

BEHAVIOR OF TRIATOMINES (HEMIPTERA : REDUVIIDAE) VECTORS OF CHAGAS' DISEASE. II. INFLUENCE OF FEEDING, LIGHTING AND TIME OF DAY ON THE NUMBER OF MATINGS, MATING SPEED AND DURATION OF COPULATION OF *PANSTRONGYLUS MEGISTUS* (BURM, 1835) UNDER LABORATORY CONDITIONS

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To determine the influence of feeding, lighting and time of day on the copulating behavior of *Panstrongylus megistus*, 480 insect pairs were divided into four groups of 120 each and tested in the following respective situations: without food deprivation (F.D.), with five days of F.D., with ten days of F.D., and with 20 days of F.D. The tests were performed between 9:00 a.m. to 12:00 a.m. and 7:00 p.m. to 10:00 p.m., with light (700-1400 lux) and in the dark (1.4-2.8 lux) and behavior was recorded by the time sampling technique. Mating speed (MS) and duration of copulation (DC) were also calculated for each situation. The maximum frequency of copulation was observed after five days of F.D., at night, in the dark ($n = 16$), and the minimum was observed for recently-fed pairs, at night, with light ($n = 4$). Males approached females more often than females approached males. MS was lowest in pairs with twenty days of F.D., at night, with light ($\bar{X} = 23.0 \pm 16.0$ minutes), and highest in recently-fed pairs, during the day, with light ($\bar{X} = 2.9 \pm 2.5$ minutes). DC was shortest in recently-fed insects, during the day, in the dark ($\bar{X} = 23.5 \pm 6.7$ minutes), and longest in recently-fed animals, at night, in the dark ($\bar{X} = 38.3 \pm 6.9$ minutes).

Key words: Chagas' disease – triatomines – *Panstrongylus megistus* – reproduction – copulation behavior – feeding – light – time of the day – mating speed – duration of copulation

After invading a new habitat, a population will colonize it only if it offers adequate and available resources which will guarantee reproductive and survival rates higher than, or at least equal to, the minimum effective size of this population. On this basis, the study of triatomine settling should also use a behavioral approach, especially in terms of determining the factors that may influence elements interfering with reproductive rates (Almeida & Almeida, 1982).

These insects are known to be active at night. Schofield (1979) has suggested that the nocturnal activity of many triatomine species is due to two factors: 1) temperatures tend to be lower at night; 2) this is the time when food sources (hosts) are usually resting. Preference for the dark, according to Wood (1964), is indicated by the negative response to phototaxis. Several investigators have studied the effect of light and nocturnal activity on the mating of triatomines. Galliard (1936) observed that copulation occurred under environmental conditions of reduced light. This author observed that *Rhodnius prolixus* copulated relatively easily during the day, but that very intense illumination was harmful and could interrupt or even prevent copulation. In contrast, Baldwin, Knight & Lynn (1971) reported that, since *R. prolixus* is a nocturnal species, the males were stimulated to copulate only in full darkness.

The objective of the present investigation was to study a few abiotic and feeding factors in the mating behavior of *Panstrongylus megistus* (Burm., 1835), such as food deprivation, lighting and time of day, as well as mating speed and duration of copulation.

MATERIAL AND METHODS

Tests were performed both during the day and at night, in a light or dark environment. Light was provided by two 40W fluorescent lamps on the ceiling and one 15W small lamp on the experimental table, the three of the daylight type, with a light intensity of 700-1400 lux. For the tests in the dark, the fluorescent lamps on the ceiling were turned off, with only the small lamp left on. The sides and back part of this small lamp were covered with black cardboard and the front part was covered with dark blue plastic film, so that the light intensity of the environment was 1.4 to 2.8 lux, i.e. the minimum to permit adequate visualization of the insects and recording

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their behavior. Observation time was 60 minutes for each experiment and behavior was recorded on a protocol sheet using the time sampling technique (Hall, 1973). The (+) sign was used to indicate pairs that copulated, the (-) sign to indicate pairs that did not copulate, and attempts at copulations were marked as AC.

A total of 480 adult virgin pairs were divided into four groups of 120 pairs each, which were allowed to have a blood meal on a pigeon and then studied as follows: 1 - One group without food deprivation (F.D.); 2 - One group with five days of F.D.; 3 - One group with ten days of F.D.; 4 - One group with twenty days of F.D.

