Chronobiological basis of thermopreference in the haematophagous bug *Triatoma infestans*

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Abstract

The chronobiological basis of the daily dynamics of thermopreference was tested in adults of *Triatoma infestans*, continuously registering the preferred temperature of insects released over a temperature gradient for 13 days. We found that the thermopreference in *T. infestans* is a dynamic process that depends on the time of the day and the post-feeding time. When submitted to a 12:12 h light/darkness cycle (L/D), the preferred temperature reached the highest and the lowest values at the end of the light and dark phases, respectively. This daily rhythm persisted under constant conditions of illumination (D/D and L/L), suggesting the existence of an internal oscillator controlling this behaviour. Statistical analysis revealed that the thermopreference of insects kept under L/D exhibited a ca. 24 h periodicity, while insects kept in D/D and L/L showed free-running periods of $t_{(D/D)} = 23.35$ and $t_{(L/L)} = 27.35$ h, respectively. The persistence of a cyclic pattern of thermopreference under constant conditions, and free-running periods, close to, but different from 24 h, demonstrate the existence of an endogenous control of the thermopreference in this species. The biological relevance of these results is discussed in the light of the hypothesis that both the length of time elapsed since feeding and the time of the day modify thermopreference in these bugs. The gradual decline in preferred temperature following feeding may be associated with energy conservation during starvation. The rhythmic modulation of thermopreference may be associated with the daily rhythm of locomotion activity shown by these bugs.

Keywords: Temperature; Rhythms; Triatominae; Behaviour; Chagas disease

1. Introduction

*Triatoma infestans* is the main vector of the flagellate parasite *Trypanosoma cruzi*, responsible for the Chagas disease in Latin America (Zeledón and Rabinovich, 1981). This blood-sucking bug is mainly restricted to domestic and peridomestic habitats and feeds on endothermic animals. During the day, the insects remain inactive inside refuges such as crevices and straw roofs of human dwellings. During the night, they leave their shelters and display their maximum activity, e.g. feeding behaviour, refuge search, etc. (Espinola, 1973; Núñez, 1987; Lazzari, 1992; Lorenzo and Lazzari, 1993, 1996). Three main factors determine the spatial distribution of *T. infestans*: temperature (Lazzari, 1991a; Lorenzo and Lazzari, 1998), relative humidity (Roca and Lazzari, 1994; Lorenzo and Lazzari, 1998) and the kind of materials used in the construction of dwellings (Gürtler et al., 1992).

Insects are ectothermic animals, i.e. they are unable to physiologically control their body temperature in order to regulate their metabolism. Consequently, their metabolic rate depends on the environmental temperature. However, the ability to choose different environmental temperatures according to their immediate metabolic needs may be regarded as a behavioural method of thermoregulation (Remmert, 1985; Wurtsbaugh and Neverman, 1988). In triatomines, a strong correlation between preferred temperature and starvation level has been demonstrated in *T. infestans* (Lazzari, 1991a), *Panstrongylus megistus* (Pires et al., 2002) and *T. brasiliensis* (Guarnieri et al., 1998), and, to a lesser extent, in *Rhodnius prolixus* (Schilman, 1998; Corchs and Lazzari,
In addition to this long-term variation in thermopreference, Lazzari (1991a) found a daily pattern of thermopreference in adults of *T. infestans* submitted to a 14:10 h light/darkness cycle (L/D). During the photophase, insects displayed a slow but constant shift towards higher temperatures, reaching the highest preferred temperature value at the time of lights-off, while during the scotophase, they moved towards lower temperatures, reaching the lowest preferred value just before lights-on.

Since life appeared on the earth, it has been exposed to daily cycles of light/darkness and seasonal cycles of climatic changes, due to the natural movements of the earth. Like many other organisms, some insects temporally restrict their activities, avoiding times when conditions are unfavourable. Such rhythmical behaviour could be either a direct response to environmental changes, i.e. consequence of illumination and/or temperature cycles, or under an endogenous control, i.e. commanded by internal oscillator/s. However, there is usually a dual action of both factors. The existence of circadian rhythms, i.e. cycles with a period around 24 h controlled by an internal oscillator, has been demonstrated in almost all kinds of organisms (Aschoff, 1989).

