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Diel rhythmicity of prey-search activity and its predominance over starvation in the lady beetle, *Coccinella septempunctata bruckii*

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ABSTRACT. The locomotor activity of the lady beetle, *Coccinella septempunctata bruckii* Mulsant (Coleoptera: Coccinellidae), was recorded during food deprivation in LD 16:8 h and continuous light. Activity was relatively low immediately after the lady beetle was fed to satiation but increased from 4 to 24 h later. It thereafter gradually decreased to a low level when the food was withheld for several days. Over a period of 10 days of starvation the beetles showed a high level of activity throughout the photophase and a low level during the scotophase in LD 16:8 h. In continuous light this rhythmic pattern persisted, with a free-running period of *c.* 22 h, significantly shorter than 24 h. The results suggest that the endogenous circadian timing predominates over 'hunger' as a determinant of search activity. The lady beetle accepted and consumed aphid prey presented at night, but the number of prey consumed was significantly lower than in the light. This suggests that, during the dark, activity related to prey-searching is suppressed, though lady beetles are able to accept and consume prey even at night.

Key words. *Coccinella septempunctata bruckii*, lady beetle, prey-search behaviour, hunger, starvation, circadian rhythm.

Introduction

The behaviour of animals follows a pattern of rhythmicity controlled by a combination of endogenous and exogenous (environmental) components. In a coccinellid lady beetle, *Coccinella californica*, the locomotory activity related to prey-search is affected both by a circadian rhythm and by 'hunger', i.e. food-deprivation (Frazer & Gill, 1981). Although 'hunger', among the internal factors, is the most pervasive in causing a predator to search for prey (Curio, 1976), the manner in which nutritional factors interact with diel rhythmicity has been rarely studied. In tsetse flies, *Glossina morsitans*, locomotor activity increased with starvation and clear circadian rhythm emerged in the activity in continuous dark (Brady, 1972). The relationship, however, between food deprivation and circadian rhythmicity still remains unknown. In the present paper the interaction between endogenous and exogenous components of diel rhythmicity is studied in the prey-search behaviour of a predatory coccinellid beetle.

Preliminary observations showed that the lady beetle, *Coccinella septempunctata bruckii* Mulsant, actively searched for prey only during the

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light period. However, it was unclear whether search activity itself shows an endogenous rhythmicity, or how 'hunger' interacts with rhythmicity. Three experiments, therefore, were conducted to delineate (1) how starvation affects the search activity, (2) whether or not search activity has a diel rhythmicity, and (3) which dominates in regulating search activity.

Materials and Methods

Lady beetles, *Coccinella septempunctata bruckii*, were collected in the university farm of Nagoya University and reared on an excess of the green peach aphid, *Myzus persicae* (Sulzer) (sixty to ninety individuals/day) in a test tube (diameter 2 cm, length 22 cm) from egg to adult. *M.persicae* were reared by the method described by Nakamuta (1983).

The rooms for experiments and rearing insects were maintained at 25±1°C and a LD 16:8 h cycle (lights-on and lights-off are at 04.00 and 20.00 hours local time, respectively).

Experiment 1

Changes in locomotor activity were monitored using an activity arena and a photocell actograph.

Activity arena experiments. Changes in locomotor activity during deprivation of food for periods less than 24 h were recorded in an experimental arena composed of a cylindrical grey PVC circle (the arena's wall) resting on a transparent acrylic plate. The arena was circular (diameter 30 cm, height 5 cm), thereby eliminating the problem of the beetles coming to rest thigmotactically in corners. The base of the arena was divided into 145 2×2 cm grids and fifty-six marginal sections (Nakamuta, 1982) used to plot the location of the beetle under observation. Each beetle was introduced into the experimental arena at 0, 4, 8, 16, 20, 24 h after being fed to satiation. The age of adult beetles varied between 5 and 10 days post-emergence. Unless otherwise stated, satiation was achieved by presenting aphids to the beetle with forceps until they were consistently rejected. The beetle was then allowed to accustom itself to the experimental conditions for 2 min. Subsequently its position relative to the grid was noted every 5 min for 1 h from 09.00 to 16.00 hours. After each recording, the acrylic base was washed with 75% ethanol.