At testing time each pair was placed in a 12 x 6.5 cm glass flask. The flasks were arranged linearly under the light fixture so that they would all be lighted homogeneously and equally visualized. The triatomines varied in age from five to thirty days after ecdysis to the adult phase.

The data obtained with the time sampling technique were also used to calculate mating speed and duration of copulation for each situation. Mating speed (MS) was considered to be the time elapsing from the moment when the pairs were formed to the beginning of copulation, and duration of copulation (DC) the period of time spent by the pair in copulation, from the joining of the genitals to complete separation.

RESULTS

The results of the influence of the tested factors (feeding, lighting, and time of day) on the copulation behavior of *P. megistus* are shown in Table I. The maximum number of copulations was 16 (53.3%) and occurred in pairs with five days of food deprivation, at night, in the dark (Fig. 1). The minimum number (four copulations; 13.3%) occurred in recently-fed pairs, at night, in the dark. For the pairs tested during the day the number of matings was always the same, i.e. eight copulations (26.6%), and for those tested during the night it varied between six and 11 (20% and 37%, respectively).

In all situations the males approached the females more often than the females approached them. The males made several attempts at copulation, for which they were almost always ready, whereas the females were not always receptive.

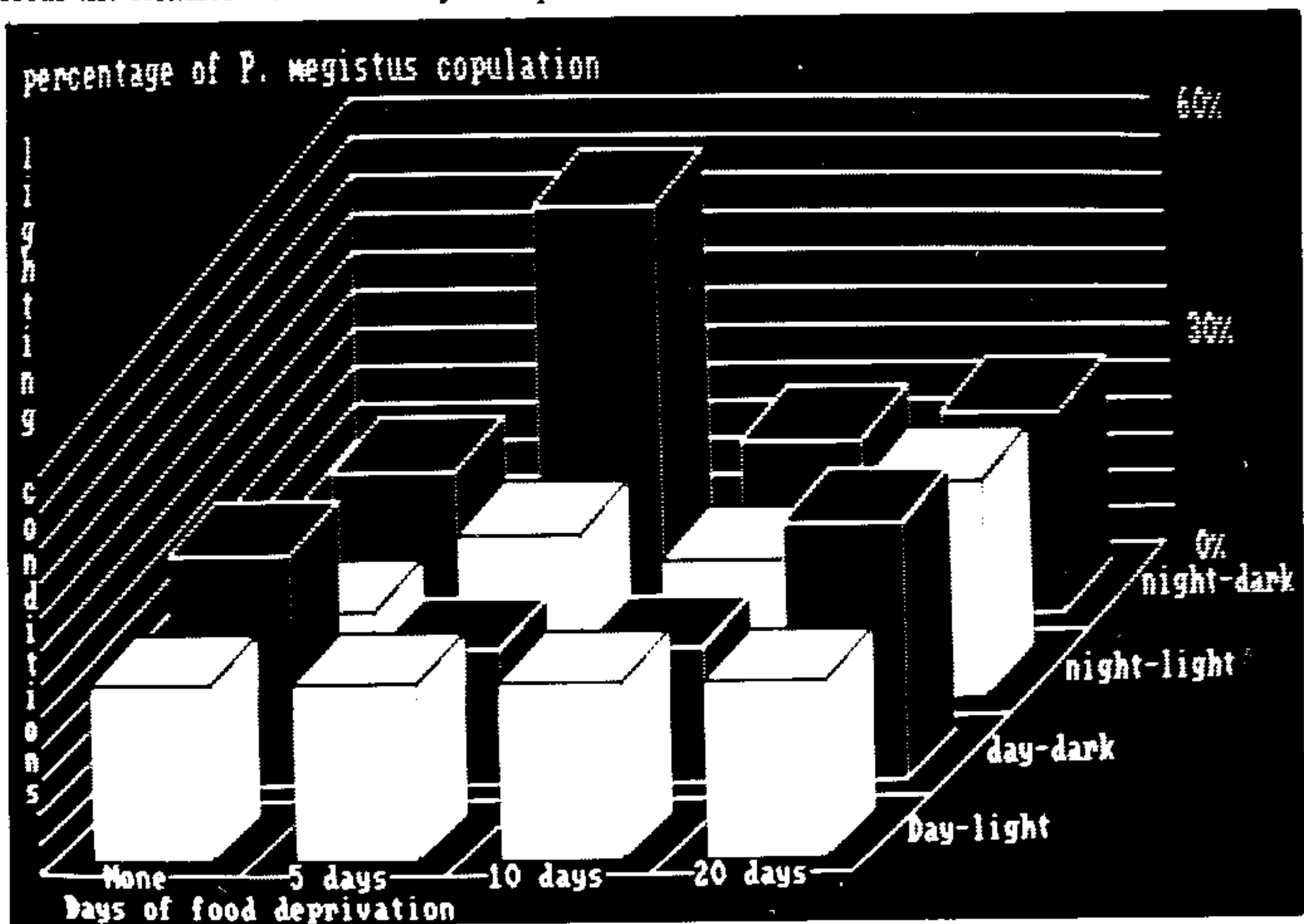


Fig. 1: percentage of *P. megistus* copulation (n = 30). Virgin pairs, recently fed or submitted to food deprivation for 5, 10 or 20 days, tested during the day or at night under varying lighting conditions (light: 700-1400 lux; dark: 1.4-2.8 lux). Significant difference: $P < 0.05$.

No significant difference between day and night testing was observed for the following situations: a) mating (day: 66; night: 63; $\chi^2 = 0.07$; $P > 0.05$); b) attempts at copulation on the part of males (day: 118; night, 108; $\chi^2 = 0.8$; $P > 0.05$); c) absence of copulation (day: 56; night: 69; $\chi^2 = 1.8$; $P > 0.05$).

As far as feeding is concerned, the correlation coefficient test showed that the number of males indifferent to copulation decreased with increased food deprivation ($r = -0.725$; $P > 0.05$) (Table I).