Being aware of the previous information available about thermopreference in triatomines and taking into account the adaptive value of biological clocks in organisms, the aim of this work was to study and describe the dynamics of the thermopreference in adults of *T. infestans*, and to understand the mechanisms involved in its temporal control. Three different hypotheses were tested in adults of *T. infestans*: (H₁) they prefer lower temperatures as the level of starvation increases, (H₂) thermopreference is a cyclic process, and (H₃) the daily rhythm of thermopreference is under endogenous control, i.e. controlled by internal oscillators, and not a direct response to environmental illumination. The three associated predictions are: (P₁) the slope of the regression line of the daily mean preferred temperature is negative, (P₂) the pattern of preferred temperature with time manifests periodicity, and (P₃) bugs kept under constant conditions (D/D and L/L) continue to show rhythmic thermopreference, and the periods of the rhythm under these conditions are close to but different from 24 h. Finally, we discuss the relationship between thermopreference, activity pattern and metabolism in this species.

### 2. Materials and methods

#### 2.1. Experimental arrangement

The adults of *T. infestans* used for the experiments were reared in the laboratory at constant 28 °C temperature and fed once a week in vivo on hens until the day before the beginning of the assays. A temperature gradient was generated over an aluminium plate using a thermostatised heater in one end and a thermostatised cooler device in the other. An experimental glass arena (length: 39.00 cm, width: 9.25 cm, height: 3.00 cm) with cover was laid over this plate. Thirteen equal cells of 3.00 × 9.25 × 3.00 cm were drawn on the cover glass and the temperature was measured in each zone (Fig. 1) before and after each experiment. In order to register the position of the insects along the gradient over time, we used an infrared-sensitive video camera connected to a video recorder controlled by a PC. An ad hoc device allowed long-term programming of the video recorder. During the experiments, bugs were kept under different illumination conditions. When the light was turned on, the intensity measured at the insect’s position was 180 lux, while during dark periods, it was 0 lux.

For each experiment, five adult males or females, fed 24 h before the beginning of each series, were released in the experimental arena. The position, and thus the preferred temperature of the insects, was registered in videotapes every 30 min for 13 consecutive days. Three experimental conditions of illumination were established: (a) bugs kept under a 12 h light/12 h darkness regime (L/D), (b) bugs that after five training days in L/D were transferred to constant darkness (D/D), and (c) bugs that after five training days in L/D were transferred to constant light (L/L). Four replicates (two with males and two with females) were carried out for each treatment, comprising a total of 12 experimental series.

#### 2.2. Variables

In each series of raw data, we averaged the thermopreference of the five bugs released together, at each interval of time, in order to obtain the mean temperature every 30 min (MT30). Only for the bugs submitted to L/D, the 48 MT30 of each day were averaged obtaining the daily mean temperature (DMT). The hourly mean deviation (HMD) was calculated by subtracting the pre-
ferred temperatures at every half-hour from the DMT of the same day, averaged for 13 consecutive days. In this way, any long-term trend was removed and only daily components merge.

2.3. Data analysis

The differences in thermopreference along the total experimental time were statistically analysed in bugs submitted to L/D, calculating the slopes of the regressions between the DMTs and the post-feeding time, and testing whether the 95% confidence interval of the mean slope included the value 0. Time series analysis was performed with the MT30s to check the occurrence of periodical components in the series of data obtained under L/D, D/D and L/L. We tested the significance of periodic components in the data by means of Lomb–Scargle periodograms (Ruf, 1999).

3. Results

3.1. The dynamics of thermopreference under L/D

Adults of T. infestans showed variations in their preferred temperature with time. Two dynamic components were identified: a periodic daily variation was superimposed on a long-term standing displacement towards lower temperatures with increasing starvation. The long-term trend to lower preferred temperature was characterised as the variation in the DMT, whereas the variation in preferred temperature within the day was characterised as changes in the HMD.

