Activity pattern in relation to refuge exploitation and feeding in *Triatoma infestans* (Hemiptera: Reduviidae)

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Abstract

The pattern of locomotion activity was studied in *Triatoma infestans*, in relation to the use of an artificial refuge offered to the bugs in an experimental arena. In assays performed with insects that had a post-ecdysis starvation period of 1 week, the activity was low and mainly related to refuge leaving or entering. Insects that had gone through a longer period of starvation (6 weeks) exhibited a high locomotory activity throughout the night. Specific temporal windows were detected for refuge leaving and entering after dusk and before dawn, which were not modified by different levels of starvation of the insects. *T. infestans* exhibits a higher motivation for feeding during the first night hours, in comparison to that observed at the end of the scotophase. The activity peaks previously reported for *T. infestans* are therefore related to host and refuge search. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

The haematophagous bug *Triatoma infestans* (Klug) (Hemiptera, Reduviidae) is the main vector of Chagas disease, one of the most important sanitary problems in Latin America. The insects spend daylight hours assembled in shelters that offer a profuse contact with the substratum, for example, inside wall crevices and vegetal roofings in human dwellings (Zeledon and Rabinovich, 1981), and actively forage at night.

The activity of triatomines has been analysed by several authors (Wiesinger, 1956; Núñez, 1982; Settembrini, 1984; Lazzari, 1992). These reports refer to the locomotory activity of bugs registered in actographs, under discrete light/dark cycles and constant temperatures. In particular, a daily rhythm has been described with two endogenously controlled peaks of activity, at dusk and dawn, (Lazzari, 1992). Both peaks have been ascribed to host and refuge search, respectively (Lazzari, 1992), a hypothesis that needed to be tested under different conditions to those of actographs. The activity pattern of *T. infestans* in such devices could have been affected by experimental conditions, such as confinement, artificial light cycle and absence of resting places. Particularly, since in previous works neither a host nor a refuge were offered to the bugs, it was desirable to test the ´host-refuge´ hypothesis in the presence of refuges, food and natural illumination. In addition, the effect of starvation on the activity pattern of *T. infestans* was conspicuous, with the expression of the activity peaks being masked in starved animals (Núñez, 1982; Lazzari, 1992). Thus, it was necessary to determine how this could affect the activity related to refuge use (Lazzari, 1992).

In the present work we address the questions of whether host and refuge searching activities by *T. infestans* are confined to different temporal windows. We studied different parameters of the locomotory activity associated with refuge use, and how these are affected by the starvation level of the insects and whether the feeding activity of bugs varies during night hours.

2. Materials and methods

Experimental animals were fourth and fifth instar larvae of *T. infestans* from our laboratory colony, reared at 28°C and 50–70% RH. They were fed through a membrane on heparinised goat blood (Núñez and Lazzari, 1990).

2.1. Refuge activity experiment

In the two experimental series, animals with a post-ecdysis starvation period of 1 or 6 weeks were tested. The behaviour of 40 bugs was recorded for two replicates in both series.

Experiments were conducted in an arena of 80 x 100 cm. This consisted of a wooden floor surrounded with glass walls that prevented climbing by bugs. The
surface of the arena was covered with a sheet of paper that was changed after each assay. In the centre of the arena bugs were offered an artificial refuge consisting of a piece of corrugated cardboard of 10 × 20 cm, folded in the midline to offer a shelter of 10 × 10 cm with two accesses. Previous experiments showed that this kind of refuge recruits ca. 90% of the bugs present in an experimental arena in 1-night assays. Room temperature was kept at 24 ± 2°C. The arena was placed at about 1 m from a large window in order to expose the animals to a natural illumination cycle.

The behaviour of the insects was recorded by means of a video camera. The image in the screen showed the refuge in the centre, and its surroundings, ca. 10 cm to each side, representing an area nine times that of the refuge. This area was considered large enough to permit a reliable assessment of the activity of the insects. During the night-time, a dim red light (25 W) located 90 cm above provided the minimum homogeneous illumination needed for recording. This was set, after preliminary assays, at about 14 lux measured on the surface of the arena.

