Activity and shelter-related behaviour in *Rhodnius prolixus*: the role of host odours

Shelter use in *Rhodnius prolixus*

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Abstract

Triatomine bugs are considered nocturnal insects that feed on the blood of vertebrates and remain hidden inside narrow shelters during daylight hours. Nevertheless, it is not clear whether these insects become active and leave their shelters on a daily basis, less frequently or, even fortnightly. Activity patterns were studied in *Rhodnius prolixus* Stål, 1859 (Hemiptera: Triatominae) associated with shelters to evaluate whether the decision to leave a shelter depends on bug starvation and the presence of host odours. Experiments were conducted with groups of 5th instar nymphs released in an experimental arena offering an artificial shelter consisting of a piece of corrugated cardboard positioned in its centre. Results indicate that host odours promoted a significant increase in shelter related activity, i.e. shelter-leaving or entering movements, and also in bug locomotion. This increase could only be observed with bugs starved for 30 or 60 days, but not for 21 days. Most *R. prolixus* nymphs that left shelters and engaged in locomotory activity were starved and in the presence of host odours. Even though *R. prolixus* is mostly considered a very active and "aggressive" triatomine, our results contradict this perspective and suggest that its main strategy regarding hosts is to wait and carefully evaluate feeding chances before becoming exposed. This behavioural strategy might have arisen through their evolution in palm trees in association with a diverse fauna that may impose predation risks.

Key-words: Triatominae, host-cues, locomotion, search, nutritional status.

Introduction

Rhodnius prolixus is the most important vector of Chagas disease in Venezuela and Colombia[1]. Chagas disease is an important neglected health condition caused by the protozoan parasite *Trypanosoma cruzi*, mostly in Latin America. Endemic to 21 countries, the disease affects approximately 6 to 7 million people, while another 28 million are exposed to risk of vectorial transmission[2,3].

Triatomines are nocturnal hematophagous insects that spend most of their life hidden inside shelters[4]. The use of shelters seems crucial to triatomine survival by protecting the insects from potential predators. During daylight hours triatomines remain hidden in akinesis, which is fundamentally promoted by physical contact with the substrate[5] and conspecifics[4]. Aggregation between *Triatoma infestans*, the species better understood in its relation to shelters, is maintained by volatile pheromones[5,6] as well as arresting factors derived from the cuticle[7]. It is unclear whether this is the case for *R. prolixus*, even though an aggregation pheromone has been described for its faeces[8,9].

The locomotory activity of triatomines has been studied by several authors[10-16]. When active, triatomines present two peaks of locomotory activity[13,14] which are endogenously controlled[14]. In the first hours of the scotophase triatomines experience a period of spontaneous and non-oriented locomotory activity[10,11,14,17] that allows them to leave the shelters and search for hosts[15]. At the end of the scotophase they present a second peak of locomotory activity that is related to their search for shelters[15]. In this study we evaluated the dynamics of shelter use, as well as the pattern of locomotory activity of sheltered *R. prolixus*. In addition, we evaluated whether bug starvation affects these behavioural profiles. Finally, we evaluated the effect of the presence of host odours on these activity parameters.

Material and methods

Insects

Rhodnius prolixus used in the experiments came from a colony maintained at our institute, which was started from insects collected in Honduras approximately twenty years ago. Insects were fed chicken blood and reared under 27±2ºC, 60±10% RH and 12:12 L/D.