Photocell actograph experiments. Locomotor activity of a single lady beetle starved for more than 24 h was recorded in LD 16:8 h using a photocell actograph. A plexiglass (perspex) box (10×30×60 mm) covered with a plexiglass plate was used as an actograph chamber. A circular dish (diameter 10 mm, height 5 mm) containing moistened cottonwool was placed in the actograph to provide water. The chamber was placed between an infrared light source (Takenaka BL-236, wavelengths exceeding 800 nm) and a detecting photocell (Takenaka BR-236). The diameter of the light beam was *c.* 1 mm. The locomotor activity of the beetle was recorded by the output of the photocell being connected to an event recorder (Model R17H12(T), Fuji Electronic Co. Ltd). When a beetle moved actively, each pen deflection could hardly be distinguished. Hence the width of the clusters of pen deflections was measured to the nearest 0.1 mm and summed for 1 h. Since the recorder paper moved at 20 mm h^{-1}, the maximum width for 1 h was 20 mm, defined as 200 scores of locomotor activity. This score thus indicates the relative time spent moving. The actograph was maintained at 25±1°C. During the photophase the actograph was illuminated from above with a fluorescent tube (at 200–300 lux at the actograph).

Activity recording was started after the introduction of a satiated lady beetle, and was continued until its death. Under these food-deprived conditions, lady beetles survived for at least 6 days (range 6–19 days).

Experiment 2

To demonstrate endogenous rhythmicity, activity was monitored in LD 16:8 h and in continuous light (LL), using the photocell actograph described above. The beetles were reared in LD 16:8 h for 5–10 days after adult emergence and fed to satiation, after which they were introduced into the actograph individually. Recording started at midnight on the second day after the introduction and continued until death. Nineteen and eight beetles were used for monitoring the activity in LD 16:8 and LL, respectively. The light regime for eight LL beetles was switched from LD 16:8 h to LL at midnight on the third day of the experiment.

In order to detect periodic components in the activity pattern, the hourly scores were used to calculate correlograms as described by Chiba (1975). A prominent peak in such a plot indicates that the corresponding period value deviates more than others and is thus likely to be a rhythmic component. The periodicity of the activity of each individual was obtained by averaging the intervals of three prominent r_k peaks (see Fig. 6).

Experiment 3

To investigate whether starvation ('hunger') or rhythmicity is the most important modulator the search activity and the number of prey consumed during the light and dark phases were compared. Seventeen and eighteen beetles were placed individually in Petri dishes (diameter 9 cm, height 1.5 cm) and each was fed to satiation on aphids at 12.00 or 20.00 hours, respectively. The beetles were then deprived of food for the next 25 h, until 13.00 or 21.00 hours (1 h into the scotophase), respectively, on the day after they had fed to satiation. An apterous adult aphid was then touched to the beetle's mouthparts with forceps, using a 6 W red lamp during observation in the scotophase. When a beetle captured and consumed the aphid, it was presented with another, repeatedly, until it consistently rejected the prey.

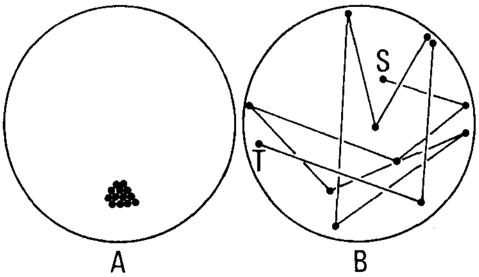

FIG. 1. Typical record of the activity of a *C.septempunctata bruckii* adult at 0 (A) and 24 h (B) after being fed to satiation in an activity arena. Minimum total distance moved is 0 and 113 2-cm units, respectively. S and T in (B) indicate the site of starting and terminating the record of the beetle's movement.

