CHRONOBIOLOGY INTERNATIONAL
https://doi.org/10.1080/07420528.2018.1486851

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Circadian system responses to nocturnal and diurnal hosts in the kissing bug, *Triatoma infestans*

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ABSTRACT

Insects express diverse behavioral rhythms synchronized to environmental cycles. While circadian entrainment to light–dark cycles is ubiquitous in living organisms, synchronization to non-photic cycles may be critical for hematophagous bugs that depend on rhythmic hosts. The purpose was to determine whether *Triatoma infestans* are capable of synchronizing to the circadian rhythms of potential hosts with temporally distinct activity patterns; and, if so, if this synchronization occurs through masking or entrainment. Precise synchronization with the food source may be critical for the insects' survival due to the specific predatory or defensive nature of each host. Kissing bugs were housed in a compartment in constant dark, air-flow-connected to another compartment with a nocturnal or a diurnal host; both hosts were synchronized to a light–dark cycle. The activity rhythms of kissing bugs were modulated by the daily activity rhythms of the vertebrates. Effects were a decrease in the endogenous circadian period, independent of the host being nocturnal or diurnal; in some cases relative coordination occurred and in others synchronization was clearly achieved. Moreover, splitting and bimodality arose, phenomena that were also affected by the host presence. The results indicate that *T. infestans* were able to detect the non-photic cycle of their potential hosts, an ability that surely facilitates feeding and hinders predation risk. Understanding triatomines behavior is of fundamental importance to the design of population control methods.

ARTICLE HISTORY
Received 1 March 2018
Revised 16 May 2018
Accepted 6 June 2018

KEYWORDS
Circadian rhythms; non-photic synchronization; Triatominae; hematophagous vectors; Chagas disease; masking; entrainment

Introduction

The kissing bug, *Triatoma infestans*, is a major insect vector of the parasite responsible for Chagas disease. Transmission of the disease often is associated with feeding events when this hematophagous insect is taking a blood meal. These insects are known to feed on diverse hosts with widely different temporal activity allocations. This includes various sylvatic vertebrate species such as bats (Thomas et al. 2007), wild guinea pigs, and mice (Bermudez et al. 1993). Moreover, Triatominae species found in human dwellings feed mainly on domestic and farm animals as well as on humans (Gürtler et al. 1997, 2009). In the laboratory, nocturnal insects also adapt well to scheduled artificial chicken feeding during daytime (Amelotti et al. 2010; Szumlewicz 1976).

Triatominae show marked temporal organization in their behavior. Different types of behaviors are expressed during dusk, night, or dawn (Lorenzo Figueiras and Lazzari 2000). At the beginning of the scotophase (dark interval of a light–dark cycle), they are active, searching for food, mating opportunities, and oviposition sites (Ampleford and Davey 1989; Lazzari 1991, 1992; Lorenzo Figueiras et al. 1994; Lorenzo and Lazzari 1998). At the end of the scotophase, they return to their refuges expressing aggregation behavior (Lorenzo Figueiras and Lazzari 2000). Valentinuzzi et al. (2013) showed that *T. infestans* is clearly nocturnal in laboratory light–dark conditions, free-runs with a stable long endogenous period (up to 28 h) in constant dark and rapidly resynchronizes when the light–dark cycle is restored.

While ambient lighting conditions can synchronize the timing of activity in hematophagous insects, the

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biological clocks in these organisms are highly responsive to other environmental conditions. This includes the activity rhythms of hosts and predators, which are roles often played by the same individuals (Barrozo et al. 2004). The combination of contrary survival factors (food/predator) suggests that for kissing bugs, the non-photic environmental cycles may provide critical signals for timing their own rhythmic expression. Valentinuzzi et al. (2013) showed that a regular daily 1-h exposure to a host can synchronize the rhythmic daily timing of activity in *T. infestans*, even in the presence of 24 h light–dark cycles.

The host may be either nocturnal or diurnal in terms of both their levels of activity and their awareness of the presence of the insect parasite. Therefore, the feeding strategy of the insects may differ depending on which host is available at any moment. Based on the literature, the foraging behavior of kissing bugs can be divided in four sequential phases: (1) dispersal, wherein animals localize potential food sources at a distance (Abrahan et al. 2011, 2016) attracted by diverse host odors (Nuñez 1982, 1987). (2) Establishment of a nearby host and identification of the best moment to approach and feed. Feeding should occur at the quiescent time of hosts not only to facilitate feeding but also to avoid being predated or killed. Detection of the best moment for this approaching behavior may rely on different stimuli generated by the hosts in a circadian pattern such as heat (Flores and Lazzari 1996; Nuñez 1987; Lazzari and Nuñez 1989), CO_2 production (Bodin et al. 2009), activity level or others. (3) Final approach, implying localization of skin and identification of the blood vessels through thermic stimuli (Ferreira et al. 2007). (4) Biting-introduction of *maxillae* for feeding (Schofield 1994).