Behavioral study

Experiments were conducted in a glass arena of 50 x 50 cm (Figure 1) maintained in a room with controlled conditions (24 ± 1°C; 43 ± 8% RH; 12:12 L/D). A piece of corrugated cardboard (10 x 20 cm), folded in the midline to offer a shelter with two accesses (shelter area: 10 cm^2) was placed in the centre of the arena[(15)] (Figure 1). In each assay 30 5^{th} instar nymphs were released in the arena. The insects were maintained in the arena for three days to allow them to acclimate to the new conditions and adopt the piece of cardboard as shelter. In the next day, 10 min before lights-off, an empty steel cage (10 x 10 x 10 cm) was suspended 12 cm above the arena substrate and 8 cm away from one of the arena walls and the insects were monitored to record their basal activity (control trials). In the following night, two female mice (30 days old; 26-30 g) were placed inside the cage and again, insect activity was recorded. Insect behavior was recorded by means of an infrared-sensitive video camera (HDL, Brazil). A panel of extra infrared light emitting diodes (LED, 900 nm) was located above the arena providing uniform illumination for video recording. This device allowed recording the position of the bugs even during complete functional darkness for the insects since infrared illumination is not perceived by triatomine bugs[(18)]. Triatomines leave their shelters in search of food in the early hours of the dark phase[15]. Therefore, video recording started 10 min before lights-off, and finished after five hours. Three different starvation levels (21, 30 and 60 days after molting to the 5th instar) were evaluated in our study (n=6 for each condition/context tested).

The number of insects leaving the refuge, as well as the level of locomotory activity expressed by them, was recorded under both situations to test whether host odors induced alterations in these parameters. In our video recordings the image of the arena occupied the whole TV monitor. To quantify the activity level of nymphs, two perpendicular lines forming a cross centered in the middle of the screen were traced and the number of times wandering bugs crossed any of the lines recorded. Data were normalized by dividing the total number of movements in each hour by the number of insects present outside the shelter at the respective hour.

Statistics

Data were analyzed by Wilcoxon and Friedman ANOVA in case of paired samples and by a Kruskal-Wallis test for independent ones. Dunn's multiple comparison test was used for pair-wise comparisons in the analysis with multiple samples. Proportion data were arc-sine transformed prior to analysis. The accepted significance level used was $p<0.05$.

Ethics

All experiments using live animals were performed in accordance to FIOCRUZ guidelines on animal experimentation and were approved by the institutional Committee for Ethics in Animal Experimentation (CEUA/FIOCRUZ, protocol number L-058/08).

Results

Extremely few *R. prolixus* nymphs left the shelters in the absence of host cues (2.2, 1.65 and 2.3% for 21, 30 and 60 days of starvation, respectively). The number of bugs coming out of the shelter increased significantly in the presence of a host only for nymphs starved for 30 (Wilcoxon, p=0.02) and 60 (Wilcoxon, p=0.04) days (2.7, 26 and

19.4% for 21, 30 and 60 days of starvation, respectively). In all cases, less than 3% of insects starved for 21 days were observed coming out of shelters (Wilcoxon, n.s.). Furthermore, in this nutritional condition no insects were observed outside shelters in three out of six assays. Nutritional status only induced a significant increase in the number of bugs leaving refuges in the presence of a host (Figure 2, Kruskal Wallis, $p=0.006$). Significantly fewer insects starved for 21 days left the shelter in the presence of a host compared to those starved for 30 days (Figure 2; Dunn's multiple comparison test, $p<0.05$). Nevertheless, a further increase in starvation did not induce a significant effect between insects starved for 30 and 60 days when exposed to host cues (Figure 2; Dunn's multiple comparison test, n.s.).

Insects starved for 30 days showed a significant variation in the percentage of them found outside shelters along the five recorded hours in the presence of a host (Figure 3; Friedman ANOVA, $p=0.0001$). In this case, the percentage of exposed nymphs increased over time, reaching 25% at the end of the interval (Dunn's multiple comparison test; 1h *vs* 4h, 1h *vs* 5h, 2h *vs* 5h, $p<0.05$ for all comparisons).

The presence of host cues also induced an increase in the locomotory activity of nymphs having a poorer nutritional condition (nymphs starved for 30 and 60 days, Figure 4, Wilcoxon; $p=0.04$ for both comparisons). Nutritional status only induced an increase in locomotory activity in the presence of host cues (Figure 4, Kruskall Wallis, $p=0.01$). Nymphs starved for 21 days showed decreased locomotory activity in comparison to both 30 and 60 day-starved ones (Dunn's multiple comparison test, $p<0.05$ for both comparisons).