Results

Experiment 1

A record was made of the various positions of the animal during the course of the experiment (Fig. 1). The individual dots represented the positions of the lady beetle at each 5 min interval; thus a cluster of more than two points indicates that the lady beetle remained at a particular site for a period of at least 5 min. The lines are the shortest distances between successive observations of the beetle and do not

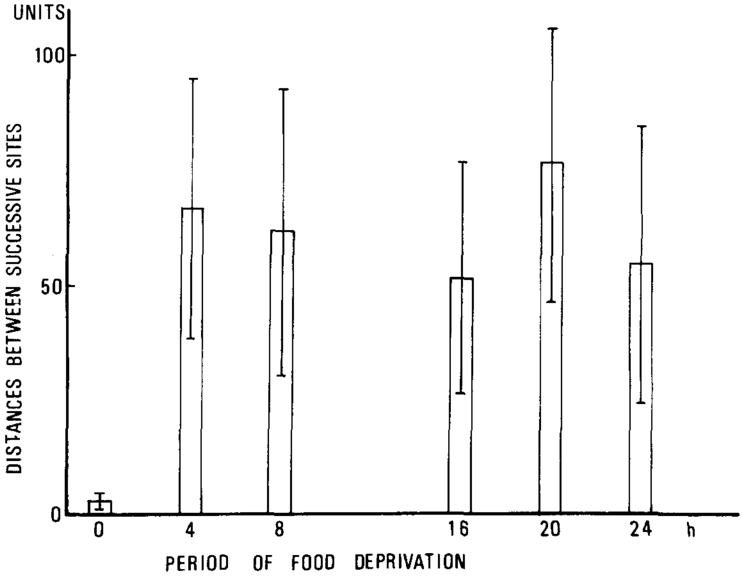

FIG. 2. Changes in distances moved between successive sites for 1 h with increasing duration of food deprivation. Mean ±SD distances moved for 1 h by ten males are shown for each period of food deprivation.

necessarily represent the animals' actual route. Immediately after satiation, the beetles became immobile and tended to remain at the site where they were introduced (Fig. 1A). After 24 h starvation, however, they changed position much more frequently (Fig. 1B).

For each record, the distances were summed in units of 2 cm corresponding to the dimensions of the grid. Fig. 2 shows the mean distance moved by ten males after various periods of food deprivation. Immediately after satiation movements were highly significantly shorter than after periods of food deprivation ($P<0.05$, Mann-Whitney *U*-test). Starvation therefore elevated locomotory activity, leading to an increase in search activity, which remained at a constant level after the first 4 h.

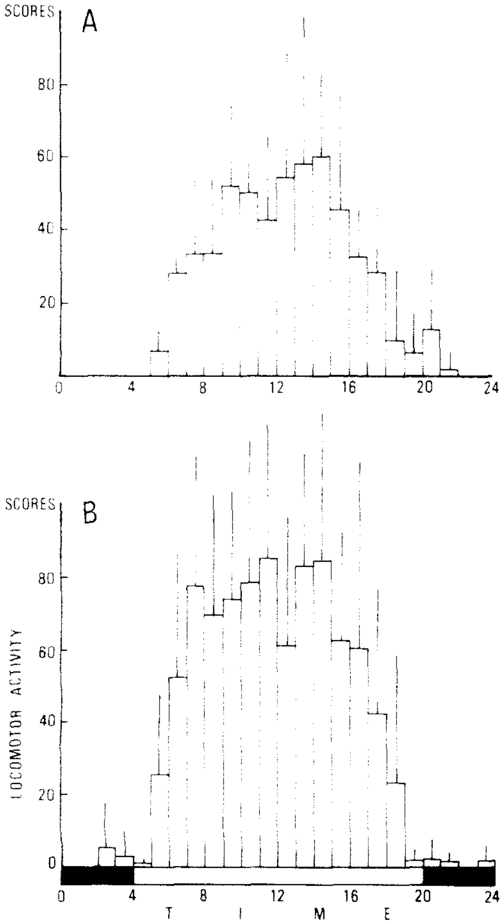

FIG. 3. Distribution of total daily locomotor activity of seven males (A) and nine females (B) on the second day of food deprivation in LD 16:8 h.

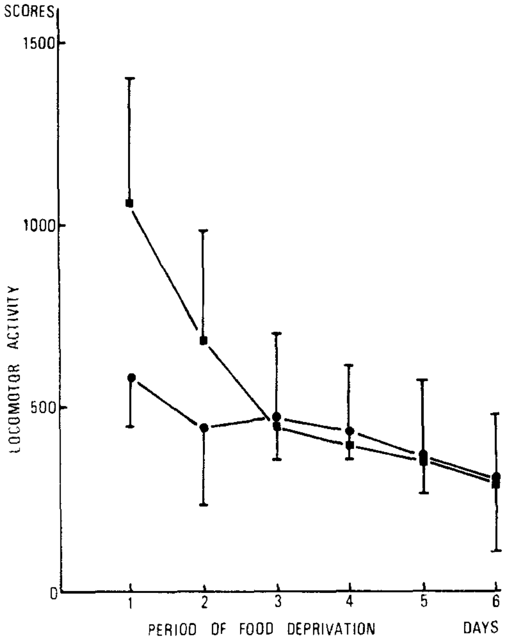

FIG. 4. Daily changes in locomotor activity of starved beetles. Circles and squares represent the mean activity of seven males and nine females, respectively. Vertical lines indicate one SD.