The second phase of foraging behavior as described implies that kissing bugs should be able to predict when a potential host/predator is likely to be active or inactive. Hypothetically, a circadian clock in the bug participates in determining an optimal time to approach. Therefore, the bug's clock must be flexible enough to enable synchronization by rhythmic signals from specific hosts. We determined whether *T. infestans* are capable of synchronizing to a potential host as a whole with either nocturnal or diurnal activity rhythms and, if so, the degree to which synchronized behavior relies on masking or circadian entrainment.

Material and methods

Insects

Triatoma infestans were maintained in the CRILAR colony in a controlled environment under a light: dark cycle 12:12 h (LD; lights on at 07:00 h), constant temperature (27 ± 2ºC) and relative humidity (40–50% RH). Feeding occurred weekly on chickens (*Gallus sp*) as part of the routine maintenance procedure. This colony consisted in insects captured in the province of Córdoba (provide by the *Coordinación Nacional de Control de Vectores*, Punilla) as well as in the province of La Rioja, both areas with similar environmental conditions. These field-captured insects were reproduced and the F1 progeny reared in the lab form eggs until they emerged to adults. Males of this F1 progeny were selected based on the weight considered to represent optimal health (260 mg ± 50 mg; Szumlewicz 1976), and used in the present experiments.

Vertebrate hosts

Two different vertebrate host species, maintained continuously in a light–dark cycle, were used as potential non-photic synchronizers. In the first experiment a nocturnal host was represented by a subterranean rodent (*Ctenomys sp*, commonly known as tuco-tuco; a 190-g adult male), while in the second experiment a diurnal host was represented by a common chicken (*Gallus sp*; a 400-g young female). These two hosts were selected because they are common species in the habitat of *T. infestans* and have clear and robust diurnal (chicken) and nocturnal (rodent) activity rhythms in the laboratory setup.

Ctenomys capture and maintenance in captivity were authorized by the Environmental Department of La Rioja (permits 028–10 and 062–08) and approved by the Ethics Committees of the Faculty of Veterinary Sciences of La Plata National University, Argentina (permit 29–2–12). Chicken maintenance was approved and authorized by the Experimental Ethics Committee (*Consejo de Ética en Investigación)* of the Public Health State Ministry of La Rioja Province, Argentina (*Ministerio de Salud Pública de la Provincia de La Rioja)* (Protocol no. 892).

Equipment and experimental conditions

A scheme of the structure used is shown in Figure 1. Two independently ventilated, light–tight wood cabinets (150 cm long × 75 cm wide × 60 cm high) were used. These were stacked one on the other. In the upper cabinet, 20 kissing bugs were housed individually in glass circular enclosures (10-cm diameter, 1.5-cm height) with a mesh covered top. These individual enclosures were distributed in the base of the compartment in a grid of 5 × 4. Each insect was identified with an alphanumeric code in which the number was related to its position in the experimental cabinet (1–20) and the letters to its exposure to a nocturnal host (N: nocturnal), a diurnal host (D: diurnal) or no host at all (C: disturbance control).

The cabinet lighting was provided by 75 white LEDs (Surface Mount Devices 5050) controlled by a digital timer (TS-ED1, Zurich). Due to the need of constant video recording as well as occasional equipment maintenance, a constant dim red background was provided by 70 red LEDs (0.5 lux) during both LD cycles as well as in "constant dark." Light intensity was kept within 43–54 lux, provided by the combined white and red LEDs, to avoid negative masking (Valentinuzzi et al. 2013). To reduce the possible introduction of extraneous non-photic stimulation from researchers or room noise, the box was closed and not opened until the end of the experiment.

The lower cabinet housed the selected host according to the experiment or no animal in the case of the control. Light was provided by white LEDs controlled with a digital timer generating an LD 12:12 h cycle. In this cabinet the LD cycle was maintained during the whole procedure of the three experiments. When the rodent was the host, it was housed in a transparent acrylic cage (43 cm long × 23 cm wide × 32 cm high) with a running-wheel (23 cm in diameter, 10 cm wide, and 1 cm between the bars). When the chicken was the host, it was