Discussion

Rhodnius prolixus is commonly found in palm tree[19-21], which present a profusion of complex structures as a consequence of the accumulation of dry leaves and palm fibers at their base offering shelters subject to small oscillations in both temperature and relative humidity[22]. These environments may grant extended resistance to starvation through helping bugs to avoid dehydration[23]. In addition, palm tree offer refuge to a vast diversity of vertebrate species[24], which in their turn can supply blood to shelter hidden bugs while resting on top of these structures. In the conditions evaluated in our study, nymphs hardly left their shelters spontaneously and starvation did not modulate the spontaneous tendency of bugs to activate and initiate foraging. Therefore, our results on spontaneous activity suggest that insects of this species wait for host cues inside resting places, probably avoiding exposure to predators.

It is important to highlight that in the present study hosts were suspended above the arena to avoid host-generated stimuli such as vibration or heat from reaching bugs. Therefore, we assume that in such a context insects could only respond to host-emitted odors (including CO_2) and, based on our results, we propose that there is a starvation threshold above which host-related chemosensory cues can induce *R. prolixus* to risk leaving shelters, even if the expected sensory signature of a host seems incomplete, i.e., no vibrations or heat are being detected. Further increased starvation (i.e., 60 days) was not able to promote increased responsiveness to host odors. It is possible to suggest that host odors are capable of activating sheltered insects of this species by breaking their akinesis.

It is known that triatomine activity can be drastically affected by nutritional status[12,14,15]. *T. infestans* nymphs starved for one week after ecdysis show a characteristic pattern of bimodal activity, that is, they leave their shelters mainly during the first night hours, and return at the end of the scotophase, but remain mostly immobile in that interval[15]. However, after six weeks of starvation nymphs become active during the whole scotophase[15]. Based on these facts and our results, we hypothesize that triatomine bugs use a nutritional resource-conservative locomotory strategy through which they rely on fasting resistance as a means of temporally extending their chances of obtaining a blood meal. Therefore, a sit-and-wait strategy seems to grant their low energy expenditure and maximize their chances of survival in ecotopes in which host presence can be poorly predictable.

A deeper understanding of triatomine sensory physiology and behavior may allow developing new control methods, which in view of insecticide resistance[25-29] problems are needed in diverse cases. This study clarifies a relevant aspect of the behavior of this important vector species by showing that it tends to remain hidden in the protection of shelters in the absence of reliable host cues. Furthermore, the effect of these cues, i.e., inducing bugs to come out of protected refuges, depends on poor nutritional status. In addition, we have shown that in such a physiological condition, host cues promote an intense locomotion on insects that left their shelters. The present study is the first to describe shelter use patterns by *R. prolixus* nymphs. Future studies are necessary to determine whether additional cues emitted by hosts, such as heat and vibration, can trigger the decision to come out from shelters.

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Figure 1. Experimental apparatus to characterize bug shelter-associated behavior and evaluate the effect of the presence of host odors. The figure depicts the arena, the camera and the cage with mice.