From the actograph experiments, a distinct diel change in activity was evident in both males and females maintained in LD 16:8 h, with high activity throughout the light period and a low activity during the dark (Fig. 3).

Fig. 4 shows the changes in activity during starvation, as measured by the actograph. On the first day of food deprivation, females showed high activity, becoming markedly less active by day 3 and then declining more gradually thereafter. The males' activity also declined, but not significantly so.

Experiment 2

Fig. 5 shows an example of the daily changes of activity in both LD 16:8 h and LL after entertainment to LD 16:8 h for 5–10 days from adult emergence. Most of the activity occurred during the light phase of LD 16:8 h, a tendency continuing to the day before the death of the beetle. Under LL, the time of activity gradually advanced as food deprivation was prolonged.

Fig. 6 is the correlogram for the animal shown in Fig. 5, with the highest peaks at 24, 49, 72 h in

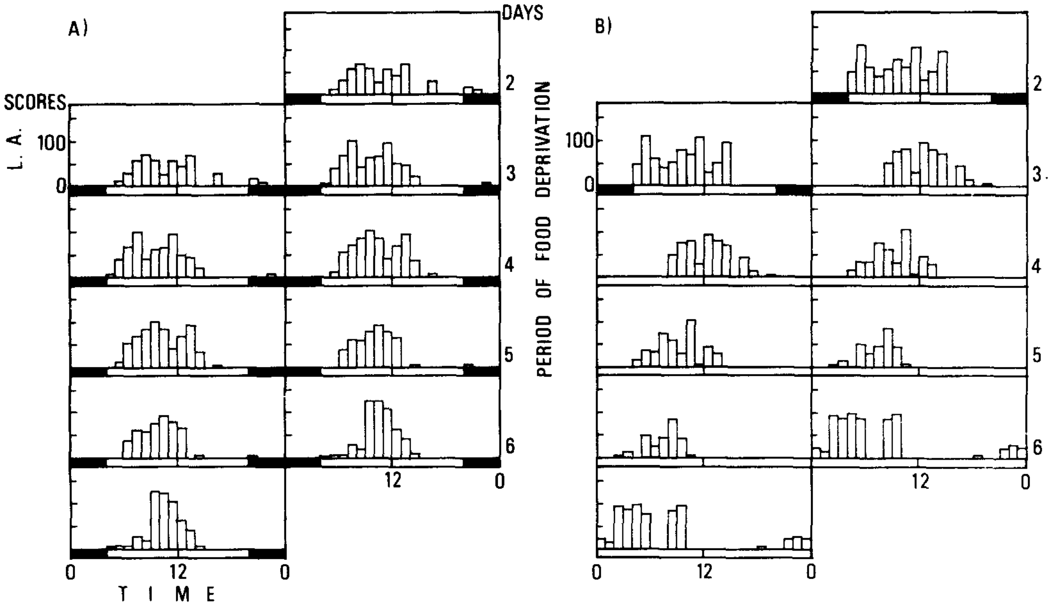

FIG. 5. Typical activity pattern of a lady beetle in LD 16:8 h (A) and in LL (B). Actograms are double-plotted. Constant light for LL beetles is begun at 00.00 hours of the third day of food deprivation.

