48 (47.4 ± 38.3) | $16 	 57 	 52 	 (23.8 \pm 7.6)$ | $11 	 25 	 32 	 (15.4 \pm 3.3)$ | | | | | |
| 3rd | $21 55 30 \\ (38.8 \pm 8.4)$ | $17 141 42$ (56.1 ± 34.2) | $26 67 49 \\ (52.2 \pm 11.2)$ | $14 66 31 \\ (26.4 \pm 18.1)$ | | | | | |
| 4th | $28 	 54 	 28 	 (35.6 \pm 7.3)$ | $15 127 35 \\ (32.4 \pm 21.5)$ | $15 83 47 \\ (49.5 \pm 15.2)$ | $19 60 31 \\ (39.8 \pm 16.1)$ | | | | | |
| 5th | $37 	 57 	 26 	 (48.7 \pm 5.1)$ | $36\ 173\ 33$ (54.4 ± 26.8) | $44 137 46 \\ (94.2 \pm 33.4)$ | $30 70 30 \\ (51.0 \pm 7.6)$ | | | | | |
| Egg hatching to adult | 124 323 | 115 677 | 112 376 | 83 246 | | | | | |

Min: minimum of days; Max: maximum of days; n: number of nymphs that molted; a: means and standard deviations.

TABLE II

Percentages of mortality in each instar of $Triatoma\ rubrofasciata$ for each group tested and result of the c^2 test

| | | | | C | Groups | | | | | |
|---------|-----------|----------------------|-------------|----|-----------|----|-------------|----|--------------|-----|
| Instars | R | Room temperature/fed | | | | | | | | |
| | Weekly | | Fortnightly | | Weekly | | Fortnightly | | _ | |
| | N % | n | N % | n | N % | n | N % | n | p c | .2 |
| 1st | 34 2.9 | 33 | 55 5.5 | 52 | 55 1.8 | 54 | 35 2.9 | 34 | 1. NS | 26 |
| 2nd | 33 6.1 | 31 | 52 7.7 | 48 | 54 3.7 | 52 | 34 5.9 | 32 | 0.78 NS | |
| 3rd | 31 3.2 | 30 | 48 12.5 | 42 | 52 5.8 | 49 | 32 3.1 | 31 | 4. NS | 03 |
| 4th | 30 6.7 | 28 | 42 16.7 | 35 | 49 4.1 | 47 | 31 | 31 | 8.83 0.05 | < |
| 5th | 28 7.1 | 26 | 35 5.7 | 33 | 47 2.1 | 46 | 31 3.1 | 30 | 1. NS | 33 |
| Total | 23.5 | | 40.0 | ı | 16.3 | | 14. | 3 | 11.11 0 | ,05 |

N: number of nymphs that started each instar; n: number of nymphs that molted; %: percentage of death; c^2 : chi-square test; p: significance level; NS: non-significant.

TABLE III

Longevity (minimum and maximum of days) of the adults of *Triatoma rubrofasciata* for each group tested

| Groups | n | Males | Females | | |
|----------------------------------|----|-------------------------------|---|--|--|
| | | Min Max | Min Max | | |
| Room temperature/fed weekly | 14 | 97 22 (202.3 ± 33.1) | 440 181 (103.1 ± 41.3) | | |
| Room temperature/fed fortnightly | 13 | $23 	 160 	 (67.7 \pm 40.5)$ | $\begin{array}{c} 29 & 122 \\ (77.3 \pm 28.1) \end{array}$ | | |
| Climatic chamber/fed weekly | 14 | $83 	 230 	 (156.9 \pm 55.8)$ | $ \begin{array}{c} 80 & 216 \\ (149.1 \pm 52.2) \end{array} $ | | |
| Climatic chamber/fed fortnightly | 11 | 48 162 (97.3 ± 36.0) | 46 91 (64.6 ± 14.6) | | |

n: number of couples per group; Min: minimum; Max: Maximum; the number in parenthesis are the mean and standard deviation for each group.

et al. 1985). It was surmised that some people of Salvador, BA, Brazil, may have been infected with *T. cruzi* transmitted by *T. rubrofasciata*, which was often found colonizing houses in the central area of this city in the 1970's (Sherlock 1979). The knowledge about its biology is still scarce, maybe due to the difficulty of maintaining an advantageous colony in the laboratory.

In order to maintain a good and fertile colony of triatomines, it is important that the nymphs have a short life cycle as well as a low rate of mortality and the adults a long life span, high fecundity and fertility, along with a high rate of hatching. In the literature, it is observed that each species has its own preference for temperature and feeding conditions.