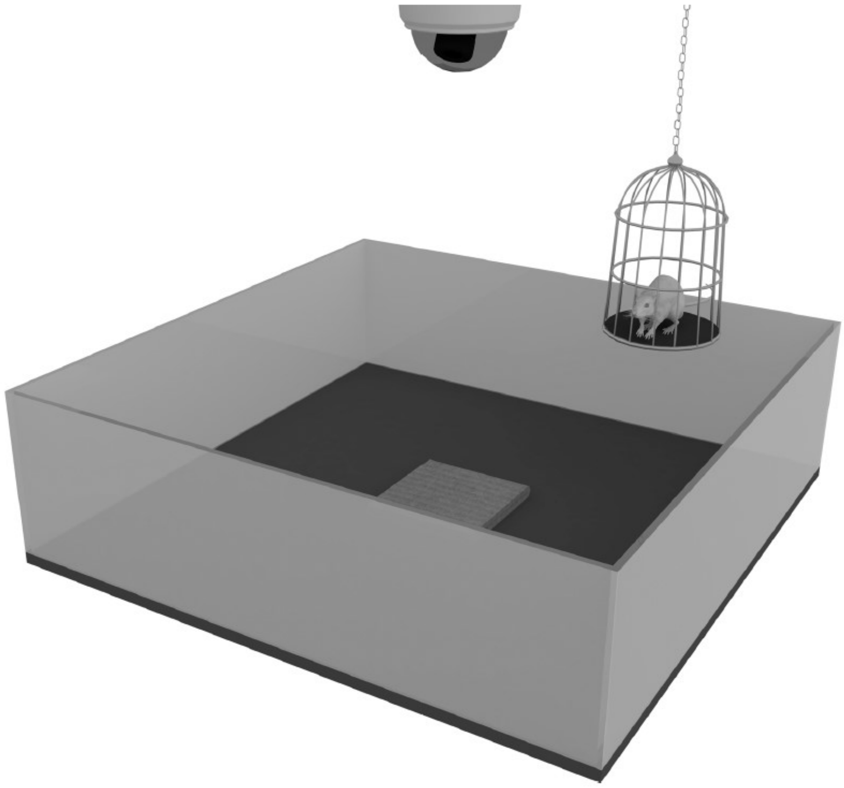

Figure 2. The effect of nutritional status and host cues on the number of *Rhodnius prolixus* nymphs coming out of shelters. Circles represent the number of insects coming out of the shelter along the recording interval of each assay. The horizontal line represents the median (n=6 replicates with 30 insects per condition/context).

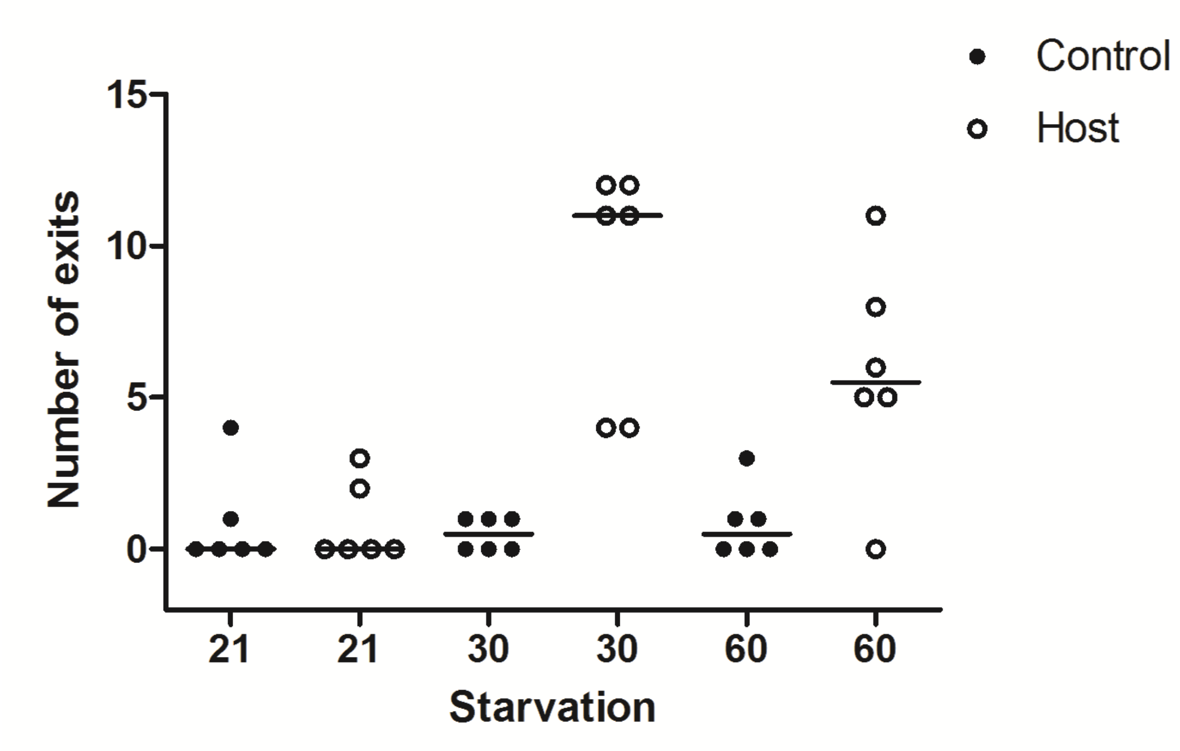

Figure 3. The effect of nutritional status and host cues on the percentage of nymphs coming out of shelters along the recording interval (n=6 replicates with 30 insects per condition/context).