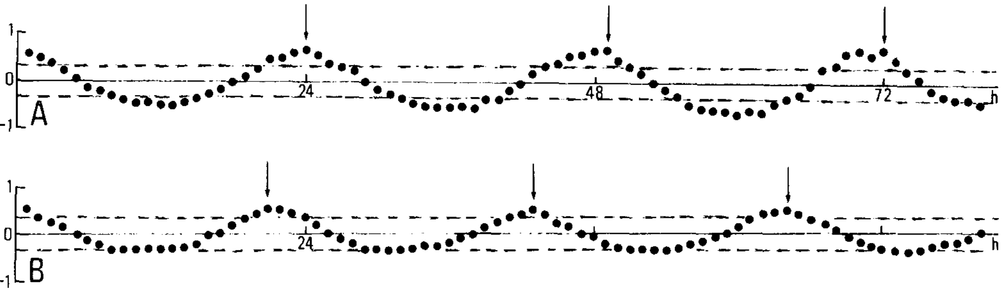

FIG. 6. Correlogram of the activity of the individual shown in Fig. 5 in LD 16:8 h (A) and LL (B). Arrows indicate the prominent peak of r_k or the circadian periodicity. Broken lines indicate the 5% significance of r_k.

LD 16:8 h and 21, 43, 64 h in LL. The mean correlogram analyses derived from the first 96 h of data in each condition shows a rhythmicity with a period of 23.9±1.0 h in LD 16:8 (n=19 beetles) and 21.7±1.3 h in LL (n=8) (Fig. 7). The rhythm persisted in continuous light for 4 or more days (10 days in some cases) with periods significantly shorter than 24 h ($P<0.01$, t-test).

Experiment 3

In the photophase, all of seventeen beetles accepted and consumed aphid prey, thereafter seeking and consuming repeatedly more than six prey (Fig. 8). In the dark, sixteen of eighteen beetles accepted and consumed aphid prey, and seven of the sixteen began to walk, searching for prey and consuming repeatedly more than four prey presented (Fig. 8). However, the number of prey consumed in the dark period was significantly lower than that in the light ($P<0.05$, Mann-Whitney U-test).

Discussion

Changes in locomotor activity during food deprivation have been observed in many insect species, which can be categorized into three classes, based on the changing pattern of activity: (i) activity increasing with the starvation period (*Glossina morsitans*: Brady, 1972; *Locusta migratoria*: Edney, 1937; *Phormia regina*: Barton-Browne & Evans, 1960; Green, 1964); (ii) activity increases several hours after the beginning of starvation, then decreases with continuing starvation (*Nauphoeta cinerea*: Ryenierse *et al.*, 1972); (iii) activity does not change with starvation (*Drosophila melanogaster*: Connoly, 1966). The changing pattern of activity in *Coccinella septempunctata bruckii* during starvation is similar to that of *N.cinerea*. Immediately after feeding to satiation the beetles' activity is low, but is then maintained at a high level from 4 to 24 h after satiation. Further deprivation then leads to a gradual decrease in activity to a low level, until death from starvation.

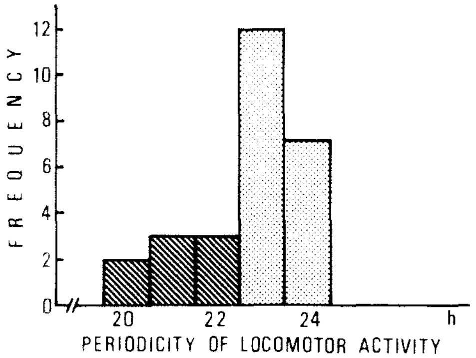

FIG. 7. Frequency distributions of circadian periodicity of locomotor activity of eight beetles in LL (cross-hatched) and nineteen beetles in LD 16:8 h (stippled). Periodicity is significantly different between LD 16:8 h and LL ($P<0.05$, Mann-Whitney *U*-test).

Similar patterns of activity correlating with the starvation period occur in other predators, e.g. in a mite, *Amblyseius largoensis* (Sandness & McMurty, 1972) and in a wolf spider, *Pardosa astrigera* (K. Tanaka, personal communication). Predators commonly face longer periods of starvation than do herbivores, since a predator's food is more discontinuous and its density fluctuates less predictably than that of herbivores. Since a lady beetle can survive for several days on its body reserves, decreased activity resulting in reduced energy expenditures is adaptive to the extent that this conserves available resources. The decline in search or locomotor activity with the passage of starvation therefore might be advantageous for a predator that waits for its prey to increase in abundance.