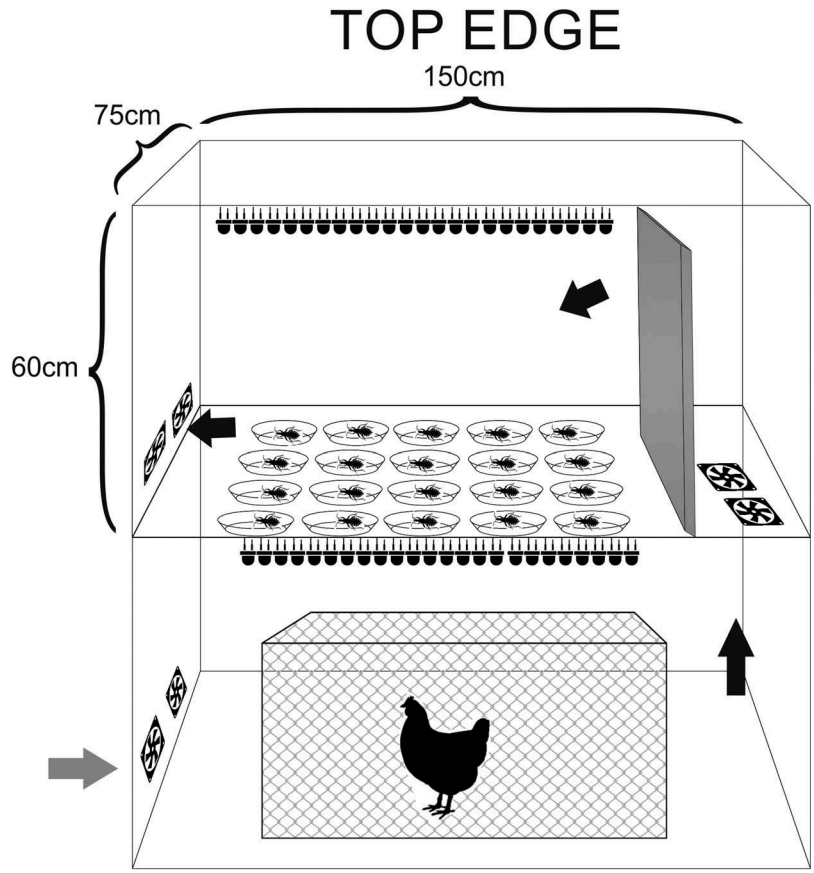

Figure 1. Schematic representation of the experimental setup for experiment II when a chicken host was used. In experiments I and III, a rodent wheel-running cage with a tuco-tuco or three empty wheel-running cages, respectively, were localized in the bottom cabinet instead of the chicken. The arrows represent the air flow.

housed in a plastic mesh cage of 95 cm in length, 48 cm height, and 48 cm to width. Entrance in the experimental room and opening of this lower cabinet occurred every 1–2 days for feeding of the hosts.

The two cabinets were connected through a ventilation system (two 80 mm × 80 mm fans) so that the air from the lower cabinet would flow continuously and steadily toward the upper kissing bug cabinet. This system intended to maximize the likelihood that volatile substances produced by the host would reach the insects. To avoid air turbulence, a wooden barrier 50 cm high was placed in the upper cabinet leaving only a 10 cm space above for air entrance. This barrier served not only as an air turbulence dissipater but also as a simple light-trap system.

Light intensity was measured in the kissing bug upper cabinet with a digital Luximeter (Model TM-201, Tenmars Electronics CO., Ltd, Taiwan). This measure was done in two opposite phases corresponding to the lower cabinet light–dark cycle confirming absence of light leaks. Ambient temperature and humidity inside the boxes were controlled with data loggers (HOBO *U10/003 Onset Computer Corporation, Bourne, MA, USA*).

Monitoring systems

The Big Brother Video tracking system (Actimetrics, Coulbourn Instruments) with video camera (Panasonic WV-BP334) was used. This software tracks the distance travelled per minute. Kissing bug activity in the upper cabinet as well as chicken activity in the lower compartment were measured through this video-tracking system. Running-wheel activity of the rodent was monitored using the ArChron Data Acquisition System (Simonetta System, *Universidad Nacional de Quilmes, Buenos Aires*) at 5-min intervals.

Experimental procedures

Three experiments were performed. Each tested different sets of 20 insects maintained in an LD cycle (as explained above). These were fed 24 h prior to the start of behavioral recording. To avoid interruption of monitoring, feeding did not occur again until finalization of the experiments. The groups were treated similarly with the exception of the potential entrainment signals: the nocturnal host, the diurnal host and mechanical disturbance independent of a living host.

Non-photic stimulus with a nocturnal host

Insects were exposed to an LD cycle for 13 days after which constant dark (DD) began and continued for the rest of the experiment. The first 8 days in DD allowed a free-run baseline serving as each insects own control for constant conditions (Pre-Host Constant Conditions: pre-host CC). On day 8, a rodent was introduced in the lower compartment remaining there for the rest of the experiment. Recording of the rodent's wheel-running activity occurred during these 23 days and was considered a marker of the host´s potential non-photic synchronizing effect.

Non-photic stimulus with a diurnal host

Insects were exposed to constant dark from the beginning to the end of the 45-day long experiment. The initial 11-day DD interval was the free-run baseline serving as each insects own control for constant conditions (pre-host CC). On day 11, a young chicken was introduced in the lower compartment remaining there during 26 days. General activity of the bird, monitored through the video-tracking system, was considered a marker of the host´s potential non-photic synchronizing effect. After removing the chicken, monitoring of the insects continued eight more days in order to determine initial phase of the free-run (post-host CC).

The initial LD cycle was avoided here since adequate synchronization to photic cycles was clear in the previous experiment as well as in Valentinuzzi et al. (2013), added to the fact that these insects had been previously exposed to an LD cycle. More importantly, this allowed not only a longer exposure to the non-photic host (increasing the probability of finding an effect) but also the addition of a constant condition interval (for elucidation of synchronization mechanisms). This is to say that, since feeding cannot occur during the experiment, and that kissing bugs are able to tolerate a maximum of a 55.2 ± 2.6 days (Szumlewicz 1976), the monitoring interval had to be limited to avoid death by starvation.

