Influence of temperature and humidity on the biology of *Triatoma mexicana* (Hemiptera: Reduviidae: Triatominae) under laboratory conditions

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Several biological parameters related to the Triatoma mexicana *life-cycle were evaluated in this study. Three cohorts were maintained under different combinations of temperature and relative humidity (RH): 25ºC/50% RH; 25ºC/75% RH; and 30ºC/75% RH. Observed hatching rates varied from 49-57.5% whereas the average time of hatching varied from 19.5-22.7 days. In the three cohorts studied, the mean time-lapse between presentation of the blood meal and the beginning of feeding was less than 5 min in all instars; the mean feeding time was longer than 10 min in all the instars; the post-feed defecation delay was over 10 min in all the instars. Less than 50% of nymphs in each cohort completed the cycle and the average time from 1st instar nymph to adult was more than 255 days for the three cohorts. The number of blood meals before molt at each nymphal instar varied from 1-9. Our results appear to indicate a lack of influence of temperature and RH on the biological parameters of* T. mexicana *that were studied, which could reflect the adaptation capacity of this species. We also conclude that* T. mexicana *can not be considered an effective transmitter of* Trypanosoma cruzi *to human populations in areas where this species is currently present.*

Key words: *Triatoma mexicana* - biology - ethology - laboratory conditions

In Mexico, at least nine of the 34 recognized Triatominae species are considered important vectors of *Trypanosoma cruzi* (Cruz-Reyes & Pickering-López 2006). The biology and behavior of most of them have already been studied using laboratory conditions considered to be favorable for development (Martínez-Ibarra et al. 2003a, b, 2006, 2007, Martínez-Ibarra & Novelo-López 2004). However, some biological behaviors are strongly influenced by conditions of temperature and humidity as previously reported for some species of triatomines (García da Silva 1989, Barbosa-Gómes & García da Silva 2001, Rocha et al. 2001a, b, Villegas-García & Santillán-Alarcón 2004) and it is thus necessary to open a wider range of studied conditions for successful rearing of those species that are distributed in areas with different temperature and humidity conditions.

Triatoma mexicana (Herrich-Scaeffer) is considered to be an epidemiologically important vector of Chagas disease in Mexico given the current presence of its populations in both domestic and wild ecotopes, its capacity to colonize human dwellings and its frequent contact with man as a blood meal source. This species usually occurs inside human houses (especially on walls and bedrooms) and in the peridomicile and is mostly collected from yards and in sylvatic foci at the center of Mexico (López-Cárdenas et al. 2005, Becerril-Flores et al. 2007, Salazar-Schettino et al. 2007, Villagrán et al. 2008). The climate in this area is mild and dry with 400-1000 mm annual rainfall and temperatures ranging from 0-40ºC (SEGOB 2005).

In spite of the important role of *T. mexicana* in the transmission of *T. cruzi* to human populations in an epidemiologically important areas of Mexico, such as the states of Guanajuato, Hidalgo, Querétaro and San Luis Potosí (Becerril-Flores et al. 2007, Salazar-Schettino et al. 2007, Villagrán et al. 2008), the biology and behavior of this species has not been documented.

As part of a larger study on the ecology of Mexican Triatominae species, studies of the biology of *T. mexicana* under different experimental conditions are described in this paper.

MATERIALS AND METHODS

A laboratory colony established in 2005 from 50 specimens captured in Tierra Blanca, Guanajuato, and identified as *T. mexicana* following the key of Lent and Wygodzinsky (1979) was used in this study. Those specimens were morphologically similar to type *T. mexicana* collected in the state of Hidalgo, but had a remarkable overall darker coloration with small yellow markings on

Financial support: PAPIIT IN 238002 from DGAPA, UNAM
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Received 3 July 2008
Accepted 25 September 2008

the connexivum. The colony was maintained at 27 ± 1ºC and 75 ± 5% relative humidity (RH) and fed weekly on immobilized New Zealand rabbits.