When the data obtained with light and in the dark were compared by the chi-square test, a significant difference was observed only for pairs with five days of food deprivation, tested at night in the dark ($\chi^2 = 5.70$; $P < 0.05$). When the chi-square homogeneity test was applied (Table II), the same situation was found to differ significantly from all others, except when compared with recently-fed pairs, during the day, in the dark ($\chi^2 = 2.40$; $P > 0.05$); with pairs with twenty days of food deprivation, during the day, in the dark ($\chi^2 = 1.2$; $P > 0.05$) and with pairs with twenty days of food deprivation, at night, with light ($\chi^2 = 3.4$; $P > 0.05$). In the remaining situations, the pairs with five days of food deprivation, when tested at night in the dark showed significant differences. Some of these were quite divergent, as, for example, when they were compared with recently-fed pairs, at night, with light ($\chi^2 = 10.8$; $P < 0.005$); when compared with pairs with five days of food deprivation, during the day, in the dark; with pairs with ten days of food deprivation, during the day, in the dark, and with pairs with ten days of food deprivation, at night, with light, the result was $\chi^2 = 7.2$; $P < 0.01$ for the three situations.

TABLE I

Influence of feeding, lighting and time of day on the mating behavior of *P. megistus*. Light: 700-1400 lux; dark: 1.4-2.8 lux.

Situations	Pairs that copulated	Attempts at copulation (males)	Pairs indifferent to copulation	Total
Recently-fed, day-with light	8	11	11	30
Recently-fed, day-in the dark	10	14	6	30
Recently-fed, night-with light	4*	12	14	30
Recently-fed, night-in the dark	6	12	12	30
5 days of food deprivation, day-with light	8	13	9	30
5 days of food deprivation, day-in the dark	7	15	8	30
5 days of food deprivation, night-with light	7	11	12	30
5 days of food deprivation, night-in the dark	16**	8	6	30
10 days of food deprivation, day-with light	8	16	6	30
10 days of food deprivation, day-in the dark	6	17	7	30
10 days of food deprivation, night-with light	6	17	7	30
10 days of food deprivation, night-in the dark	7	16	7	30
20 days of food deprivation, day-with light	8	16	6	30
20 days of food deprivation, day-in the dark	11	16	3	30
20 days of food deprivation, night-with light	9	15	6	30
20 days of food deprivation, night-in the dark	8	17	5	30
Total	129	226	125	480

*= minimum number of copulations; **= maximum number of copulations. T = 25.5°C; RH = 79%.

