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Daily locomotor activity of the parasitoid wasp *Meteorus pulchricornis* (Hymenoptera: Braconidae) that attacks exposed lepidopteran larvae

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Abstract The braconid parasitoid wasp that attacks exposed lepidopteran larvae, *Meteorus pulchricornis*, is a common natural enemy of pest caterpillars and may serve as a biological control agent. Recently spreading nighttime lighting for the control of noctuid moths may affect behavior of the parasitoid if it is nocturnal, but little is known about its daily activity patterns. This study investigated the locomotor activity of *M. pulchricornis* under various light conditions. Over a daily 16 h light and 8 h dim light cycle (16L8Dim), the wasps were inactive during most of the photophase (16L), gradually becoming active late in the photophase, with activity levels peaking just after the switch to the scotophase (8Dim). Subsequently, during the scotophase, activity decreased to a moderate level, and continued at that level until the switch to the photophase. In addition, after transfer from daily light/dim light cycles (16L8Dim), daily fluctuations in activity persisted for at least one day under continuous light or dim light conditions. This is the first report describing the nocturnal activity rhythm of parasitoid wasps and the suppression of their activity under strong light conditions.

Key words Circadian rhythm ・Nocturnal ・Crepuscular・Nighttime artificial lighting・Ichneumonoidea

Introduction

Many natural enemies of insects, including small parasitoids that attack eggs, motionless larvae, and pupae, exhibit diurnal peaks in their behavioral activity (Jervis et al. 2007). For instance, several chalcidoid wasps such as *Trichogramma brassicae* Bezdenko (Trichogrammatidae), an egg parasitoid of Lepidoptera (Pompanon et al. 1995, 1999), *Brachymeria intermedia* (Nees) (Chalcididae), a pupal parasitoid of the gypsy moth (Barbosa and Frongillo Jr. 1977), *Encarsia formosa* Gahan (Aphelinidae), a parasitoid of greenhouse whitefly (Ekbom 1982), and *Nasonia vitripennis* (Walker) (Pteromalidae), a pupal parasitoid of Diptera (Bertossa et al. 2010), are known to be active mainly during the photophase, whereas *Eretmocerus warrae* Naumann & Schmidt (Aphelinidae), a parasitoid of greenhouse whitefly, is active throughout the day (Hanan et al. 2009). Also in larval parasitoids, *Cotesia kariyai* (Watanabe) (Braconidae) is known to be diurnal, whereas it attacks nocturnal hosts hiding in folding leaves in the daytime (Sato et al. 1983).

However, many ichneumonoid parasitoids of Lepidoptera and Symphyta larvae are known to be captured in light traps (e.g., Gauld and Huddleston 1976; Short et al. 2006), suggesting that they may exhibit nocturnal or crepuscular activity. To understand host-parasitoid interactions in the field, it is necessary to know their daily activity patterns, although few laboratory studies investigated those of parasitoid wasps attacking exposed and free-living lepidopteran caterpillars.

In recent years, artificial lighting technologies have been developed for use in the control of pest insects (Johansen et al. 2011). The suppression of the nocturnal activity of adults using yellow or green lamps at night is an increasingly common method of preventing damage to vegetables and ornamental plants in Japan (see review in Shimoda and Honda 2013). However, nighttime artificial lighting may affect the behavior of the nocturnal natural enemies of pest insects. Thus, it is important to elucidate the daily activity of natural enemies and their response to and tolerance of nighttime light conditions.

Meteorus pulchricornis (Wesmael) (Braconidae) is a solitary endoparasitoid of the exposed larvae of various lepidopteran species (Fuester et al. 1993; Huddleston 1980; Maeto 1989), and is a common natural enemy of *Spodoptera, Helicoverpa, Mythimna* and other noctuid pests in East Asia (e.g., Liu and Li 2006; Takashino et al. 1998). According to Gauld and Huddleston (1976) and our personal observations, this and other congeneric wasps are often captured in light traps, and are thus suspected to be nocturnal; however, the daily activity patterns of these species have not yet been examined.

In order to elucidate the daily activity pattern of adult *M. pulchricornis*, we recorded and analyzed the locomotor activity of wasps under constant light or dim light conditions, as well as under a daily light/dim light cycle in the laboratory.

Materials and Methods

Insects

In this experiment, we used a thelytokous strain of *M. pulchricornis* originating from *Spodoptera litura* (Fabricius) larvae that were collected from soybean fields in Kagawa Prefecture, Japan, in 2001 (Nguyen et al.