Eggs were grouped by date of oviposition to initiate three cohorts of 200 eggs each. After eclosion, 1st instar nymphs were separated into groups of 10 individuals; each nymph was marked with an indelible red ink marker on different areas of the pronotum, thorax or abdomen with the objective of distinguishing one individual from another. Each group was placed into a plastic container (10.5 cm diameter x 20.5 cm height) that was identified with a progressive Arabic numeral and containing a central support of absorbant cardboard. Three days after eclosion, nymphs were individually transferred to small plastic containers (3 cm diameter x 5 cm height) and offered New Zealand rabbits on which to feed during a 1 h period until the first blood meal; subsequently, they were offered weekly. Nymphs were individually observed from the beginning of the feeding process until an hour post feeding to record feeding and defecation behaviors. Afterwards, each nymph was returned to its assigned large container. Each of the three cohorts of *T. mexicana* was maintained in a dark incubator at different conditions of temperature and RH, as follows: Cohort 25/50: 25 ± 2ºC and 50 ± 5% RH; Cohort 25/75: 25 ± 2ºC and 75 ± 5% RH and Cohort 30/75: 30 ± 2ºC and 75 ± 5% RH. Lower temperature (25ºC) and humidity (50%) levels were selected because they can be recorded in the study area where most specimens of *T. mexicana* have been collected, as was previously established (Salazar-Schettino et al. 2007). Higher temperature (30ºC) and humidity (75%) were employed to determine whether the increase in those conditions would shorten the life cycle, decrease mortality and decrease number of meals to molt, as recorded for two related Mexican triatomine species (Barbosa-Gomes & Garcia da Silva 2001, Villegas-García & Santillán-Alarcón 2004). Feeding and defecation behaviors were also studied to test the influence of different conditions of temperature and humidity on these parameters. Studied specimens were checked daily for ecdysis or death.

The variables that showed a normal distribution were compared by Student's *t*-test or analysis of variance (ANOVA). In the case of ANOVA tests, *post hoc* comparisons were made using the Scheffé test. The Wilcoxon nonparametric test was used for variables with a non-normal distribution. The differences were considered to be significant when $p < 0.05$.

RESULTS

The egg eclosion rate was under 60% for the three studied cohorts. This was lower for the cohort 25/50 (49%); however, no significant ($p > 0.05$) differences were recorded when the three cohorts were compared. The average incubation period was approximately 20 days (range 16-28 days) for the three cohorts.

The average time of egg-to-adult development for the three studied cohorts was over 8.5 months (Table I). Significant ($p < 0.05$) differences were recorded between the total mean times of the two cohorts reared at 25ºC and different RH (25/50 and 25/75) and the cohort reared at 30ºC (75% RH). No significant ($p > 0.05$) differences were recorded when the development times of the cohorts reared at 25ºC (25/50 and 25/75) were compared. Mean total number of blood meals to molt for each cohort was significantly ($p < 0.05$) higher for cohort 25/50. No significant ($p > 0.05$) differences were recorded when the numbers of meals of the cohorts 25/75 and 30/75 were compared (Table II).

Mortality rates were significantly ($p < 0.05$) higher for 1st instar nymphs than for the rest of studied instars in each of the three studied cohorts independent of temperature and humidity conditions under which the studied cohorts were reared (Table III). No significant ($p > 0.05$) differences were recorded when the same instars of different cohorts were compared, nor when mean total mortality rates of the three studied cohorts were contrasted (Table III).

The mean time-lapse between presentation of the blood meal source and the beginning of feeding was between 2.8-5 min for nymphs of all instars. No significant ($p > 0.05$) differences were recorded between instars of the same cohort, nor between instars of different cohorts.

TABLE I

Egg to adult development cycle of *Triatoma mexicana* developed under different conditions of temperature and relative humidity (RH)

	Duration in days					
	25ºC/50% RH		25ºC/75% RH		30ºC/75% RH	
Instar	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Egg-NI	98	22.7 ± 6.3	110	20.4 ± 5.7	115	19.5 ± 3.2
NI-NII	71	37.6 ± 10.7	78	35.2 ± 10.2	85	32.1 ± 11.4
NII-NIII	67	32.1 ± 8.8	72	31.3 ± 6.5	78	28.3 ± 8.5
NIII-NIV	66	39.1 ± 12.6	71	36.9 ± 6.6	75	32.7 ± 9.5
NIV-NV	66	44.5 ± 13.7	67	47.1 ± 17.1	70	43.4 ± 8.9
NV-AD	57	112.6 ± 40.4	60	115.8 ± 39.4	64	108.7 ± 28.9
Total	57	285.5 ± 56.6[a]	60	282.7 ± 67.8[a]	64	255.7 ± 49.7[b]

similar letters indicate no significant ($p > 0.05$) differences.