Bugs maintained under L/D cycles showed a marked significant diminution of the DMT with increasing time since they were fed, i.e. with increasing level of starvation (Fig. 2, mean $b = -0.275$, 95% confidence interval $[-0.362, -0.187]$, DF = 3). Given that the mean slope confidence interval did not include the 0 value, a significant effect of the post-feeding time is evinced. Both males and females showed a drift towards lower temperatures as the starvation time increased.

The daily rhythm of thermopreference, shown in Fig. 3, is expressed as the HMD. The removal of the slow trend by depicting the deviation of the preferred temperature from the DMT allows a better visualisation of the daily pattern of thermopreference. During the photophase (8:00–20:00 h), the insects displayed a slow but continuous movement towards higher temperatures, preferring the warmest place just before the beginning of the night. During the scotophase, they showed a preference for lower temperatures, preferring the minimum temperature at the end of the scotophase. Both males and females exhibited a significant daily variation in thermopreference (see below for statistics).

3.2. Circadian control of the thermopreference

Analysis of MT30 values revealed that under the three different treatments (L/D, D/D and L/L), both sexes showed a continuous cyclic pattern of thermopreference throughout the whole experimental time (Fig. 4a,b). The statistical analysis of the six series of data (males and females under either L/D, D/D or L/L) evinced periodicity. We computed the most repeated periods of thermopreference for each group and represented them by means of Lomb–Scargle periodograms (Fig. 5a–f). The periods of the rhythm of thermopreference of the insects were ca. 24 h for L/D, ca. 23.35 h for D/D and ca. 27.35 h for bugs maintained under L/L (in all cases $p < 0.05$).

Fig. 2. Daily mean temperature (DMT) preferred by bugs submitted to L/D along the 13 experimental days. Each point represents the average of four DMTs. The averaged slope of the regression lines (straight line) was significantly lower than 0 (mean $b = -0.275$, 95% confidence interval $[-0.362, -0.187]$, DF = 3).

Fig. 3. Hourly mean deviation (HMD) from the daily mean preferred temperature exhibited by insects submitted to L/D. Each point represents the average of the 13 deviations from the DMT (one per day) for each half-hour. The horizontal dashed line represents the DMT. Dark and white areas indicate the periods of lights-off and -on, respectively.
Fig. 4. (a,b) Raw data of thermopreference along the 13 experimental days. Each point represents the average of the preferred temperature of five bugs every 30 min. (a) males; (b) females.

![Fig. 4](image)

Fig. 5. (a–f) Periodogram analysis of the daily rhythm of thermopreference of males and females of *T. infestans* kept under L/D, D/D or L/L. The horizontal dotted line indicates the critical value (Lomb–Scargle periodogram, $p = 0.05$). The vertical dashed line indicates the 24 h period. Callouts denote the most repeated significant period.

![Fig. 5](image)

4. Discussion

The results presented in this work confirm previous findings by other authors that thermopreference in triatomines is not a static phenomenon, but a dynamic process. The preferred temperature of these insects varies according to the time elapsed since last feeding and also to the time of day. Given that these bugs exhibit a marked circadian organisation of different physiological and behavioural processes, the question arises as to whether the rhythm of thermopreference is controlled by an endogenous oscillator or appears as a direct response to external variables.

As shown in Fig. 2, the DMT was different at the beginning and at the end of each assay. Just after feeding, both males and females preferred to stay at rela-
tively higher temperatures and moved towards lower values with increasing starvation. The same has been observed in larvae and adults of other triatomine species (Lazzari, 1991a; Schilman, 1998; Pires et al., 2002; Corchs and Lazzari, 2000; Corchs et al., 2001). This behaviour has been interpreted as a behavioural reaction by which the insects can modulate their metabolic rate. Evidently they expose themselves to higher temperatures when the blood storage is maximal and move towards colder places with increasing starvation. In this way, the bugs can attain a relatively higher metabolic rate when active transformation of food is needed and save energy when food is not available.