Control for constant conditions and mechanical vibrations

Insects were submitted to similar settings but in the absence of a host. Initially, they were exposed to an LD cycle during 3 days. Afterwards, constant dark began and continued for the rest of the

experiment. In this dark background, on day 15 a mechanical cycle imitating the vibrations generated by the host's activity in the contiguous cabinet was established. For this, three running wheels were activated through a timer-controlled system in a 12:12 h cycle during a 13-day interval. Finally, the timer was disconnected and DD continued for 6 more days. This protocol is a control for the particular vibrations generated by turning of the wheel which could be considered an artifact of the activity-measuring method used for the rodent host.

Data analysis

Kissing bugs as well as host activity were depicted in double-plotted actograms using the software *El Temps* (A. Díez-Noguera, Universitat de Barcelona, 1999). Actograms allowed visual

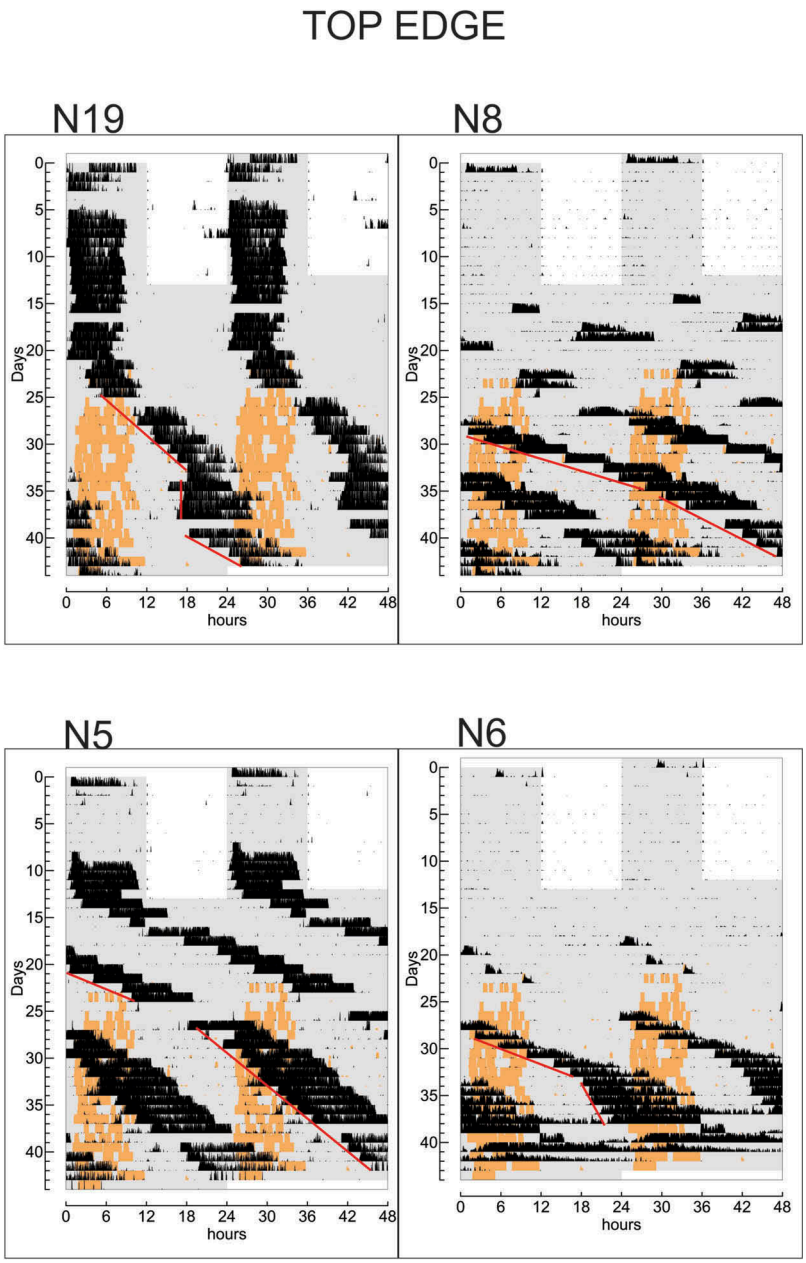

Figure 2. Kissing bugs activity during the presence of a rhythmic nocturnal host. Left: two representative actograms of insects that showed activity from the beginning of the recording interval. Right: two representative actograms of delayed-activity insects. Actograms are double-plotted, light–dark conditions (white–grey) or constant dark (grey) are represented in the background. Kissing bugs' and the host's activities cycle are represented in black and orange respectively. Eye-fitted lines used for free-running period calculation are shown in red.

estimation of phase and rhythmic patterns. Analyses of actograms were done through visual inspection by two independent observers assisted by the "point-and-read" tools of *El Temps*. Period (τ) was the response variable measured. The tendency of these insects in constant conditions is to conserve or even gradually increase their free-running period, always much longer than 24 h. Due to this, a decrease in τ was used as an indicator that the bug´s activity rhythm is affected by an external stimulus. For this, activity onset was determined as in Valentinuzzi et al. (2013), defined as the first bout of activity of at least 60 min of continuous activity after a previous 60-min or longer interval of inactivity. Importantly, this definition had to be in accordance with a group pattern; that is, this