TABLE II

Number of blood meals for *Triatoma mexicana* developed under different conditions of temperature and relative humidity (RH)

	Number of blood meals					
	25ºC/50% RH		25ºC/75% RH		30ºC/75% RH	
Instar	n nymphs	Mean ± SD	n nymphs	Mean ± SD	n nymphs	Mean ± SD
NI-NII	71	3.3 ± 1.3	78	1.1 ± 0.3	85	1.2 ± 0.4
NII-NIII	67	3.4 ± 2.2	72	1.5 ± 0.6	78	2.1 ± 1.4
NIII-NIV	66	3.3 ± 2.1	71	1.3 ± 0.5	75	1.1 ± 0.7
NIV-NV	66	3.3 ± 1.1	67	1.9 ± 0.6	70	1.3 ± 0.9
NV-AD	57	7.3 ± 2.4	60	7.5 ± 2.1	64	6.9 ± 1.7
Total	57	20.6 ± 6.4[a]	60	12.7 ± 5.2[b]	64	12.8 ± 3.1[b]

similar letters indicate no significant ($p > 0.05$) differences.

TABLE III

Instar mortality for *Triatoma mexicana* developed under different conditions of temperature and relative humidity (RH)

	25ºC/50% RH		25ºC/75% RH		30ºC/75% RH	
Instar	n nymphs	Mortality %	n nymphs	Mortality %	n nymphs	Mortality %
I	98	27.6	110	29.1	115	26.1
II	71	4.1	78	5.5	85	6.1
III	67	1.0	72	0.9	78	2.6
IV	66	0	71	3.6	75	4.3
V	66	9.2	67	6.4	70	5.2
Total	57	(41.9)[a]	60	(45.5)[a]	64	(44.3)[a]

similar letters indicate no significant ($p > 0.05$) differences.

Mean feeding times were over 14 min for all instars and even over 20 min for 2nd, 3rd and 4th instar nymphs of the three studied cohorts. No significant ($p > 0.05$) differences were found when the same instars of different cohorts were compared.

Post feed defecation delay was over 10 min for all instars with longer mean defecation delays (over 18 min) recorded in adults of both sexes in the three cohorts. No significant ($p > 0.05$) differences were recorded when the three cohorts were compared.

DISCUSSION

T. mexicana has been recently proposed to be included the Phyllosoma complex (Martínez et al. 2006). Recorded hatching rates of those species, reared under similar laboratory conditions as those of the current study, are usually over 70% except in the cases of *Meccus bassolsae* (Alejandre-Aguilar, Nogueda-Torres, Cortés-Jiménez, Jurberg, Galvão & Carcavallo) (Martínez-Ibarra et al. 2003b, 2006, Martínez-Ibarra & Novelo-López 2004) and *T. mexicana* in this study. Low egg hatching rates could explain why *T. mexicana* is not as abundant as some other Mexican triatomine species in those species' respective distribution areas.

A significantly ($p < 0.05$) shorter average egg-to-adult development time was recorded in the cohort of *T. mexicana* at the highest temperature and RH compared with the two other cohorts. Different results were observed in two distinct studies on *Rhodnius robustus* Larrouse and *Rhodnius neglectus* Lent where the longest average development periods from egg to 5th instar nymph were recorded on the cohorts reared at the highest temperature and RH (Rocha et al. 2001a, b). In the case of *T. mexicana*, there was an apparent influence of temperature increase on the shortening of development time. This was also recorded for two species of the Phyllosoma complex, *Meccus picturatus* (Usinger) and *Meccus pallidipennis* (Stål) (Barbosa-Gomes & García da Silva 2001, Villegas-García & Santillán-Alarcón 2004).

The average development times of the three studied cohorts of *T. mexicana* fed on rabbits were over 255 days. They were similar to the average development time (270.5 ± 44.0 days) of *M. bassolsae* reared under similar laboratory conditions (Martínez-Ibarra et al. 2006). Those development times from *T. mexicana* were longer than most of those from four species of the Phyllosoma complex. All those species were reared under similar laboratory conditions with recorded development times from 176.8 ± 34.8 days for *Meccus phyllosomus* (Burmeister) to 204.6 ± 17.3 for *M. pallidipennis* (Martínez-Ibarra et al. 2003b, 2006, Martínez-Ibarra & Novelo-López 2004).