As we know, several factors may influence triatomine biology in the laboratory, such as environmental conditions, food sources or even a prolonged endogamy to which most colonies of these insects are submitted. In our study, nymphs of *T. rubrofasciata* were able to develop and the females

| TABLE IV |
|--|
| Reproductive patterns (minimum and maximum) of adults of <i>Triatoma rubrofasciata</i> for each group tested |

| | Groups | | | | | | | |
|---------------------------------|----------------------|---------------------|--------------|----------------|----------------------|-----------------------|---------------|------------------|
| | Room temperature/fed | | | | Climatic chamber/fed | | | |
| | Weekly | | Fortnightly | | Weekly | | Fortnightly | |
| | Min | Max | Min | Max | Min | Max | Min | Max |
| Number of couples | 14 | | 13 | | 14 | | 11 | |
| Preoviposition period (in days) | 02 (15.2 | 27 $2 \pm 7.7)^a$ | 03 (20.5 | 42 ± 13.6) | 06 (13. | 22 1 ± 5.7) | 07 (11.3 | 15 3 ± 2.4) |
| Number of eggs | 132 (242.9 | 410 9 ± 77.4) | 05 (55.3 | 109 ± 37.8) | 136 (281.3 | $480 \\ 5 \pm 114.3)$ | 108 (159.0 | 220 0 ± 35.6) |
| Number of fertile eggs | 70 (181.4 | 332 4 ± 74.6) | 04 (42.8 | 103 ± 35.6) | 133 (277.0 | 478 0 ± 115.7) | 70 (140.4 | 218 4 ± 47.6) |
| Number of eggs that hatched | 63 (162.9 | 26 9 ± 69.7) | 101 (40.8 | 98 ± 35.5) | 132 (271.9 | 470 9 ± 114.3) | 63 (127.5 | 209 8 ± 48.8) |
| Number of matings | 02 (3.9 | 07 ± 1.5) | 01 (2.0 | 05 ± 1.2) | 01 (3.4 | 08 ± 2.1) | 03 (6.4 | 11 ± 2.8) |

a: means and standard deviations.

lay eggs under controlled or RT and fed weekly or fortnightly. However, the climatic conditions were more favorable, since the life cycle from egg hatching to the imaginal ecdyses was shorter, and the mortality rate of nymphs was lower; the differences observed were in relation to the feeding interval. When fed weekly, the life cycle of both sexes was longer; with respect to fecundity, fertility and the number of hatching, a higher rate was observed. In the group fed fortnightly, the development from eggs to adult was faster and the mortality rate was lower, maybe due to the lesser manipulation of the insects.

Therefore, we conclude that constant temperature (CC) and weekly (for adults) or fortnightly (for nymphs) feeding intervals are the appropriate conditions for the maintenance of a successful colony of *T. rubrofasciata*.

Triatomines with peridomestic habitats are very important in the epidemiology of Chagas disease, because they cannot be readily controlled; particular species such as *T. rubrofasciata* can attack workers in port cities or reinvade houses that were sprayed with chemical insecticides, becoming specially dangerous when infected with *T. cruzi*. Thus, it is important to know their biology under laboratory conditions and to seek means to control them.

ACKNOWLEDGMENTS

To Antonio Henrique A de Moraes Neto, for the critical revision of manuscript.

REFERENCES

Brazil RP, Da Silva AR, Albarelli A, Valle JF 1985.

Distribuição e infecção de triatomíneos por *Trypa-nosoma* do tipo *cruzi* na Ilha de São Luís - Maranhão. *Rev Soc Brasil Med Trop 18*: 257-260.

Dias E, Neves O 1943. Determinação da infecção natural por *Schizotrypanum* em *Triatoma rubrofasciata* no Estado de Pernambuco. *Mem Inst Oswaldo Cruz* 39: 331-334.

Leal JM, Sherlock IA, Serafim EM 1965. Observações sobre o combate aos triatomíneos domiciliares com BHC em Salvador, Bahia. Rev Bras Malar D Trop 17: 65-73.

Lent H, Wygodzinsky P 1979. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. *Bull Am Mus Nat Hist 163*: 125-520.

Lucena D, Magalhães Netto B 1939. Infecção natural de *Triatoma rubrofasciata* pelo *Trypanosoma cruzi*. *Rev Assoc Paul Med 15*: 177.

Mac Cord JR, Jurberg P, Lima MM 1983. Marcação individual de triatomíneos para estudos comportamentais e ecológicos. Mem Inst Oswaldo Cruz 78: 473-476.

Sherlock IA 1979. Vetores, p. 42-88. In *Trypanosoma* cruzi e Doença de Chagas, Ed. Guanabara Koogan, Brasil

Sherlock IA, Serafim EM 1972. Fauna Triatominae do Estado da Bahia, Brasil. I. As espécies e distribuição geográfica. VI. Prevalência geográfica e infecção dos triatomíneos por *Trypanosoma cruzi. Rev Soc Bras* Med Trop 6: 265-297.

Sherlock IA, Serafim EM 1974. Fauna Triatominae do Estado da Bahia, Brasil. VI. Prevalência geográfica da infecção dos triatomíneos por *T.cruzi. Rev Soc Bras Med Trop* 8: 129-142.

Siegel S 1956. Nonparametric Statistics for the Behavioral Sciences, New York, McGraw-Hill, 350 pp.