Insects were released near to the centre of the arena, and given 3 days to habituate to their environment. The recording started at the 3rd day, approximately 1 h before dusk, and finished at least 3 h after dawn. The recording period of approximately 16 h was established from preliminary assays.

Video records were analysed for the following parameters: (a) spontaneous locomotion activity; (b) the number of insects going out from refuges; and (c) the number of insects coming into refuges. All parameter values were determined for every recorded hour, in order to quantify the variation in activity with time. Spontaneous activity was measured by tracing two perpendicular lines forming a cross centred in the middle of the screen, and counting each time the animals crossed any of the lines.

Continuous records with a digital lux meter (TES 1330) during the complete dark phase showed no significant change in light intensity (± 0.5 lux) during the night, i.e. between what we here call dusk and dawn.

2.2. Analysis of feeding motivation

The insects used were fourth instar larvae of *T. infestans*. The assays corresponding to this experiment were developed as in the refuge activity experiment, except that the refuge was placed in one end of the arena. Room temperature was kept at 22 ± 2°C.

During the 3rd day an artificial feeder controlled by a timer was mounted at the opposite end of the arena. In the two series of assays the feeder was switched on 1 h after dusk for 3 h or during the last 3 h of the night. In both series blood was put in the feeder 7 h before it was switched on.

The number of bugs that ingested a meal was determined after each assay by direct counting.
3. Results

3.1. Refuge activity experiment

In both assays carried out with insects starved during 1 week after ecdysis, the locomotory activity of the bugs was low (Fig. 1). Although the insects showed little activity through the night, minor activity peaks occurred near dusk and dawn. The tendency of the insects to leave and enter the refuge was restricted to definite temporal windows (Fig. 2). The insects left the refuges mainly during the first night hours, whereas the main refuge entering activity was at the end of the dark phase. The latter took place preceding sunrise, before any detectable change in the illumination occurred. Comparison of the number of bugs leaving and entering the refuge (number of insects outgoing – number of insects incoming), rendered a positive value for the first half of the night (6 h) and a negative one for the last half.

In the two assays performed with insects starved for 6 weeks, the locomotory activity displayed by the bugs was higher than that observed in the first experimental series. Insects were active during the entire night, without any discernible peak (Fig. 1). Activity in the accesses to the refuge showed a pattern similar to that observed in the first series, but more intense. The bugs left the refuge in the first hours of the night, and then, mostly during the 4 h previous to dawn, the insects returned inside. Comparison between the number of insects going out and coming into the refuge showed a similar profile to the first series (Fig. 2).

![Fig. 1. Mean locomotory activity recorded from bugs that had gone through a short starvation (1 week, black bars) or a prolonged one (6 weeks, white bars). Depicted values represent the number of bugs that crossed the arbitrary axis per hour, and are the means from two assays for each starvation state. Arrows indicate the time for dusk and dawn, respectively.](image-url)
Fig. 2. Comparison of the number of bugs leaving the refuge and entering it per hour (number of insects going out – number of insects coming in). Depicted values represent means from two assays for each starvation state. Black bars represent the insects starved for 1 week, white bars represent the insects that had gone through a prolonged starvation (6 weeks). Arrows: as in Fig. 1.

3.2. Analysis of feeding behaviour

A greater proportion of fed bugs was found in those experiments in which the feeder was turned on 1 h after dusk. In this series, 23 ± 6.4% (mean ± S.E.) of the insects were found to be engorged, whereas in assays corresponding to the second series, in which the feeder offered blood during the last 3 night h, only 9.5 ± 1% of the insects engorged (t-test for independence on transformed data, $P = 0.043$, df = 4).

4. Discussion

The experimental design allowed conditions of illumination, refuge availability and spatial dimensions different to those of actographs (Lazzari, 1992). The results showed that the insects left their refuges mainly during the first night hours, and returned at the end of the scotophase. In between, those bugs which had previously left the refuge would remain outside. The activity related to shelters, i.e. leaving or entering movements, was displayed during the same temporal windows in both experimental groups, independent of the starvation level, although the overall number of individuals leaving or entering the refuges was higher for starved insects.