2005). The strain is light in body color and apomictic ("KAGAWA_01_U" of Abe et al. 2013 and Tsutsui et al. 2014), and has been successively maintained on *S. litura* larvae reared on an artificial diet (Insecta LFS, Nosan, Kanagawa). Adult wasps were reared on 50% honey solution under a 16L8D photoperiod (16 h light and 8 h complete darkness) at 15°C. After being permitted to oviposit on *S. litura* larvae for three days under the aforementioned photoperiod at 20°C, female wasps 14 to 17 days old were used to observe locomotor activity.

Photoperiod conditions

The 14–17 day old female wasps were transferred to a photoperiod of 16L8Dim (a 16 h light photophase, followed by a 8 h dim light scotophase to imitate full moon conditions) at 20°C. After about two days under the 16L8Dim cycle, the wasps' locomotor activity was video-recorded for 192 hours (eight cycles) from the beginning of the scotophase (Dim).

To investigate the presence of an endogenous circadian system, we also observed the locomotor activity of wasps under LL (constant light) and DimDim (constant dim light) conditions at 20°C. After three cycles of 16L8Dim, the photoperiod was switched to LL or DimDim, and video recording continued for 144 hours.

Experimental devices

Within a plastic experimental booth (Fig. 1, A, 70cm × 45cm × 45cm) covered with a blackout curtain, one of three photoperiod conditions was established: 16L8Dim, LL, or DimDim. The light condition (L, ca. 4.0 W m^{-2}) was created using two fluorescent lamps (FL20SS・W/18, Panasonic, Osaka), while the dim light condition (Dim, ca. 0.0003 W m^{-2}) was created using a dimmable bulb-type fluorescent lamp (EFD15EL/14/E17/C, Panasonic, Osaka). To reduce the light intensity, the bulb was covered laterally with aluminum foil, attached to three dimmer filters (ND400 PROFESSIONAL, PRO ND2, PRO ND4 (W), Kenko Tokina, Tokyo), and was controlled with a dimmer (NE-740, NOA Enterprises, Shizuoka, Japan). For the 16L8Dim photoperiod, we lighted the dimmable lamp always and the two fluorescent lamps during the 16 h photophase.

We placed a piece of white Kent paper (17 cm × 8 cm), as an experimental arena, on which six black circles 35 mm in diameter with a black centerline were drawn (Fig. 1, F). A reversed transparent plastic plate (35mm diameter × 10mm height, 1000-035, AGC TECHNO GLASS, Chiba) was placed on each circle. One wasp was placed within each plastic plate with honey solution in a pierced 0.2ml PCR tube and three host feces arranged across the centerline (Fig. 2).

The locomotor activity of the wasps was simultaneously recorded using two video cameras (HDR-SR12 and HDR-SR1, Sony, Tokyo) connected to an HDD recorder (RD-S600, TOSHIBA, Tokyo). The HDR-SR12 was set for recording during the photophase (L), and HDR-SR1 was set in Night Shot mode, with radiating infrared rays, for recording during the scotophase (Dim).

Measurement of locomotor activity and data analyses

While reviewing the recorded video, the locomotor activity was quantified by counting the number of times per hour the wasp touched the centerline.

To elucidate the daily pattern of locomotor activity under 16L8Dim, we used the generalized estimating equation (GEE), which was proposed to model correlated repeated-measures data (Zeger and Liang 1986). Recently it has been used for analyzing daily patterns of biological phenomena (e.g., Nota et al. 2014; Tupper et al. 2013). Using GEE, we tested the effect of time (24 hours) on the locomotor activity (number of times that a wasp touched the centerline within one hour, in counts per hour). Thus, locomotor activity was designated to be the responsible variable, with a Poisson distribution and Log link function. We considered the wasps (8 individuals) to be a subject variable and time (24 hours) and days (8 days) to be within-subject variables. An independent working correlation matrix was specified since it exhibited the smallest QIC model fit index. Then, we compared the locomotor activity at every time point with the total average using a sequential Bonferroni correction.

To demonstrate the effects of constant light on the level of locomotor activity, we used the paired sample *t*-test (two-tailed) to compare the overall average hourly locomotor activity between the 72-h period of 16L8Dim (three cycles) and the 120-h period of LL or DimDim, excluding the first 24 h after transfer from the 16L8Dim conditions. We also observed the temporal changes in mean locomotor activity that occurred after transfer to LL or DimDim.

