

Biologic Development of *Triatoma mexicana* (Herrich-Schaeffer 1848) (Hemiptera: Reduviidae: Triatominae) under Laboratory Conditions

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Abstract: *Triatoma mexicana* is one of the triatomine species whose biologic development is yet unknown, but it is frequently reported to be infected by *Trypanosoma cruzi*. In this research, we explore the life cycle and reproductive and feeding patterns of *T. mexicana* under laboratory conditions. Ten female and 12 male insects were collected to make up a artificial colony. The deposited eggs were maintained in the laboratory under 3 different conditions: 27°C and 66% humidity, 26°C and 35% humidity and 19°C and 45% humidity (these last were room temperature and humidity). Nymph and adult insects were fed on CD-1 mice and chicken once a week in order to study the alimentary preference of this triatomine species. Our observations were that 71.5% of successful hatching took place at 27°C and 66% humidity, therefore were the better environmental conditions for *T. mexicana*. Out of successfully hatched ones, 8% survived till adulthood, in which 2.5% were males and 5.5% were females, at the end of 338.66±35.38 days. In the adult phase, the insects survived between 100 and 190 days. In 1st to 4th instars, *T. mexicana* was fed for 1-3 times; in 5th instar for 1-4 times and in adulthood for 3-7 times. Each feeding occurred at an interval of 15-34 days. The morphologic characteristics of every instar phase are described by photographs. Through this research, biologic cycle and survival conditions of *T. mexicana* were explored for the first time.

Key words: *Triatoma mexicana*, biologic cycle, development, vectors, triatomine, morphologic characteristics, insects, transmission, chagas disease

INTRODUCTION

Triatomine are vectors of *Trypanosoma cruzi*, which is the causative agent of Chagas disease, a significant problem on public health (WHO, 2002; Moncayo and Ortiz-Yannie, 2006). They are classified within Triatominae family (Hemiptera: Reduviidae) which is composed by 137 species (Galvao *et al.*, 2003). This research explores the biologic development, feeding and defecation patterns of these insects in order to determine their capacity to transmit *T. cruzi* infectivity and to better understand their general reproductive behaviour. It has been determined that several species of triatomine can be seen present in different countries. In Mexico, biologic behaviour has been studied of *Triatoma barberi* (Zarate, 1983; Zarate *et al.*, 1984), *Triatoma pallidipennis* (Martinez and Katthain-Duchateau, 1999) and *Triatoma dimidiata* (Zeledón *et al.*, 1970). However, for other species of triatomine, no description of their behaviour is available. Among them, one of the prospective species of research was *T. mexicana*, which has been reported with natural infection by *T. cruzi* in Guanajuato, San Luis Potosí and Hidalgo states (Vidal-Acosta *et al.*,

2000; López-Cárdenas *et al.*, 2005; Salazar *et al.*, 2007; Becerril-Flores *et al.*, 2007). Although, several development parameters have been studied of *T. mexicana*, e.g. molting between one instar to the other, no exact account is available (Salazar *et al.*, 2007). Thus, we undertook to study the behaviour and development patterns of *T. mexicana* under laboratory conditions.

In this research, we determine several development parameters of this species, including hatching, molting, biologic cycle as well as feeding patterns.

MATERIALS AND METHODS

Triatomine collection: Triatomine were captured from the community of Caltimacan, municipality of Tasquillo, Hidalgo state, Mexico (20°32'N, 99°22'W, 1720 m above sea level) (Becerril *et al.*, 2007). We collected 12 female and 10 male insects, which were identified and classified according to Lent and Wygodzinsky keys (1979). The insects were placed in plastic containers covered with nylon netting bound. A filter paper was placed at the bottom and another one folded and placed vertically inside the container.

Triatomine maintenance: Adult insects were placed into a recipient in different matching combinations (female-male): 1:3, 1:2, 1:1, 2:1, 3:1, 1:0 and 1:0. The females deposited 600 eggs, which were divided into 3 groups of equal size; each group (containing 200 eggs) was incubated at stipulated temperature conditions: 27°C and 66% humidity, 26°C and 35% humidity and 19°C and 45% humidity. Colour changes, visibility of eyes in the eggs as well as hatching time were recorded along time. The insects were fed on CD-1 mice and chicken. Better feeding was shown on mice, since first instar nymphs were twice a week.