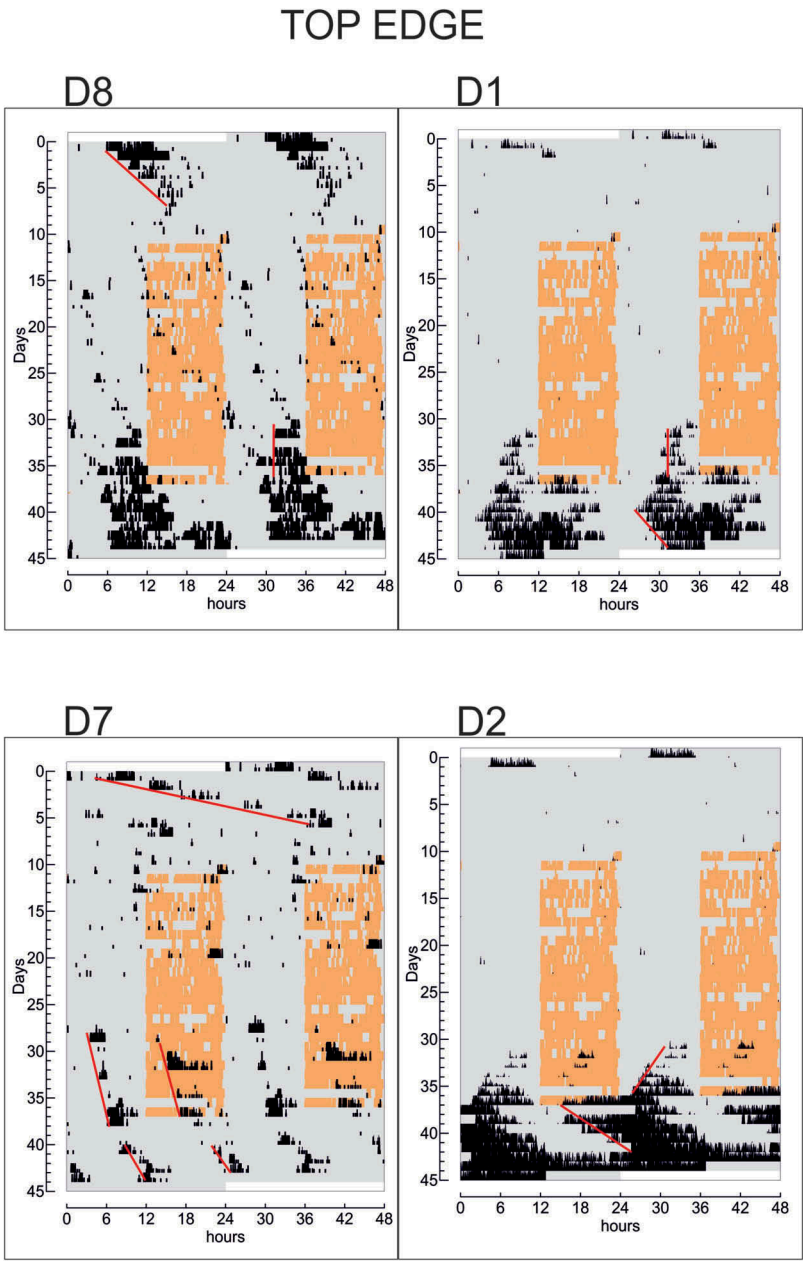

Figure 3. Kissing bugs activity during the presence of a rhythmic diurnal host. Left: two representative actograms of insects that showed activity from the beginning of the recording interval. Right: two representative actograms of delayed-activity insects. Actograms are double-plotted, light–dark conditions (white–grey) or constant dark (grey) are represented in the background. Kissing bugs´ and the host's activities cycle are represented in black and orange respectively. Eye-fitted lines used for free-running period calculation are shown in red. The insect D8 expressed *splitting* and D7 expressed bimodality. All insects synchronized during the last days of the non-photic stimuli.

should occur for a minimum of two consecutive days and in a coherent way (showing a 24-h period related to synchronization or a consistently increasing or decreasing period in the case of constant conditions).

Intervals for period calculation were during the pre-host constant conditions; that is, in the absence of light and non-photic stimuli ($\tau_{\text{pre-host CC}}$). According to the experiment, a second constant condition interval was the post-host one ($\tau_{\text{post-host CC}}$). During the non-photic cycle, a τ host was calculated (τ_{host}). When synchronization occurred, phase angle of entrainment was calculated with respect to the non-photic cycle, defined as the number of hours that insect´s activity onset preceded the beginning of the host´s activity onset. Data were expressed in mean values ± standard error (SE). Periods during constant conditions ($\tau_{\text{pre-host CC}}$) were compared with periods during host presence (τ_{host}) with a paired *t*-tests in the Excel software.