Statistical analyses were performed using IBM SPSS Statistics Version 19 for Windows.

Results

Daily pattern of locomotor activity under 16L8Dim

Under the 16L8Dim photoperiod condition, wasps exhibited a daily periodic pattern of locomotor activity (Fig. 3), which was significantly affected by time (GEE, *Wald Chi-square* = 1680.4, $df = 7$, $p < 0.001$). On average, the activity peaked at the beginning of the scotophase (Dim), remained at a moderate level during the scotophase, decreased after the scotophase, and remained low until the middle of the photophase (L), before increasing gradually in the last third of the photophase (Fig. 4).

Persistence of daily locomotor rhythm under the constant light (LL) or dim light (DimDim) conditions

As shown in Fig. 5, the locomotor activity tended to decline after transfer from 16L8Dim to the constant light (LL) condition. The total average of the hourly locomotor activity was significantly different between the 16L8Dim (50.03 ± 5.00, ± SE) and LL (6.63 ± 1.65) treatments ($n = 6$, $t = -11.525$, $df = 5$, $p < 0.001$). In contrast, as shown in Fig. 6, the wasps maintained the same level of locomotor activity after transfer to the constant dim light (DimDim) condition. The total average of the hourly locomotor activity was not significantly different between the 16L8Dim (37.50 ± 5.99) and DimDim (45.86 ± 13.59) treatments ($n = 8$, $t = 0.809$, $df = 7$, $p = 0.445$). Daily fluctuations in average activity persisted for one day under LL conditions and for two days under DimDim conditions after transfer from the 16L8Dim treatment (Figs. 5, 6). Under LL conditions, all six individuals exhibited the first peak of activity around 16 h after transfer from 16L8Dim and two individuals exhibited the second ambiguous peak around 24 h after the first one (Online Resource 1). Under DimDim conditions, all eight individuals exhibited the first peak of activity around 24 h after transfer from 16L8Dim and the second ambiguous peak in 16－32 h after the first one (Online Resource 2).

Discussion

The diurnal activity of parasitoid wasps that attack the eggs, motionless or hidden larvae, and pupae of insects is well known (Barbosa and Frongillo Jr. 1977; Bertossa et al. 2010; Ekbom 1982; Pompanon et al. 1995; Sato et al. 1983); however, our study is the first to describe the nocturnal and partly crepuscular activity of the parasitoids of exposed lepidopteran larvae in the laboratory. The results of our experiment support previous field observations of the nocturnal activity of ichneumonoid parasitoids that display “ophionoid facies” (e.g., Short et al. 2006). The ”ophionoid facies” was first proposed by Gauld and Huddleston (1976), who pointed out that many ichneumonoid wasps collected in light traps (thus presumably nocturnal) were common in having a brown-yellow body coloration, long antennae and large ocelli. The name came from an ichneumonid genus *Ophion*, but this suite of morphological characters associated with nighttime collection has evolved many times independently in the family Braconidae and Ichneumonidae (Quicke 2015).

Female *M. pulchricornis* wasps, having the typical “ophionoid facies”, exhibited a predominantly nocturnal activity pattern, with a peak in activity at the beginning of the scotophase in the daily 16L8Dim cycle. Their locomotor activity, however, did not simply depend on light intensity, but increased anticipatorily during the late photophase, suggesting the presence of a circadian system to control behavior, as has been observed in many other arthropods (e.g., Chiesa et al. 2010; Helfrich-Förster 2001). This may be supported by the fact that daily fluctuations in activity persisted for at least one day under continuous light or dim light conditions after transfer from daily light/dim light cycles. However, our results only indicate (1) persisting oscillations in constant conditions with a period of ～24 h, one of three requirements of the circadian rhythm (e.g., Izumo et al. 2003); further investigations on (2) entrainment to environmental cycles and on (3) temperature compensation are necessary to test the control of locomotor activity by the circadian system. Such before-dark anticipatory increases in locomotor activity may ensure crepuscular foraging during the twilight hours at dusk.

Gauld (1987) has suggested three possible advantages of nocturnal foraging of parasitoid wasps: (1) escape from diurnal predators, (2) access to nocturnal hosts, and (3) avoidance of high temperatures and drying in sunlight. Predation of small wasps, like *Meteorus*, by hunting spiders is probably common enough to serve as a selective force for evolution of mimicry in parasitoids (Malcicka et al. 2015), but many wolf spiders are nocturnal rather than diurnal according to Suter (2014); thus their forces on daily activity pattern of prey parasitoids seem limited, if any. The second hypothesis is more likely supported by the fact that larvae of *Mythimna separate* (Walker), a common host of *M. pulchricornis*, are nocturnal and they conceal themselves in folding leaves in the daytime (Sato et al. 1983).