Biologic cycle determination: We determined the following parameters: Fecundity (number of eggs in the lifetime of a female), fertility (number of eggs with embryos), hatching, life span of each instar and adult, number of days between repasts, number of days between instars, time to grow into adults.

Reproductive patterns: One female specimen from each recipient was separated when each recipient had eggs and then were monitored until they died or left to laid eggs. The visibility of eyes in every egg was observed daily.

Statistics analysis: We used the student's statistic t-test to compare the environmental conditions for the development of eggs. In addition, we used the z-test to compare the sizes of populations hatched at 3 different environmental conditions (Daniel *et al.*, 1999).

RESULTS

Reproductive patterns: Mean number of eggs per female was 271.5 ± 14.4 . Temperature and humidity are 2 important factors to the embryogenesis of *T. mexicana*. Table 1 shows the development phases of eggs. The most favourable environmental conditions were 27°C and 66% humidity; eyes became visible in the eggs by 15.61 ± 4.29 days and on average, hatching took place after 27.02 ± 2.89 . Comparatively, in other test conditions, hatching was observed after 19 and 41 days, respectively. A significant difference was noted when humidity was lower than 66% but temperature was conserved between 26°C and 27°C ($t = 7.68$, $p > 0.001$). The duration of development and hatching was higher if both temperature and humidity lowered ($t = 51.94$, $p > 0.001$).

The success of hatching depended much on temperature. When eggs were incubated at 26°C or 27°C, the results were similar (68.5% and 71.5%, respectively) without significant differences ($D = 48.75$, $z = 0.66$), but at 19°C or lower, the hatching outcome was 45.5%. As an

Table 1: Embryogenic and reproductive behavior of *Triatoma mexicana* at three environmental conditions

Environmental conditions	66% humidity (27°C)	35% humidity (26°C)	45% humidity (19°C)
Minimum time for observation of presence of eyes in the eggs (days)	15.61 ± 4.29	19.78 ± 6.37	27 ± 5.59
Time to hatching	27.02 ± 2.89	41.54 ± 9.51	63.21 ± 9.42
Percentage of hatched eggs (No. of hatched eggs/No. of eggs studied)	71.5% (143/200)	68.5% (137/200)	45.5% (91/200)

Student's t-test for: (1) Time to visibility of eyes in the eggs, $t(27-26^\circ\text{C}) = 7.68$, $p > 0.0001$; $t(26-19^\circ\text{C}) = 12.05$, $p > 0.0001$; $t(27-19^\circ\text{C}) = 22.86$, $p > 0.0001$. (2) Time to hatching, $t(27-26^\circ\text{C}) = 20.66$, $p > 0.001$; $t(26-19^\circ\text{C}) = 22.89$, $p > 0.001$; $t(27-19^\circ\text{C}) = 51.94$, $p > 0.0001$. Z analysis for percentage of hatched eggs: $D(27-26^\circ\text{C}) = *48.75$, $z = 0.66$; $D(26-19^\circ\text{C}) = 99.99$, $z = 4.77$; $D(27-19^\circ\text{C}) = 99.99$, $z = 5.47$. D = difference, * = There is not a significant difference

Table 2: Nymphal development

Molt	*Percentage of molted instars	Elapsed time b/w nymphal stages (days)	Accumulative time since hatch (days)
From 1st-2nd	47.55% (68/143)	45.59	45.59 ± 12.86
From 2nd-3rd	77.94% (53/68)	41.15	86.74 ± 25.36
From 3rd-4th	75.47% (40/53)	38.65	125.39 ± 34.75
From 4-5th	62.50% (25/40)	54.17	179.56 ± 38.17
From 5th to adulthood (female or male)	64.00% (16/25)	159.10	338.66 ± 35.38
From 5th to female	44.00% (11/25)	166.62	346.18 ± 32.60
From 5th to male	20.00% (5/25)	151.59	331.15 ± 33.134

*(No. of molted triatomine/No. triatomine before molt)