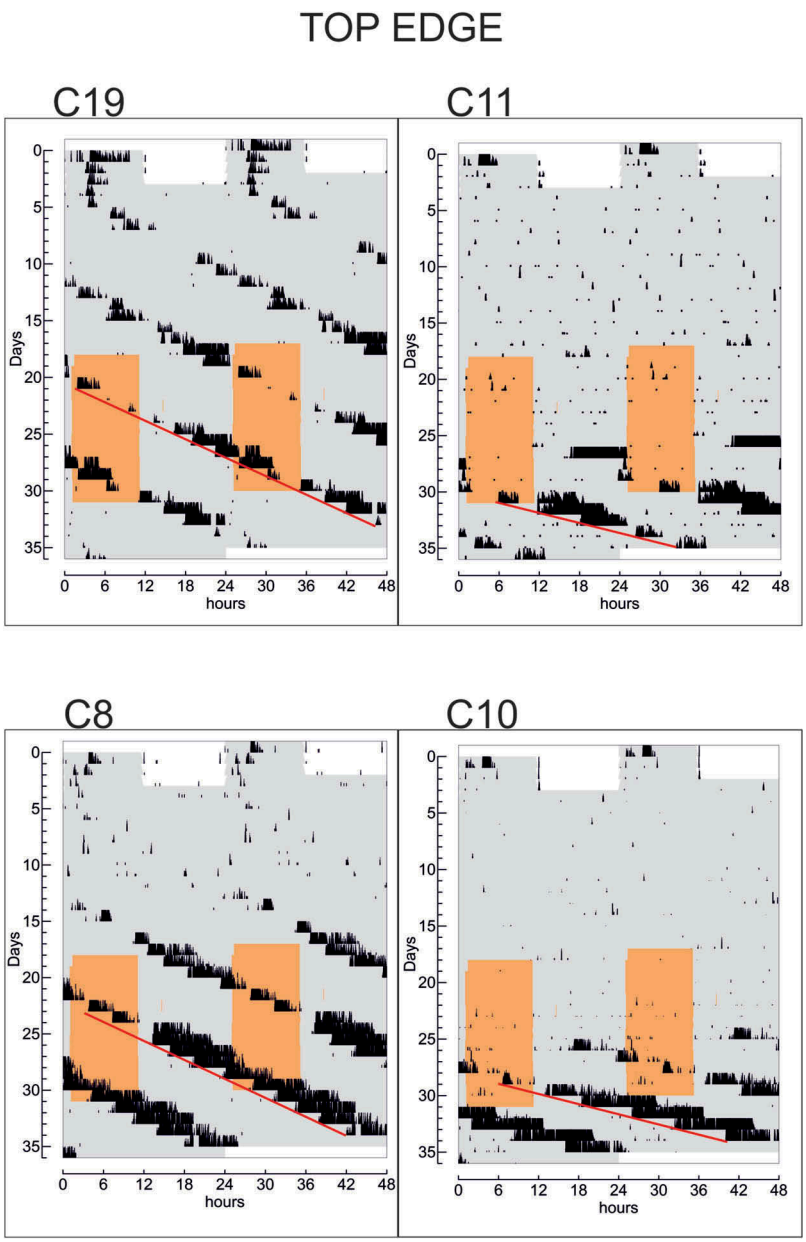

Figure 4. Kissing bugs activity during constant conditions and mechanical vibrations in the absence of a host. Left: two representative actograms of insects that showed activity from the beginning of the recording interval. Right: two representative actograms of delayed-activity insects. Actograms are double-plotted, light–dark conditions (white–grey) or constant dark (grey) are represented in the background. Kissing bugs activity and the non-photic cycle are represented in black and orange respectively. Eye-fitted lines used for free-running period calculation are shown in red.

Results

Of the insects that presented sufficient activity for data analysis, 60% were active throughout the recording interval and are shown in the left column of each figure. The remaining 40% showed a delay to express consolidated activity rhythms. This delay varied according to the host presence (nocturnal or diurnal hosts) or absence (disturbance control) from 29.38 ± 2.10 to 23.43 ± 1.95 days, respectively (t = 2.06; p = 0.03). Nonetheless, the actograms of these delayed-activity insects still allowed a good detection of host-influence and are shown in the right columns of each Figure.

Non-photic stimulus with a nocturnal host

Figure 2 shows representative actograms of insects exposed to a rodent host. The tuco-tuco showed a robust nocturnal wheel-running rhythm clearly synchronized to the LD cycle (orange background). When exposed to the host, the majority of the insects showed a significant decrease in the free-running period from 27.38 ± 0.31 ($\tau_{\text{pre-host CC}}$) to 25.22 ± 0.31 h ($\tau_{\text{host}}$) ($t$ = 7.58; p = 0.00006).