TABLE II

Homogeneity chi-square test comparing the effect of feeding, lighting and time of day on the copulation behavior of *P. megistus* (n = 30). RF = recently-fed; DL = day, with light; DD = day, in the dark; NL = night, with light; ND = night, in the dark; DFD = days food deprivation.

	RFDL	RFDD	RFNL	RFND	5DFD (DL)	5DFD (DD)	5DFD (NL)	5DFD (ND)	10DFD (DL)	10DFD (DD)	10DFD (NL)	10DFD (ND)	20DFD (DL)	20DFD (DD)	20DFD (NL)	20DFD (ND)
RFDL																
RFDD	0.32															
RFNL	1.70	1.93														
RFND	0.36	1.36	0.48													
5DFD (DL)	0.00	0.30	1.66	0.36												
5DFD (DD)	0.36	1.36	0.48	0.00	0.36											
5DFD (NL)	0.08	0.72	1.00	0.10	0.08	0.10										
5DFD (ND)	4.44*	2.44	10.8***	7.18**	4.44*	7.18**	5.70*									
10DFD (DL)	0.00	0.30	1.68	0.36	0.00	0.36	0.10	4.44*								
10DFD (DD)	0.36	1.36	0.48	0.00	0.36	0.00	0.10	7.18**	0.36							
10DFD (NL)	0.36	1.36	0.48	0.00	0.36	0.00	0.10	7.18**	0.36	0.00						
10DFD (ND)	0.08	0.72	1.00	0.01	0.01	0.01	0.09	5.70*	0.08	0.10	0.16					
20DFD (DL)	0.09	1.11	2.30	0.36	0.08	0.36	0.18	4.44*	0.09	0.09	0.47	0.18				
20DFD (DD)	0.72	0.15	4.44*	2.13	0.77	0.94	1.35	1.20	0.78	2.13	2.13	1.01	0.18			
20DFD (NL)	0.08	0.15	2.55	2.55	0.16	0.94	0.43	3.40	0.16	0.94	0.89	0.43	0.16	0.38		
20DFD (ND)	0.09	0.40	1.77	0.47	0.09	0.36	0.18	4.44*	0.09	0.36	0.47	0.18	0.09	0.78	0.16	

*= P < 0.05; **= P < 0.01; ***= P < 0.005.

TABLE III

Mating speed of *P. megistus* (n = 30). Virgin pairs, recently-fed or submitted to food deprivation for 5, 10 or 20 days, tested during the day or at night under varying lighting conditions (light: 700-1400 lux; dark: 1.4-2.8 lux).

Situation	Day		Night	
	Light	Dark	Light	Dark
Recently-fed	2.9 ± 2.5*	9.0 ± 15.5	12.5 ± 13.5	5.8 ± 8.4
5 days of food deprivation	12.1 ± 17.5	2.9 ± 3.6	6.4 ± 7.9	6.9 ± 15.2
10 days of food deprivation	6.9 ± 11.2	6.7 ± 8.0	13.3 ± 5.5	17.9 ± 17.1
20 days of food deprivation	8.4 ± 8.6	8.6 ± 15.5	23.0 ± 16.0	11.3 ± 16.2

*Mean and standard deviation.

Mating speed was lowest in pairs with twenty days of food deprivation, at night, with light ($\bar{X} = 23.0 \pm 16.0$ minutes) and highest in recently-fed pairs, during the day, with light ($\bar{X} = 2.9 \pm 2.5$ minutes) (Table III).

Duration of copulation was longest in pairs with twenty days of food deprivation, during the day, in the dark ($\bar{X} = 38.3 \pm 6.9$ minutes) and shortest in recently-fed pairs, during the day, in the dark ($\bar{X} = 23.5 \pm 6.7$ minutes). One of the thirty pairs tested after twenty days of food

deprivation during the day, in the dark (eleven copulations) mated for eight hours and forty-five minutes. Since this appeared to be an isolated situation, it was not considered for the average. The DC of the other ten pairs which copulated was $\bar{X} = 27.5 \pm 8.6$ minutes (Table IV).