Table 3: Feeding patterns of each nymphal stage

Instar stage	Mean number of feedings for each instar	Mean time between each feeding (days)
1st	1.72 ± 0.78 (N = 92)	18.15 ± 8.23 (N = 113)
2nd	1.74 ± 0.82 (N = 46)	17.03 ± 12.86 (N = 68)
3rd	1.82 ± 0.94 (N = 50)	14.27 ± 12.02 (N = 48)
4th	2.53 ± 1.26 (N = 36)	15.10 ± 9.06 (N = 31)
5th	3.28 ± 1.09 (N = 18)	18.89 ± 5.98 (N = 27)
Female	6.49 ± 0.03 (N = 9)	25.66 ± 6.44 (N = 9)
Male	3.40 ± 0.94 (N = 5)	34.00 ± 8.84 (N = 6)

N: Number of measurements

outcome of this result, the development of instars was registered only when the insects were maintained at 27°C and 66% humidity (Table 2).

Development of instars and adults: Out of 143 hatched eggs, only 16 survived into adulthood (11 females and 5 males) at the end of 338.66 ± 35.38 days (Table 3). Females had longer lifespan than males (346.18 ± 32.60 and 331.15 ± 33.134 days, respectively). Almost 6 months elapsed from 1st to 5th instar and 6 months from fifth instar to adulthood. The time of development from one instar to the next was around one and a half months; however, they molted to the adult phase in 159.10 and 166.62 days (> 5 months). The number of instars was descending along time; only 62-79% survived to the next phase (3 of 4 triatomine). The time to survival of females and males were 166.54 ± 25.98 (N = 11) and 115.6 ± 15.12 (N = 5) days, respectively.



Fig. 1: Morphological phases of *T. mexicana*. (a) Eggs with the presence of eyes (embryonic eggs) Size: $1 \times 1.5 \text{ mm} \pm 0.03$. (b) Hatching from egg. (c) 1st instar Size: $3 \times 1.2 \text{ mm} \pm 0.1 \text{ mm}$. (d) 2nd instar Size: $6.5 \times 2.7 \text{ mm} \pm 0.1 \text{ mm}$. (e) 3rd instar Size: $10 \pm 0.1 \times 0.5 \pm 0.1 \text{ mm}$. (f) 4th instar Size: $14 \pm 0.2 \times 0.8 \pm 0.2 \text{ mm}$. (g) 5th instar Size: $10 \pm 0.1 \times 0.5 \pm 0.1 \text{ mm}$. (h) Female Size: $28 \pm 0.3 \times 13 \pm 0.3 \text{ mm}$. (i) Male Size: $23 \pm 0.3 \times 14 \pm 0.3 \text{ mm}$. Scale bars (A, C and D) = 1mm; (E-I) = 1 cm

Feeding patterns: The number of feedings increased along time; from 1st to 3rd instar, the number varied from 1-2 times, while in the 4th instar, the number was 2 or 3 and in the 5th instar, the number was 3 or 4. At adulthood, the number of feedings was 3 in males and 6 in females. The feeding interval in instars varied from 14-19 days, but in adults it was longer (34 for males and 25 for females). Survival was longer in females than males (166.54 ± 25.98 and 115.6 ± 15.12 , respectively).

Morphologic features: Since the first instar, *T. mexicana* showed features different than other species of triatomine. For instance, the colour of eyes was pale yellow; the size was $1.2 \times 3 \text{ mm}$. In this phase, we observed that the insect can stand easily on its 2 legs; however, the colour of eggs never changed, they were always yellow (Fig. 1). The size of eggs was always $1 \times 1.5 \text{ mm}$. Length of adult phase were 2.5 and 2.9 cm in males and females, respectively, as measured from the posterior edge to the clypeus and the humeral angles were observed only in these stages.

DISCUSSION

T. mexicana has been found infected by *T. cruzi* in studies carried out by several authors (Salazar *et al.*, 2005; Becernil *et al.*, 2007); therefore, it is an important triatomine species of research interest. Its habitat is predominantly peridomestic, although, in Hidalgo State, Mexico, it has been found colonizing in the houses. Therefore, the knowledge about its biology is vital to seek means to control this species. The biologic cycle and behaviour of this species was explored for first time. The results were amazing that this species demonstrated different features than the associated species; for example, the colour of its eggs was yellow and never changed, whereas in most other species, change in colour from white to orange or from yellow to brown was frequently observed. Typically, a newborn appears yellow and as it gets older, it turns brown.