Non-photic stimulus with a diurnal host

Figure 3 shows actograms of insects exposed to a bird host. Activity–inactivity rhythm of the diurnal host was clear and robust representing the diurnal non-photic stimulus (orange background) which was in addition precisely synchronized to the LD cycle. The majority of the insects showed a significant decrease in the free-running period from 29.12 ± 0.83 ($\tau_{\text{pre-host CC}}$) to 26.70 ± 0.87 ($\tau_{\text{host}}$) h ($t$ = 7.58; p = 0.00006) (left panel of Figure 3). Moreover, other insects ended up adjusting their activity to the host only after 31 days and despite the previous absence of activity (right panel of Figure 3). During these days, insects tended to concentrate their active phase during the host´s sleep phase. The mean phase relationship with the host activity onset was −5.65 h ± 81.7 min. Once in constant conditions, two effects can be described. On one hand, the above-mentioned component starts its free-run with the same phase of previous synchronization during the previous inactive phase of the bird (i.e., the bird's night). Initially, τ is shorter than 24 h but 3–4 days later, it increased. On the other hand, the bugs expressed activity suddenly during the interval in which the host had been previously active.

The particular rhythmic expression of insects D8 and D7 entail a further description. In insect D8, from days 5 to 9, activity duration decreased, almost disappearing, while the period also decreased. During the presence of the host, splitting in two clear components 12 h apart occurred, both with similar periods (24.88h and 24.56h). Toward days 32 and 36, both components tended to merge reaching a phase relationship of 3.53 h before the bird's activity onset. Once in constant conditions, the free-run initiated in the same phase. With insect D7, after a clear long free-run period (30.2 h) in constant conditions, in the bird´s presence, activity decreased and became arrhythmic. Afterwards, inactivity occurred between days 23 and 27, reappearing in a bimodal pattern with two components approximately 12 h apart. The first component occurred 8.76 h before the chicken´s activity onset and the second, 3.04 h after. Once in constant conditions the components free ran with shorter periods (the mean of two components was as follows: $\tau_{\text{post-host CC}}$ = 25.6h ± 0.1 h) compared to the initial free-run.

Control for constant conditions and mechanical vibrations

Figure 4 shows the insects that were not exposed to a host. Clear free-runs occurred throughout the whole experiment, in the absence of any type of cycle ($\tau_{\text{pre-host CC}}$ = 27.96h ± 0.41 h) as well as in the presence of the mechanical cycle (τ_{host} = 28.04h ± 0.51 h) (t = − 1.08; p = 0.34). Moreover, when the non-photic cycle was removed the endogenous period continued invariable. These free-run values were the same as those found in kissing bugs maintained in constant conditions in Valentinuzzi et al. (2013).

Discussion

Considerable variations (individual differences) were found among the animals' responses to confinement in the experimental apparatus. Some animals (~40%) never moved, although they

remained alive. The others ranged from being active throughout the recording period, or becoming active after many weeks. A similar range of responses to the test environment has been reported previously (Valentinuzzi et al. 2013). Clearly, unspecified differences among individuals contribute to their responses to the experimental context. One possibility is the motivation to find food, which is reduced with recent feeding. Although all animals were fed before the start of the experiment, it is not certain that all were equally sated. Therefore, movement or response to a potential host could vary. In addition, the likelihood of foraging movements could be reduced upon the recognition of the host as a potential predation risk (Chelini et al. 2009; Misslin 2003). It is possible that the responses of individuals could differ depending on whether the bug detects the host from a distance, and moves toward it (i.e. host = food = approach), or whether the host approaches the bug (i.e. host = danger = freeze), as might occur in the experimental situation.

For those animals that produced interpretable activity rhythms, regardless of how long the activity expression was delayed, we found that individuals responded to the non-photic activity–inactivity rhythms of both nocturnal and diurnal hosts housed in close proximity. The long period activity rhythms (>24 h) of these kissing bugs were decreased in the presence of the hosts and, in many animals complete entrainment was evident. When the host was present, entrainment and period effects were confirmed from the timing of the subsequent free-run following removal of the host. When entrainment occurred, activity was concentrated during the host´s sleep phase; active hosts seem to inhibit activity in kissing bugs. Splitting and bimodality also occurred in a two animals, and these patterns were similarly affected by the host presence.

In the presence of a nocturnal host, the endogenous period in the majority of the insects was reduced. This could be quantified not only in the animals with complete records but also in the delayed-activity ones (right panel of Figure 2). In some cases, this period reduction reached values close to 24 h (indicating entrainment); in other cases, the reduction was expressed as a phase-dependent shortening, which was followed by a second period change from 24.46 ± 0.34 to 26.15 ± 0.19 h, indicating the occurrence of relative coordination or partial entrainment (Refinetti 2006; Swade and Pittendrigh 1967) (e.g., N19 in Figure 2).

A similar decrease in the endogenous period was also produced in the presence of a diurnal host. Period adjustment is indicated in the rhythms of the insects shown in Figure 3, with concentration of activity during the host´s sleep phase. When the host was removed and constant conditions began, the free runs started at the same phase as in the host presence. This suggests that the expression of the real phase of the biological clock was occurring during the non-photic cycle. Negative masking is also suggested by the data since activity in constant conditions appears during the phase that corresponds to the previous chicken`s alert phase. An active host tends to inhibit the activity of the insects, suggesting that an alert bird is perceived as a potential predator.