When compared with activity records obtained with actographs (Lazzari, 1992), our arena experiments did not reveal a clearly defined bimodal pattern, restricted to
dusk and dawn. It is worth mentioning that the bimodal activity pattern recorded in actographs vanishes with increasing starvation (Lazzari, 1992). In both experimental series presented here, spontaneous activity was observed during the whole night. In the first series, there were slight increases in locomotory activity after dusk and before dawn, which was not observed in the second series in which the insects walked steadily during the scotophase. As in the experiments performed with actographs, prolonged starvation could have masked the expression of defined peaks of locomotory activity. However, for the same starvation, the dynamics of refuge exploitation revealed that during the night two different activities are split in temporal windows, i.e. leaving and entering the refuges, during the first and second night halves, respectively (Fig. 2). This pattern could not be demonstrated by the measure of spontaneous locomotion activity using actographs, but is consistent with the hypothesis raised by Lazzari (1992) about the role of the activity peaks observed in actographs at the same temporal windows.

The second peak of activity described by Lazzari (1992) occurred when light was turned on. In our experiments, however, the activity related to refuge entering occurred mostly during those dark hours preceding dawn, even though no change in light intensity was detectable by us. Avoiding exposure during daylight hours would seem important for insects that associate to organisms that are ambiguously hosts and potential predators.

The results obtained in the feeding experiment suggest that bugs exhibit a variable motivation for food search during night hours. Bugs were reluctant to feed close to dawn, even though food was available and, as shown in the refuge activity experiment and also in previous work by other authors (Lazzari, 1992), T. infestans display a marked activity at this moment. If these results are considered together with activity assays in relation to refuges, we can conclude that these insects show different activity peaks related to temporal windows at which motivation for host and refuge searching are split. This means that the endogenous bimodal pattern of circadian activity exhibited by T. infestans would have a component that corresponds to food search at the beginning of the night, and another one preceding dawn, fundamentally directed to the search of a refuge in which to find protection during daylight hours.

Preliminary assays performed using a guinea-pig instead of an artificial feeder, showed the same tendency of bugs to feed during the first half of the night (Lorenzo, unpublished) even though the food offer was not restrained to different time intervals as in the feeder experiments, i.e. the host remained at the arena during the whole assay. However, the variable level of activity, as well as irritability, displayed by hosts, restricts the reproducibility of these assays. Here, these problems were avoided by means of the artificial feeder which allowed a better control of the experimental conditions.

The results reported here are also consistent with previous reports on the assembling signal present in the dry faeces of these insects (Schofield and Patterson, 1977; Lorenzo Figueiras and Lazzari, 1993; Lorenzo Figueiras et al., 1994). The aggregation-inducing substance is effective on fed bugs only when insects ingested a blood meal at least 8 h before, but not for shorter periods of time (Lorenzo
This interval is similar to the one elapsed between the leaving and entering activity shown here. Although not yet tested, it seems reasonable to assume that food may be obtained rapidly after the insect leaves the refuge, but then, it would be useful for the bug to remain motionless for the longest time possible after blood ingestion, taking into account the poor movement capacity of engorged bugs and their requirement for diuresis. For about 5 h after ingesting a blood meal, triatomines eliminate ca. 40–50% of the ingested fluid as water excess (Wigglesworth, 1931). As a consequence, a great amount of urine containing nitrogenous derivatives would be eliminated outside the resting places. Afterwards, *T. infestans* would become active and look for any cue orienting them to a refuge, with the faeces acting as a chemical marker of the accesses to the shelter (Lorenzo and Lazzari, 1996).

Our results add new data to the knowledge of the circadian organisation of triatomiine activity. The available information reveals the existence of temporal windows, in which bugs may be more exposed to risk. In fact: (1) ecdysis and egg hatching occur mostly at dawn (Ampleford and Steel, 1982; Lazzari, 1991), newly-emerged insects being more vulnerable; (2) bugs feed at night, when their cuticle is more permeable to certain insecticides (Fontán and Zerba, 1992); (3) bugs remain outside refuges most of the night (this study); (4) they display their maximal activity at dusk and dawn, reaching the highest metabolic rate and, consequently, increasing their sensitivity to xenobiotics; and (5) females oviposit mostly at dusk (Constantinou, 1984). Control of these insects might be improved if available techniques could be adapted to exploit these periods of maximal sensitivity.

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