Adults of *M. pulchricornis* need visual cues from moving larvae for oviposition (Yamamoto at al. 2009) and thus they could not attack the concealed larvae in the daytime. However, other common host species, *Spodoptera exigua* (Hübner) and *S. litura*, are nocturnal only in middle or late larval instars (Griswold and Trumble 1985; Parasuraman and Jayaraj 1983). Therefore, host's nocturnality should be a potent but not exclusive factor for the evolution of nocturnal activity of this species. The third factor should be also considered. Adult body temperature of *M. pulchricornis* went up to 35°C within several minutes in a sunny day in May when ambient air temperature was about 25°C (Abe et al. 2013). Inactivity in the daytime seems reasonable to avoid heating up in the strong sunshine, although despite this many chalcid wasps are known to be diurnal (see Introduction). Our present knowledge is still poor to understand the ecological significance of the daily activity patterns of parasitoid wasps.

Our study implies the possibility of the combined use of nighttime artificial lighting and nocturnal biocontrol agents. When nighttime lighting is used to control nocturnal moths, the leaves of crops are irradiated with a yellow or green light of 1–50 lux in night (Kosaka and Yase 2003; Yamada et al. 2006); such lighting might to some extent affect the activity of *M. pulchricornis*, which was markedly reduced by exposure to constant nighttime light conditions in our experiments. Instead, nighttime lighting might be able to promote oviposition by this species, as the process uses visual cues (Yamamoto et al. 2009), but further experiments under 1–50 lux light are necessary to confirm that. The effects of nighttime lighting on the foraging and oviposition behavior of nocturnal parasitoids should be elucidated in order to improve IPM systems of protecting vegetable crops.

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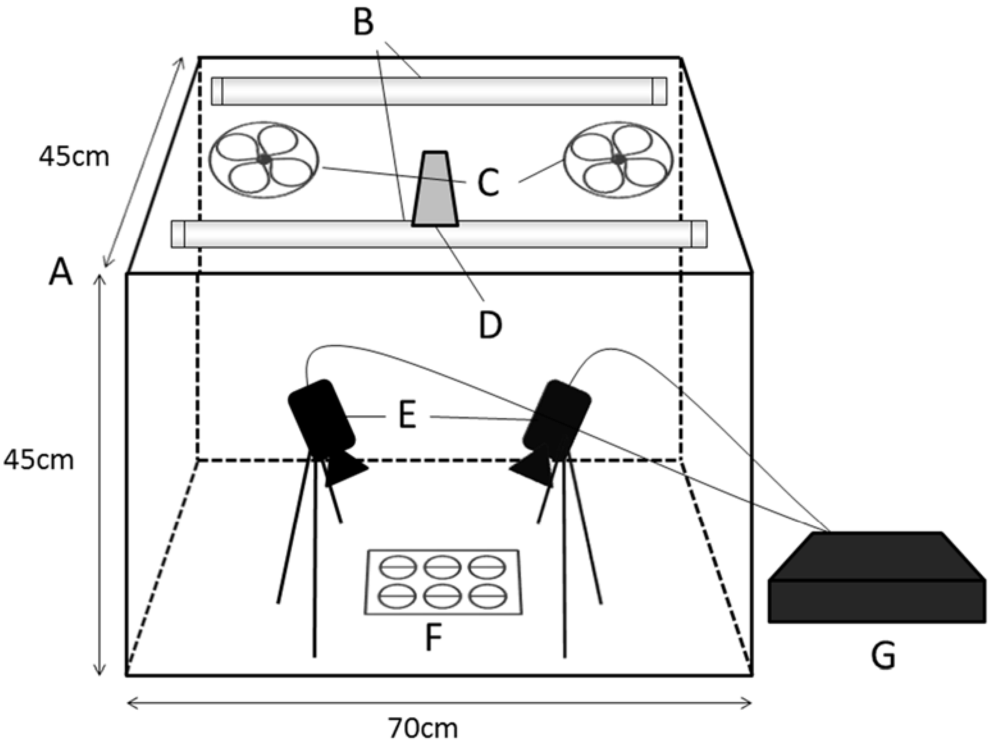

Fig. 1 Diagram of experimental devices. **a** Experimental booth. **b** Fluorescent lamps for photophase. **c** Air infiltration fan. **d** Dimmable fluorescent lamp for scotophase. **e** Video cameras. **f** Experimental arena. **g** HDD recorder.