In this research, the ideal environmental conditions for development phases were 27°C and 66% humidity;

findings for other species are available for comparison: *M. pallidipennis* ($27\pm 2^{\circ}\text{C}$; $60\pm 5\%$ humidity) (Martínez-Ibarra and Katthain-Duchateau, 1999), *T. barberi* (27°C ; $60\pm 10\%$ humidity) (Zárate, 1983), *T. nitida* ($28\pm 1^{\circ}\text{C}$; $80\pm 5\%$ humidity) (Galvao *et al.*, 1995) and *M. mazzottii* ($27\pm 2^{\circ}\text{C}$; 70% humidity) (Malo *et al.*, 1993). However, hatching outcome varied among different species; for example, in this research, *T. mexicana* (71.5%) had higher hatching outcome than *M. mazzottii* (58.7%) and *M. pallidipennis* (60%) (Malo *et al.* 1993; Martínez-Ibarra and Katthain, 1999); however, the hatching outcome was lower as compared to *T. dimidiata* (>80%) (Zeledón, 1970) and *M. picturatus* (82.1%), which were fed with rabbit flesh (Martínez-Ibarra *et al.*, 2003). Securely, these variations arise from differences in their genetics, biochemical mechanisms, adaptation capacity, food sources, etc. It is interesting to note that humidity showed no considerable effect on hatching because the difference in hatching outcome at 35 and 66% humidity is not significant statistically. On the other hand, when temperature was lowered to 19°C , the hatching outcome reduced to 45.5%, which confirms the results of Villegas-García and Santillán-Alorcon (2004) on *M. pallidipennis*; this species developed better at 30°C than at 25°C , the hatching outcome was 78 and 84%, respectively. Therefore, temperatures higher than 27°C help the development of triatomine. On the other hand, humidity affected the time to hatching; thus, hatching took place at 27, 41.5 and 63.2 days, at 66, 35 and 45% humidity, respectively. In general, water helps respiration in insects (Williams *et al.*, 1998). The visibility of eyes in the eggs is another indicator of the embryonic state of the eggs.

The rate of mortality was higher as triatomine developed from first to second instar. Along the time, percentage of molted instar is decreased. Thus, at the end, only 20% could survive; this finding was in contrast to *T. barberi* (81.08%) (Zárate, 1983) and *T. pallidipennis* (66.2%). However, the authors also observed that as the bugs try to molt from 1st to 2nd and from 5th to adulthood, they confront more struggle (Martínez *et al.*, 2003). For example, the triatomine die if they starve for 3-7 days of birth; on the other hand, they do not sometimes molt from fifth instar to adulthood because they do not reach sexual maturity and then they die off. Early phases of *T. mexicana* are less resistant because they need to be adapted; *T. mexicana* reach its adaptability when it reached the last phases.

From our observations, *T. mexicana* reach adulthood in almost 1 year from first instar when they are maintained at 27°C and 66% humidity, but it is interesting to note that the insect reaches the fifth instar in 6 months and then

takes almost 6 months to reach adulthood, which is the time it needs for the maturity of sex organs. In other environmental conditions, the results are fruitless for *T. mexicana*. It means that favourable climatic conditions are very important for *T. mexicana* to survive. It is also concluded of this species that time of development is the longest (almost 1 year), which is almost same as in *T. spinolai* (285-372 days) and *T. nitida* (897.5 days), whereas for other species it ranges between 140 and 240 days; for example, *T. pallidipennis* 168 days (Martínez-Ibarra and Katthain-Duchateau, 1999) or 221-229 days (Martínez-Ibarra *et al.*, 2003), *M. dimidiatus* 161.7 days (Martínez *et al.*, 2001), *M. picturatus* 272.3 days, *M. dimidiatus* 240 days, *M. mazzottii* 235.77 days (Malo *et al.*, 1993), *T. barberi* 143 days and *T. infestans* 141 days. Some research hypothesis states that the time of development may be related to the kind of biologic cycle, sylvatic or domestic; for example, in sylvatic cycle, the time of development is longer than in domestic habitats. *T. mexicana* has been reported from peridomestic habitats, probably its biologic cycle is mainly sylvatic (Salazar *et al.*, 2007).