In the insects submitted to mechanical disturbance independent of a living host, free-running occurred normally with increasing values as time in constant dark continued (after 33 days, period was 28.57 ± 0.47 h). This was opposite to what happened in the experimental groups, where, in the presence of a rhythmic host, deceleration of the kissing bug´s clocks was the tendency. Clearly the insects do not synchronize to any mechanical cycle even to this more intense cycle (three cyclically moving wheels, instead of only one). The wheel movement is external to the host, generating vibrations different from those generated by the hosts´ normal behavior such as waking around, grooming, or more species-specific activities. The latter are probably part of the combo of stimuli originated in a host (Barrozo et al. 2017).

These data suggest that the timing of kissing bugs´ activities can be modulated by various hosts with distinct temporal niches. The implication is that the circadian system in the bug is flexible and strongly reactive to food sources. Diverse output patterns were observed here, including phenomena as splitting and bimodality that are evidence of multiple oscillators tied to dawn and dusk or sensitive to non-photic stimuli. These two phenomena are additional examples of how adjustable this circadian system might be. Splitting of activity was detected in insect D8, a

phenomenon revealing internal desynchronization and caused by a variety of treatments being constant light exposure of various intensities one of them (Christensen and Lewis 1982; Wiedenmann 1980, 1983). Since the splitting insect began showing signs of occurrence before the host presence (decreasing of period and decreasing α), we attribute this effect to the constant red light background. Even though we cannot say that the host stimuli determined splitting, we can surely say that it had an effect on the evolution of its expression. This is visible in the clear stop of the first component close to the hosts´ activity onset and the posterior fusion of the second component. In other words, the non-photic stimuli recoupled the oscillators or, at least, accelerated the process. When the host was removed, the posterior free-run at the same phase was evidence of entrainment.

Bimodality occurred in insect D7, with one component in the middle of the host´s sleep phase and a second in the middle its wake phase. Bimodality in triatomines had been described previously (Lazzari 1992); however, in a photic cycle where activity occurred at dusk and dawn. Valentinuzzi et al. (2013) also observed bimodality but associated with shorter constant dark periods and always followed by entrainment to a 1-h non-photic exposure. The very long period ($\tau_{\text{pre-host CC}}$ = 30.21 h) measured in this insect at the beginning of the experiment was importantly reduced after the non-photic cycle ($\tau_{\text{post-host CC}}$ = 25.06 h; mean of both components). Additionally, the initial phases of $\tau_{\text{post-host CC}}$ are the same as during the host presence. These are signs of non-photic entrainment. The free run of both components, once in constant conditions, also indicates the involvement of two oscillators.

Splitting and bimodality reflect the existence of at least two functional oscillators or group of oscillators, which in certain conditions uncouple and begin to express themselves independently, manifesting two overt behavioral components (Saunders 2002).

The preponderance of data indicates that *T. infestans* in constant darkness is capable of adjusting its activity to the host´s activity rhythm, regardless of whether the host is diurnal or nocturnal. The bottom line is that the insects were able to detect the different stimuli generated by an active animal in opposition to those generated by an inactive animal. These hematophagous bugs are able to detect the daily changes of different stimuli such as CO_2 (Nuñez 1982), heat (Lazzari and Nuñez 1989), and other metabolic products (Guerenstein and Guerin 2001) that result from the activity rhythm of the hosts and consequently allowing time-adjustment. We acknowledge that these experiments may not be directly comparable due to the different taxa of the hosts used (that already imply in numerous physiological dissimilarities, e.g., birds have higher body temperatures), different host weights (consequently different stimuli levels), and/or the insects acquaintance to the host (insects here were accustomed to feed on chickens in the lab). Despite these differences, the bottom line is that the presence of either of the hosts affected kissing bugs activity rhythms.

Synchronization was manifested in overt behaviors through a combination of entrainment and masking. The circadian system of the kissing bug, therefore, is capable of synchronizing to potential hosts with varied temporal expressions. The diverse patterns of behavior that are exhibited among insects raised in identical conditions suggest that their circadian system is highly adaptable to individual immediate needs. Since hematophagous insects depend on hosts that express their own temporally defined rhythms, adjustments to multiple food sources implies in significant survival advantages.

Acknowledgments

The authors thank D. Gorla for the initial support and discussion in planning. The authors also thank I. Amelotti, ML Hernandez, MJ Cavallo, and C. Nieto for kissing bug field captures and laboratory analysis of *T. cruzi*. *Centro Regional de Vectores*, Córdoba, for facilitating some insects. A word of thanks also goes to C. Nieto for assistance with animal maintenance and J. Barros for experimental support; and G. Oda, M. Gomes, G. Improta, and T. Yamamoto for collaboration with data analysis.

Declaration of interest

The authors declare that there is no conflict of interest.

Funding

This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica [grant number PICT 2013/2753], Consejo Nacional de Investigaciones Científicas y Técnicas [grant number PIP-11220120100415CO], and Natural Sciences and Engineering Research Council of Canada [grant number 170040].

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