Superimposed on this long-term variation in thermopreference, bugs showed a daily variation in their preferred temperature (Fig. 3). This daily change can be related to the rhythm of spontaneous activity in this species (Lazzari, 1992). We found that the bugs select the warmest place that they visit on a given day (the absolute value of the chosen temperature depends on the time elapsed since feeding) just before the beginning of the scotophase, when they become active (Fig. 3). We asked, then, whether this daily rhythm could be controlled by an endogenous factor, i.e. an internal circadian clock, or by external cues, such as the illumination cycle. Animals maintained in L/D cycle displayed a thermopreference rhythm that varied with the same period as the external Zeitgeber (24 h). However, in the absence of external periodic cues, i.e. under D/D or L/L, the internal oscillator could run freely, revealing its own periods (τ), which were \( \tau_{D/D} = 23.35 \) and \( \tau_{L/L} = 27.35 \) h for D/D and L/L, respectively. The persistence of this rhythm under constant conditions, and the fact that the free-running period of the rhythm had a value close to, but different from 24 h, demonstrate the existence of an endogenous circadian rhythm of thermopreference in \( T. infestans \).

The adaptive value of circadian endogenous clocks in organisms has been profusely discussed in the literature (e.g. DeCoursey, 1983; Aschoff, 1989). In the case of \( T. infestans \), the circadian system controls, amongst others, processes like locomotion activity (Lazzari, 1992), egg-hatching (Lazzari, 1991b) and ecdysis (Ampleford and Steel, 1982). Concerning thermopreference, its daily variation has been reported in \( T. infestans \) (Lazzari, 1991a), \( Rhodnius prolitus \) (Schilman, 1998) and \( Panstrongylus megistus \) (Pires et al., 2002). However, the control of this rhythm has never been investigated previously. The key finding of the present work is that, at least in \( T. infestans \), this behaviour is under the control of an endogenous circadian clock.

The question arises as to the nature of the adaptive value or the selective pressures involved in the occurrence of a clock controlling such behaviour in triatomines. These insects inhabit human dwellings, peridomestic structures and nests of sylvatic animals. They usually spend the daylight hours inside shelters, where light cannot easily reach them. At night, they leave their refuges in search of food, sexual partners, etc. The possession of circadian clocks would make bugs less dependent on environmental conditions to establish the appropriate time of the day to leave the refuges. At first glance, thermopreference does not seem to be a process that would need precise temporal control, since an animal could move to a different place, either warmer or colder, depending on physiological demands at a given moment. We can speculate about the need for an internal program controlling the change in the exposure of bugs to environments at different temperature along the day. If we consider that these insects remain immobile for most of the day, the temporal modulation of the thermopreference would allow them to finely tune their metabolic demands in order to prepare themselves for their maximum and minimum periods of activity, which occur at the beginning of the night and during the day, respectively.

On the other hand, provided that the free-running periods observed for thermopreference under D/D (\( \tau_{D/D} = 23.35 \)) and L/L (\( \tau_{L/L} = 27.35 \) h) are similar to those found in spontaneous locomotion experiments (Lazzari, 1992), and that a temporal association between both activities occurs, the same oscillator/s could be controlling both behaviours.

Many reports on thermopreference in insects can be found in the literature. However, most of them are based on short-term static analysis, in which insects released at a certain time of the day have to decide where to stay, either between two different temperatures or along a temperature gradient (Lactin and Johnson, 1996). Others are dynamic studies, aimed at analysing the thermopreference during a certain time period (Corchs and Lazzari, 2000; Corchs et al., 2001; Guarneri et al., 1998; Lazzari, 1991a; Pires et al., 2002). Our work asked specifically whether changes in the preferred temperature at different day times are endogenously controlled or not. This paper constitutes the first report of a circadian control of thermopreference in insects. This fact becomes particularly important in individuals that are not able to physiologically sustain a constant body temperature independent of the ambient temperature, as insects. Moreover, as the water loss and the metabolic rate of most insects depend on ambient temperature (Sanborn et al., 2002), this kind of control ensures a rhythmic expression of activities associated with energetic needs, independent of the actual environmental conditions.

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