TABLE IV

Duration of copulation of *P. megistus* (n = 30). Virgin pairs, recently fed or submitted to food deprivation for 5, 10 or 20 days, tested during the day or at night under varying lighting conditions (light: 700-1400 lux; dark: 1.4 - 2.8 lux).

Situation	Day		Night	
	Light	Dark	Light	Dark
Recently-fed	31.0 ± 9.4*	23.5 ± 6.7	23.8 ± 5.5	38.3 ± 6.9
5 days of food deprivation	30.0 ± 7.1	25.0 ± 3.8	24.3 ± 6.8	28.1 ± 13.6
10 days of food deprivation	31.9 ± 7.0	26.7 ± 7.5	28.3 ± 4.7	29.3 ± 3.2
20 days of food deprivation	29.4 ± 9.0	27.5 ± 8.6	25.0 ± 2.9	32.5 ± 10.4

*Mean and standard deviation.

DISCUSSION

The preference of triatomines for darkness is more marked for their feeding activity (Correa, 1954), although several authors have observed that, under natural conditions, these insects may leave their hiding places and feed during the day in environments with reduced light (Neiva, 1910; Deane & Deane, 1957). According to Wiesinger (1956), the activity of *Triatoma infestans* can occur at any time and not only at night, and, according to Espínola (1973), permanent illumination acts as an inhibitor of locomotion in this insect, causing more intense disturbances in the rhythm of females, even though this rhythm is not completely eliminated. These data agree with those reported by Mac Cord, Jurberg & Raymundo (1983), who observed that, under laboratory conditions, *T. infestans* only left its shelter if stimulated by a food source, regardless of luminosity. Marsden (1980) observed that *P. megistus* is more active at night between 5:00 p.m. and 1:00 a.m.

Under laboratory conditions, *P. megistus* was able to copulate in all situations tested, indicating that food deprivation, luminosity and time of day did not have much influence on its mating behavior. Only one situation was considered relevant: pairs with five days of food deprivation copulated more often in the night, in the dark. On the basis of the results, it can be stated that the success or failure of copulation is linked to female behavior, since, as shown in Table I, males attempted copulation in all situations tested.

In *R. prolixus*, copulation was observed during the day, in dark environments or in environments with reduced light (Galliard, 1936). Silva (1982), without reporting light intensity, noted that *T. infestans* performs the largest number of matings in the afternoon during the warmest hours of the day. According to Furtado & Queiroz (1978), the fecundity of insects may be affected by external factors such as hygrometry, temperature and feeding. The results of this study are close to those reported by Buxton (1930) and Galliard (1936), who observed that *R. prolixus* was able to copulate even when the pairs had not received a blood meal, and to those reported by Hase (1932), who obtained matings between recently-fed males and food-deprived females and between fed females and starved males of *Panstrongylus geniculatus*, indicating that feeding was not a fundamental prerequisite for copulation to occur. Other investigators, such as Baldwin, Knight & Lynn (1971) observed that feeding was a prerequisite for *R. prolixus* to produce sex pheromones, and therefore these insects only copulated after a blood meal. Zárate (1983) observed that in *Triatoma barberi* blood intake was practically indispensable for copulation to occur, since copulation was observed only sporadically between feedings.

An interesting fact was that the number of males looking for copulation increased with time of food deprivation. Another factor that should be taken into account is that in the experiments performed in the present study, males and females were kept in separate jars until the time for the test. In this situation, the males tried so hard to mate that, in the absence of females, they made attempts at copulation even with other males. This isolation may possibly be more important

in terms of the number of matings than the factors tested (food deprivation, lighting and time of day) and may have been responsible for the decrease in indifference to copulation, since, as the time of food deprivation increased (five, ten and twenty days), so did the time of separation between males and females. With respect to the situation of five days of deprivation, at night, in the dark, when the largest number of matings occurred the hypothesis was raised that this is the situation in which the female is ready to receive the male, because she is not sated with food and not yet starved and in an ideal biological situation such as night and dark.

In studies started in 1982, Lima, Jurberg & Almeida (1986) used a red lamp to investigate and describe the copulation of *P. megistus*. After they started the tests, they read the study of Ward & Finlayson (1982) who had concluded that blue light, after infrared light, was the light that least affected the behavior of *Triatoma infestans*, because of its wavelength. Thus, the authors of the present paper used blue light to obtain 1.4-2.8 lux. The blue plastic film used, evaluated with a QV-50 spectrophotometer, showed a wavelength closer to that obtained by Ward & Finlayson (1982). When the results obtained in the presence of blue light were compared to those obtained in the presence of red light, no difference was detected in insect behavior.