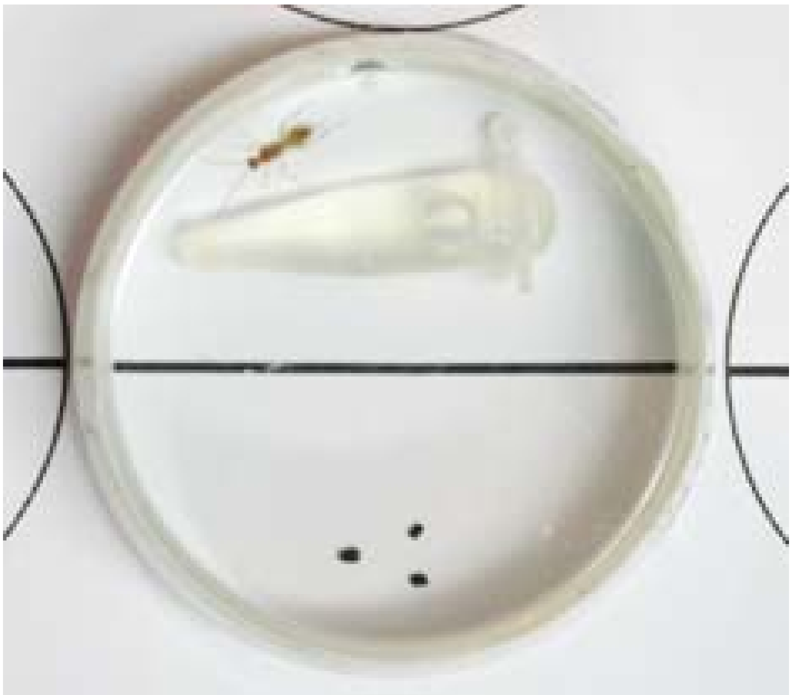

Fig. 2 A parasitoid wasp, the PCR tube containing the honey solution, and three host feces within a reversed plastic plate placed on the paper with a black centerline.