The total mean number of blood meals before molt for each instar was significantly ($p < 0.05$) higher for the co-

hort with the combination of the lowest temperature and RH than the other two cohorts. A similar behavior was reported for *M. pallidipennis* from two cohorts reared under similar conditions of these two latest cohorts from our study (Villegas-García & Santillán-Alarcón 2004). Apparently, RH had a higher influence than temperature since lower humidity increased the necessity of specimens to take blood as a way of compensating for water loss.

Lower mortality rates were observed in the molt from 3rd-4th instar in the three studied cohorts of *T. mexicana* as well as in the molt from 4th-5th instar in the cohort 25/50. On the other hand, the highest mortality rate was in the molt from the 1st-2nd second instar in the three cohorts. As recorded in other studies on these species of the Phyllosoma complex (Martínez-Ibarra et al. 2003a, b, 2006, Martínez-Ibarra & Novelo-López 2004), the mortality of the youngest nymphs (1st, 2nd and 3rd instar) appeared to mainly be due to an incapacity to feed since dead bugs generally had no significant intestinal content whereas mortality in the older nymphs occurred mainly during molting.

The total mortality rates for *T. mexicana* were lower than those for *M. bassolsae* (78.6%), *M. picturatus* (71.9%) and *M. pallidipennis* (60.8%), which is similar to those for *M. phyllosomus* (45.1%). All of them were reared under similar laboratory conditions to those of *T. mexicana* (Martínez-Ibarra et al. 2003b, 2006, Martínez-Ibarra & Novelo-López 2004).

The recorded periods of time to the start of feeding were similar to those registered for *Triatoma dimidiata* (Latreille), *Triatoma infestans* (Klug) and *Rhodnius prolixus* (Stal) (Zeledón et al. 1977), which are considered to be some of the most important Chagas disease vectors in America (WHO 2002). *Meccus longipennis* (Usinger) and *M. picturatus* (Martínez-Ibarra et al. 2003a, b) were also similar and are considered two of the most important Chagas disease vectors in Mexico (Cruz-Reyes & Pickering-López 2006).

The mean feeding times observed in the current study (over 14 min) were similar to those reported for most instars of *M. picturatus*, adults of *M. pallidipennis* and *M. phyllosomus*, and all instars of *Meccus mazzottii* (Usinger) and *M. bassolsae*. All of them were reared under similar laboratory conditions as *T. mexicana* in this current study (Martínez-Ibarra et al. 2003b, 2006, Martínez-Ibarra & Novelo-López 2004).

Similar to some previously published studies (Martínez-Ibarra et al. 2003a, b, 2006) regarding the biology of some related species, the parameters of Zeledón et al. (1977) were followed in order to obtain a valid comparison between those species and *T. mexicana*. Those authors proposed that a species of triatomines that defecates during first 5-10 min after feeding could be considered potentially effective transmitters of *T. cruzi* to humans since assumption of contact with their victim is along that period. Consequently, no instars of the three studied cohorts could be considered as effective transmitters. The longest mean defecation delay was recorded for adults (females and males) of *T. mexicana*, similar to the reported data in some previous studies on species of the Phyllosoma complex, such as *M. pallidipennis*, *M. picturatus* and *M. bassolsae* (Martínez-Ibarra et al. 2003b, 2006, Martínez-Ibarra & Novelo-López 2004).

An apparent lack of influence of temperature and RH was recorded on most researched biological parameters (mortality, feeding and defecation) in the three studied cohorts. These results could be due to an adaptive response to different climatic conditions, such as those recorded in the natural distribution area of *T. mexicana* in Mexico (López-Cárdenas et al. 2005, Becerril-Flores et al. 2007, Salazar-Schettino et al. 2007, Villagrán et al. 2008). According to most of the studied parameters in this study, *T. mexicana* cannot be considered to be an effective transmitter of *T. cruzi* to humans, which is different from other similar species, such as six species in the Phyllosoma complex (Martínez-Ibarra et al. 2003a, b, 2006, Martínez-Ibarra & Novelo-López 2004).

ACKNOWLEDGEMENTS

To Gloria Rojas Wastavino, for support on field collections of triatomines.

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