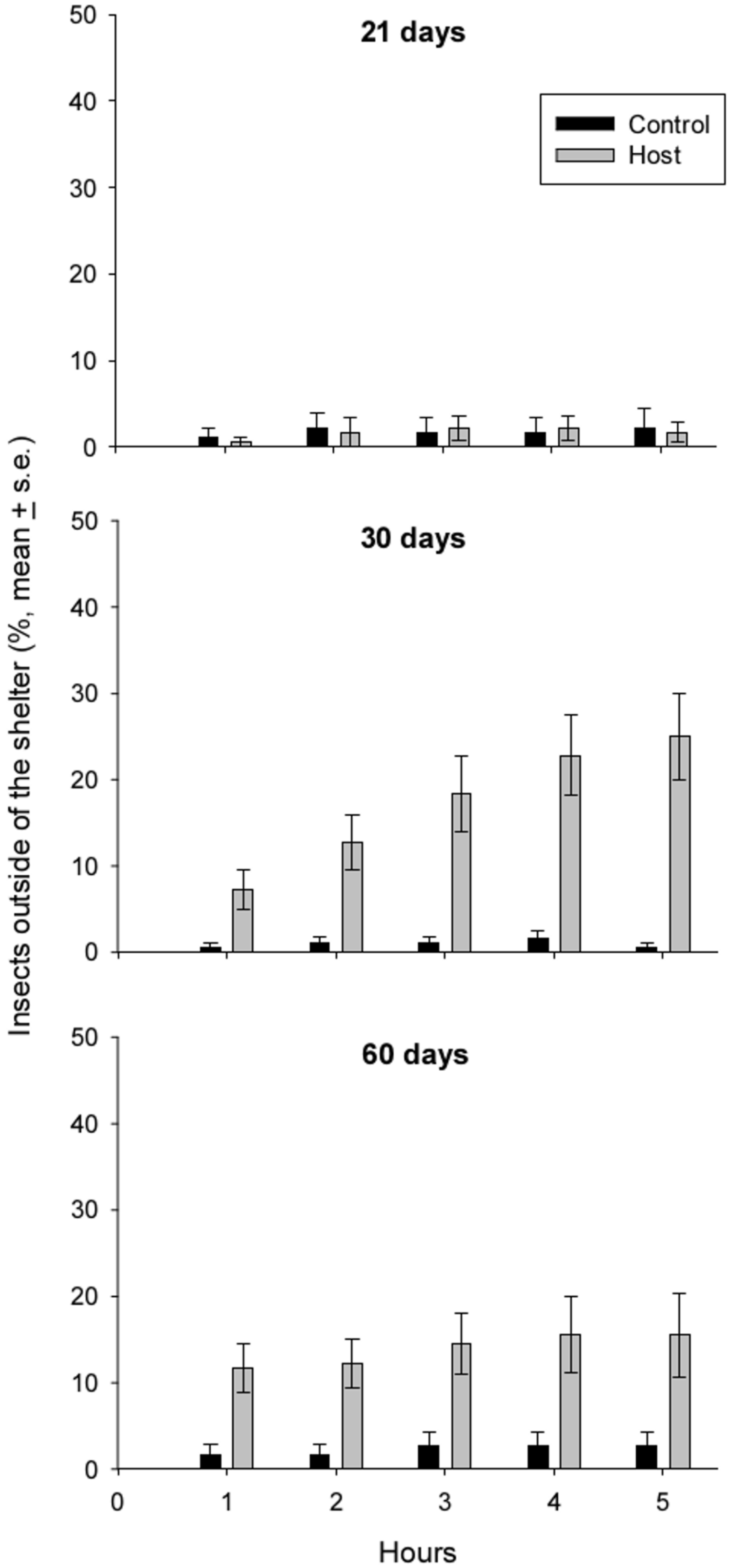

Figure 4. The effect of nutritional status and host cues on the intensity of locomotory activity of *Rhodnius prolixus* nymphs. Circles represent the total number of locomotion events recorded for each assay. The horizontal line represents the median (n=6 replicates with 30 insects per condition/context).