Mating speed (MS) showed discrepant data (a minimum of $\bar{X} = 2.9 \pm 2.5$ minutes and a maximum of $\bar{X} = 23.0 \pm 16.0$ minutes) in the different situations studied. This discrepancy seems to be linked to other factors not related to those studied here, because the increased time until the beginning of copulation was not influenced homogeneously by food deprivation, luminosity or time of day.

Duration of copulation was homogeneous in all situations tested, except for that involving twenty days of food deprivation, during the day, in the dark. Even so, this was due to the fact that one of the pairs copulated for eight hours and forty-five minutes, which, on the basis of the frequency observed, can be considered as an isolated fact.

Mating speed is an expression which indicates a period of time and not a space elapsed in the time unit. Even though in this respect it is inadequate, MS is universally used by investigators who study the genetics of *Drosophila*, and for this reason it was maintained in the present study.

In *Drosophila*, MS as well as DC is linked to genetic factors (Parsons, 1964; Fulker, 1966), and has been used to separate strains with long MS from strains with short MS (Spiess & Langer, 1968; Brncic & Koref-Santibañez, 1964). Maning (1961, 1963, 1968) and Kessler (1969) launched the idea of polygenic heredity through the study of artificial selection for faster or slower MS in *Drosophila*.

As to DC, Hildreth (1962) concluded that in *Drosophila* it is also regulated genetically, and later MacBean & Parsons (1966) calculated the heritability of this trait for *D. pseudoobscura* and *D. melanogaster*.

This type of analysis has not been performed in other species of triatomines. In the present study, great variability in MS was observed in the situations tested. This may indicate that the period until the beginning of mating is not controlled by genetic factors, but may be considered an appetitive behavior of the male. If not, genetic variation is extremely wide. Comparative studies of other genera or species of this groups, as well as selected crosses would permit the elucidation of this aspect. This could be of great importance, since, under field conditions, a lower MS would be selectively advantageous for males bearing this trait, since they would invest less time in attempts at copulation and would test a larger number of females faster, thus increasing their chances of finding females ready to copulate (Fulker, 1966; Prakash, 1967). On the other hand, if longer DC increased the chances of spermatophore transfer from male to female (Loher & Gordon, 1968), then genotypes bearing a longer DC would have greater adaptive value. However, if the process of spermatophore transfer does not depend on DC, then the genotype with longer DC would have a greater selective advantage, since a male would have the possibility of fecundating larger numbers of females with a lower investment of time and energy (Mizugushi & Almeida, 1983).

RESUMO

Para determinar a influência da alimentação, luminosidade e horário sobre a cópula de *Panstrongylus megistus*, foram utilizados quatro grupos de 120 casais, testados nas seguintes situações: um grupo sem privação alimentar (P.A.); um grupo com cinco, um com dez e um com vinte dias de P.A. Os testes foram feitos entre 9-12h e 19-22h, no claro (700-1400 lux) e no escuro (1,4-2,8 lux). Registrou-se o comportamento pela técnica de amostragem de tempo. Foram calculadas ainda a velocidade de cópula (VC) e a duração da cópula (DC) para cada situação. A frequência

máxima de cópula observada foi com cinco dias de privação alimentar, à noite, no escuro ($n = 16$). A mínima foi com casais recém-alimentados, à noite, no claro ($n = 4$). Os machos deslocaram-se mais em direção às fêmeas do que estas em direção àqueles. A VC foi menor nos casais com 20 dias de privação alimentar, à noite, no claro ($\bar{X} = 23,0 \pm 16,0$ minutos) e maior nos recém-alimentados, de dia, no claro ($\bar{X} = 2,9 \pm 2,5$ minutos). A DC foi menor nos recém-alimentados, de dia, no escuro ($\bar{X} = 23,5 \pm 6,4$ minutos) e maior naqueles recém-alimentados, à noite, no escuro ($\bar{X} = 38,3 \pm 6,9$ minutos).

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