Locomotor activity has two components: distance covered per unit time (rate), and percentage of time spent moving. In *Musca dosmestica*, although the former was rather constant for 80 h of starvation and then declined with death, the percentage of time spent moving increased exponentially to a maximum at *c.* 90 h of starvation and then declined (Green, 1964). Similar results have been obtained in the fruit fly, *Drosophila melanogaster*: the percentage of time spent moving increased to a maximum at 3 to 12 h of starvation and then declined with further starvation (Bell *et al.*, 1985). From the present study, activity scores of lady beetles were highest during the first day of starvation and then decreased with continued food deprivation, suggesting that the rate of time spent moving was highest on the first day of starvation but then decreased with food deprivation, since this index indicates the relative time spent moving even though it is not an actual

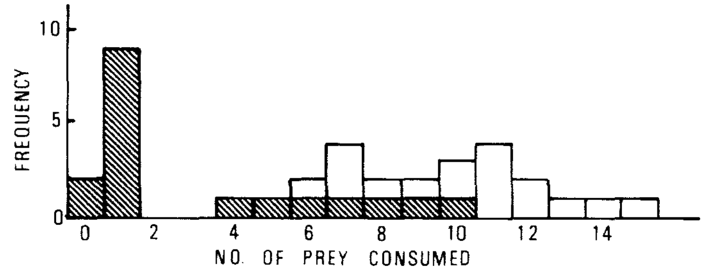

FIG. 8. Number of aphid prey consumed by the 24-h-starved lady beetles in the light (open region) and dark (cross-hatched region). Numbers are significantly different between light and dark ($P<0.05$, Mann-Whitney *U*-test).

percentage of time spent moving. This suggests that the beetles' behaviour differs from that of unfed flies (Green, 1964; Bell *et al.*, 1985). This might be due to the differences of food habit. As discussed above, the decline in search or locomotor activity with the passage of starvation might be advantageous for a predator that waits for its prey to increase in abundance.

In LD 16:8 h most of the beetles' activity occurred during the photophase. In LL the activity was likewise confined to the subjective 'day', until death. This persistence of the rhythm in LL clearly indicates that it is endogenous. The free-running period in LL is shorter than 24 h, thus conforming to 'Aschoff's rule' (Aschoff, 1960) for a diurnal animal.

Predaceous coccinellid beetles (*Hippodamia convergens, Coccinella transversoguttata* and *Coleomegilla maculata*) are captured in greatest abundance from 09.00 to 17.00 hours in the field (Mack & Smilowitz, 1979). *Harmonia axyridis*, another lady beetle, is active throughout the daytime and consumes about half the prey eaten per 24 h from 10.00 to 16.00 hours (Miura & Nishimura, 1980). The present results show that locomotor activity in *C.septempunctata bruckii* is likewise highest from 09.00 to 16.00 hours. This activity may therefore represent searching for prey, with the pattern determined by an endogenous circadian rhythm.

Curio (1976) suggested that the pattern of daily predatory activity is more or less adjustable to the opportunities to feed rather than being rigidly linked to an activity pattern in terms of 'diurnal', 'nocturnal' or 'crepuscular'. The activity of the lady beetle seems to be adjustable to feeding opportunities in the sense that the starved beetles will consume prey even at night when they are normally inactive. Circadian modulation of spontaneous activity still continued after prolonged periods of starvation, however, suggesting that even under extreme 'hunger' the activity pattern is determined by circadian control. This circadian timing therefore apparently largely overrides the effects of 'hunger' as a determinant of locomotor activity.

Lady beetles locate their prey visually from close range (Nakamuta, 1984, 1985). Even in complete darkness they can capture prey after contacting them, but in the dark the beetles frequently pass by aphids without finding them (Nakamuta, 1984). To optimize prey-finding and the conservation of food resources, it seems that search activity is then suppressed at night to avoid less successful hunting in the darkness.

When prey was presented to the lady beetle at night, most beetles consumed prey and many then began searching for prey. This suggests that although search activity is suppressed during the dark period, the beetle is able to search for and consume prey if prey are available. However, the number of aphids consumed by a 24-h-starved lady beetle was significantly smaller in the dark period than in the light period, suggesting that feeding threshold is lower by day than at night and it may change with circadian timing. In conclusion, starved lady beetles will consume readily available prey at night, even though they are normally inactive at this time, but circadian rhythmicity dominates over 'hunger' as a determinant of locomotor activity related to search behaviour.

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