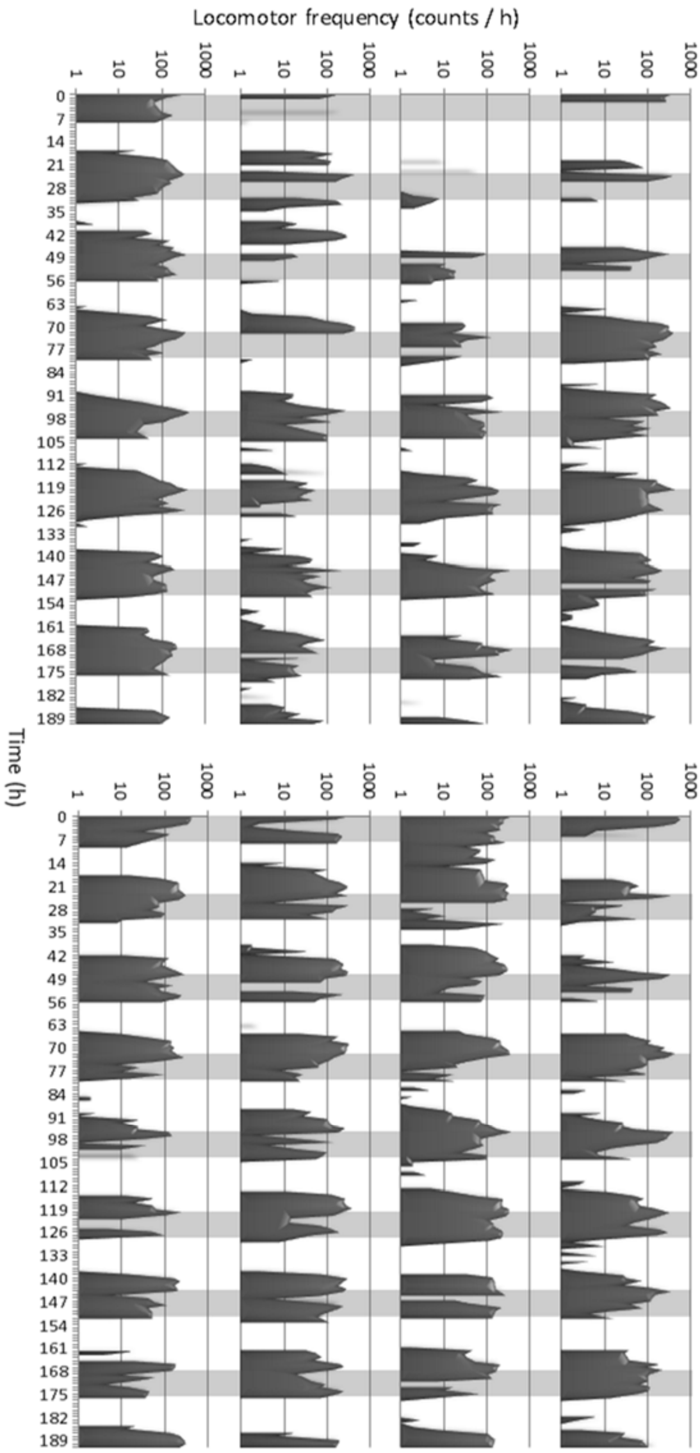

Fig. 3 The locomotor activity of eight female wasps under 16L8Dim. The gray background represents the scotophase (Dim), and the white background represents the photophase (L).

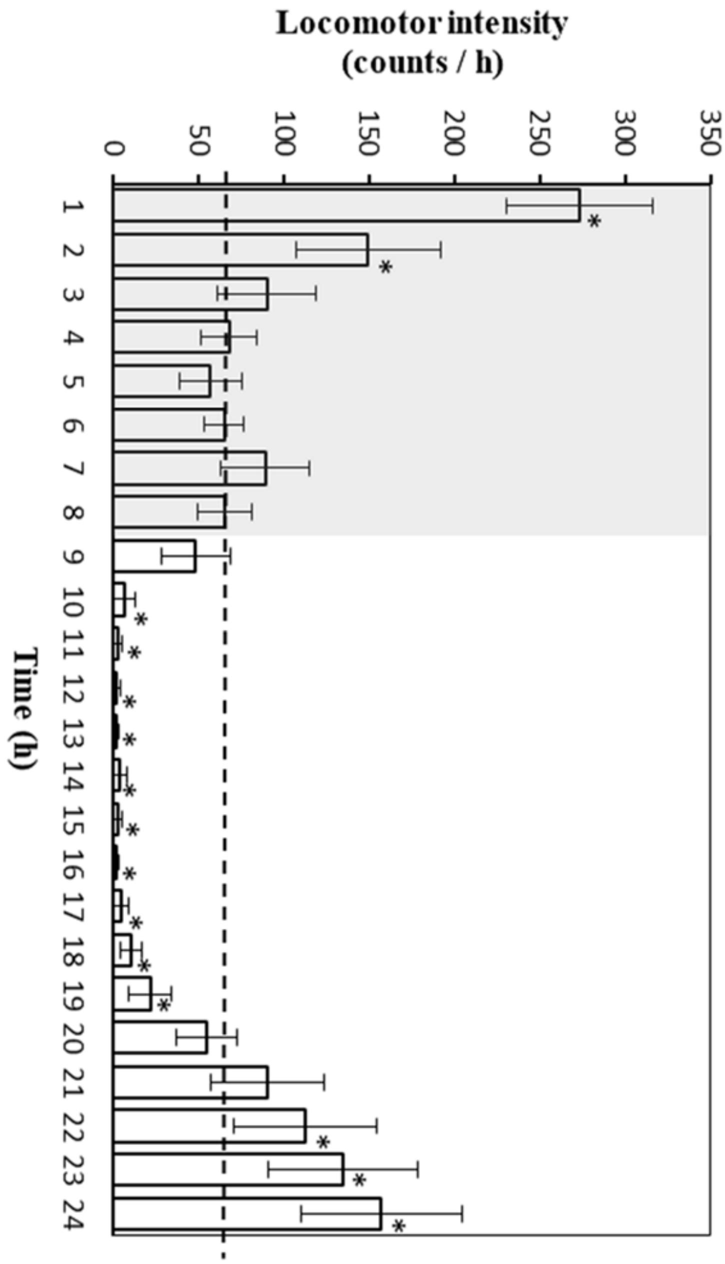

Fig. 4 Estimated mean locomotor activity (± 95% confidence intervals) of the eight female wasps under 16L8Dim. The gray background represents the scotophase (Dim), and the white background represents the photophase (L). The dashed horizontal line indicates the level of the total average (62.9 counts / h), from which the means marked with an asterisk (*) are significantly different after sequential Bonferroni correction ($p < 0.05$).