In each molt, 2 or 3 out of 4 insects develop to the next instar. However, for other species such as *T. barberi*, *T. dimidiata* and *T. pallidipennis*, almost 90% of insects reach to the next phase. Thus, *T. mexicana* is clearly a species whose dissemination is poor or limited. However, with respect to feeding patterns, *T. mexicana* can spend up to 2-4 weeks without food, because they eat 1-3 times before molting to the next phase, which takes about 45 days. Certainly, *T. mexicana* are tolerant to starvation. However, feeding is important to development in *T. mexicana* as it has been reported of other species. We experimented with chicken and mice flesh; in the latter, the insects took 2-18 min to ingest (results not shown), whereas in the former, they took 3 h to ingest. Moreover, only 25% of triatomine showed preference to chicken. Some triatomine starved for 34 days after the last feeding. Generally, triatomine can starve 2 or 3 weeks after a repast. Between 1st and 4th instars, *T. mexicana* feed 1-3 times at each instar; however, in 5th instar, they feed up to 4 (for males) or 6 times (for females). Especially for females, feeding is critical for the maturity of gonad, which is necessary for the synthesis of hormones (Ibañez *et al.*, 2004). Molecules such as amino acids and lipids are found in triatomine blood. Triatomine produce vitellogenin, which helps develop the oocytes and lipophorin, a major lipoprotein that helps the transport of lipids to organs like ovaries to produce ovules (Atella *et al.*, 2005). In insects like *Drosophila melanogaster*, a diet rich or poor in proteins will have an effect on the development of follicles during the oogenesis (Drummond-Barbosa and Spradling, 2001).

It is important to note that the preferential food source of *T. mexicana* is mammalian rather than avian. From our observations, when they were fed with the first source, they could molt in the registered time and gain weight, whereas on avian source, most of them did not gain weight. This concludes to an interesting finding that *T. mexicana* can survive in peridomestic or domestic habitats with the presence of at least rodents. However, we observed that the insects took 5 min or up to 3 h to initiate feeding and several bugs needed up to 12 h to feed and they need around 15 días between each food, then they can spend many days without feeding. This means that *T. mexicana* need to be in safe place with the prey to bite it and if they could not find the prey, they can survive for about 2 weeks. The places with this features are in the field for instance in the burrows as it has been observed in other species (Zeledón *et al.*, 1977). This can explain the peridomestic and sylvatic cycle. We observed cannibalism among first instar triatomine, they feed oneself on other full blood triatomines.

Understanding the behaviour and biologic development of *T. mexicana* is an important aspect of public health in the populations of Mexico because its extension encompasses several states, including Guanajuato and Hidalgo and because it is a potential vector of *T. cruzi* (López, 2005; Vidal *et al.*, 2000; Becerril *et al.*, 2007). In this research, the biologic development and behaviour of *T. mexicana* have been described for the first time under laboratory conditions. The ideal conditions for their survival and development are 16-20°C (Salazar *et al.*, 2007) and 30°C and 70% humidity (Becerril *et al.*, 2007). In the present research, the optimal conditions were similar to the conditions described in Hidalgo State, i.e., 27 and 35°C (Becerril *et al.*, 2007; Vidal *et al.*, 2000). However, it cannot be concluded that Guanajuato is a poor habitat; our results indicate that *T. mexicana* should be found in regions with 27°C and humidity higher than 65%.

The morphophysiological features of *T. mexicana* suggest they are perfect vectors of *T. cruzi* (Almeida *et al.*, 2003, 2005). Salazar *et al.* (2005) proposed that *T. barberi* is the best vector among triatomine and *T. pallidipennis* and *T. dimidiata* are the potential ones. Its role as a vector of *T. cruzi* is suggestive of potential research on its pathogenic behaviour.

CONCLUSION

T. mexicana is a species that survives in strict feeding and environmental conditions. Although it is prevalently found in the fields, it moves into the houses

of rural places to feed on human blood and is a potential carrier of *T. cruzi* infection to the humans. However, it is challenging to control *T. mexicana* because they live in burrows, which are difficult to be accessed by humans.

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