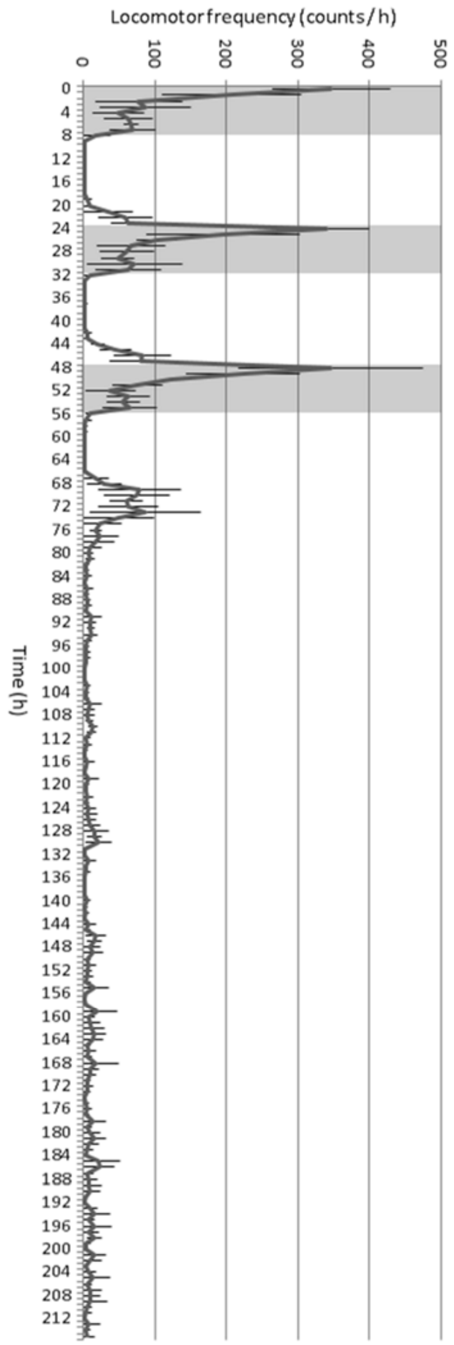

Fig. 5 The mean locomotor activity (± SD) of the six female wasps transferred from 16L8Dim to LL. The gray background represents the scotophase (Dim), and the white background represents the photophase (L).

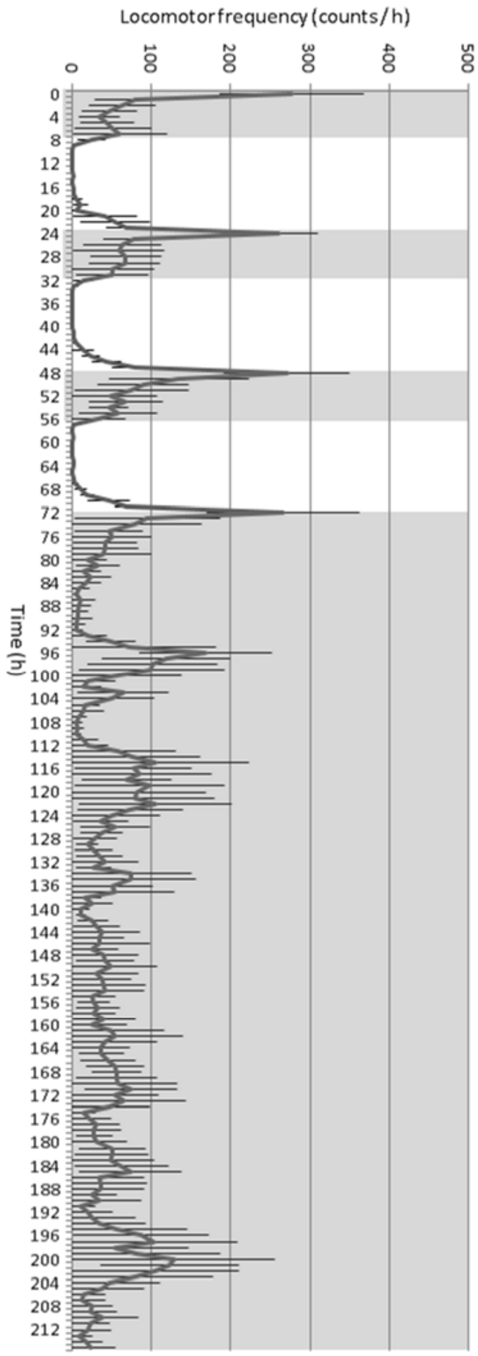

Fig. 6 The mean locomotor activity (± SD) of the eight female wasps transferred from 16L8Dim to DimDim. The gray background represents the scotophase (Dim), and the white background represents the photophase (L).

Online Resource 1. Locomotor activity of *M. pulchricornis* wasps transferred from 16L8Dim to LL.

"Daily locomotor activity of the parasitoid wasp *Meteorus pulchricornis* (Hymenoptera: Braconidae) that attacks exposed lepidopteran larvae" on Applied Entomology and Zoology by Nishimura T. et al.

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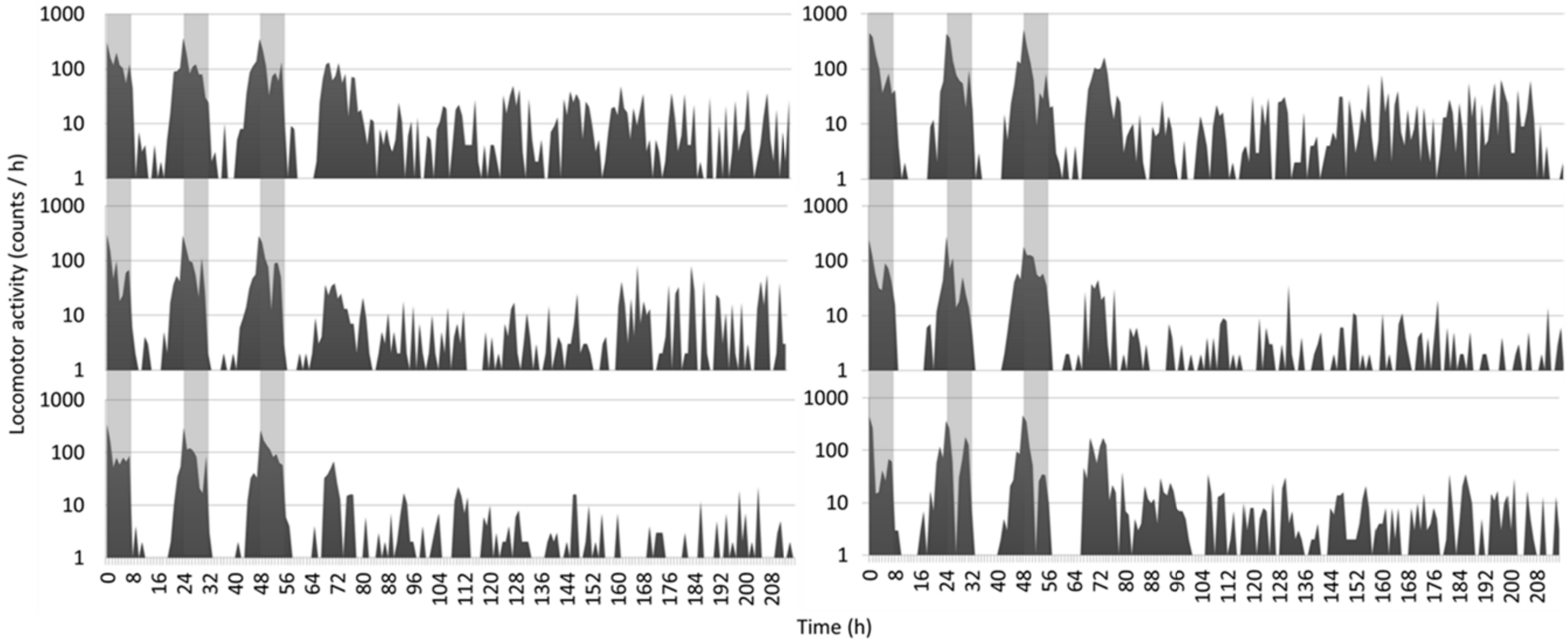

Online Resource 2. Locomotor activity of *M. pulchricornis* wasps transferred from 16L8Dim to DimDim.

"Daily locomotor activity of the parasitoid wasp *Meteorus pulchricornis* (Hymenoptera: Braconidae) that attacks exposed lepidopteran larvae" on Applied Entomology and Zoology by Nishimura T. et al.

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