

ORIGINAL ARTICLE

Effect of temperature on circadian rhythm controlling the crepuscular activity of the burying beetle *Nicrophorus quadripunctatus* Kraatz (Coleoptera: Silphidae)

Tomoyosi NISIMURA¹, Hideharu NUMATA¹ and Eiji YOSHIOKA²

¹Department of Biology and Geosciences, Graduate School of Science, Osaka City University, Osaka and ²Kobe Yamate University, Kobe, Japan

Abstract

Locomotor activity rhythm was examined at various temperatures under a 16 h light : 8 h dark photoperiod (LD 16:8) or LD 12:12 using adults of the burying beetle *Nicrophorus quadripunctatus*. At 20°C, the locomotor activity of the beetles showed a bimodal daily pattern with two peaks around lights on and lights off under both photoperiods. This bimodal activity rhythm persisted under constant darkness; therefore, the activity of adult *N. quadripunctatus* is controlled by a circadian clock. Adults showed a bimodal activity pattern for temperatures ranging from 15 to 25°C. The evening peak of the daily activity was earlier at lower temperatures. These findings suggest that in the field, *N. quadripunctatus* shows crepuscular activity, and is active earlier in the afternoon in cooler seasons. In this species, therefore, temperature appears to play an important role in the determination of daily activity patterns.

Key words: bimodal daily rhythm, circadian clock, locomotor activity, photoperiod, thermoperiod.

INTRODUCTION

Burying beetles (*Nicrophorus*) use carcasses of small vertebrates for reproduction. A single carcass is often found by several beetles, but it is eventually monopolized by a single male–female pair. The pair conceals the carcass and treats it for consumption by the pair's young (Pukowski 1933; Wilson & Fudge 1984; Scott 1998). Carcasses are nutritionally valuable and their availability is unpredictable, therefore are exploited by various organisms (Tallamy & Wood 1986; Scott 1998). Competition for carcasses is often intense among many organisms, including *Nicrophorus* species. Investigation of silphid fauna has revealed that differences in habitat use and in seasonal activity can be attributed to resource partitioning by sympatric species (Anderson 1982;

Wilson *et al.* 1984; Katakura & Ueno 1985; Ohkawara *et al.* 1998). These studies have examined the daily activity patterns of *Nicrophorus* species within a community to understand intraspecific competition. The determination of daily activity patterns of *Nicrophorus* was based on the number of trapped beetles and the number of females that were attracted to males emitting pheromones in the field (Wilson *et al.* 1984; Müller & Eggert 1987; Ohkawara *et al.* 1998). The mechanism controlling the daily activity of these organisms has not been determined but it is either exogenous (a direct response to the environment) or endogenous (an underlying circadian clock). Endogenous circadian rhythms of behavior have been recorded in the laboratory for many insects (Saunders 2002).

In males of the moth *Antheraea pernyi* (Guérin-Ménéville), temperatures in the pupal stage had an effect on the daily pattern of flight activity that is controlled by an endogenous circadian clock (Truman 1973). Exposure to low temperature advances the onset of the activity, which allows the males to better adjust to seasonal change in temperature. In the burying beetles, it

Correspondence: Hideharu Numata, Department of Biology and Geosciences, Graduate School of Science, Osaka City University, Osaka, 558-8585 Japan.
Email: numata@sci.osaka-cu.ac.jp

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is known that sympatric *Nicrophorus* species in the field show different patterns of daily activity, and intraspecific competition for carcasses depends on temperature (Wilson *et al.* 1984). In the laboratory, however, it has not been examined whether temperature affects the timing of activity in *Nicrophorus* species.

In the present study, we observed the daily activity of *N. quadripunctatus* under various controlled conditions in the laboratory to determine if this species' daily activity is controlled by an endogenous or exogenous mechanism.

MATERIALS AND METHODS

Insects

Adults of *N. quadripunctatus* were collected by baited traps in Kyoto City (35°01'N, 135°44'E) in September 1996 and May 1997, and their progeny were used for the experiments. The progeny were reared under a short-day photoperiod of 12 h light : 12 h dark (LD 12:12) or a long-day photoperiod of LD 16:8 at $20 \pm 1^\circ\text{C}$. Adults were kept as single male–female pairs and were provided with five third instar larvae of the blowfly *Protophormia terraenovae* (Robineau-Desvoidy) as food every 3 days (see Nisimura *et al.* 2002 for details).

Activity recording

Adults of the beetles were individually housed in plastic boxes (68 × 39 × 15 mm). An infrared beam (EE SPW-321; Omron, Kyoto, Japan) was passed across the chamber and the number of interruptions of the beam was recorded at 6 min intervals on a personal computer (Yoshioka 1992). During recording, water was provided through a cord of cotton from outside the chamber, although no food was given. Ten recording chambers were set on a plate in an incubator (MIR-153; Sanyo, Osaka, Japan) equipped with a 15 W fluorescent lamp (FL15W-B; Hitachi, Tokyo, Japan), and the light intensity in the chamber was 1.4 W m^{-2} . Lighting schedules were controlled by the computer for activity recording. When adults were kept under a square-wave type thermoperiod, the computer changed the temperature without disturbance of the activity recording. We used adults that had emerged 12 or more days earlier to record locomotor activity rhythms for 3–9 days. The hourly amount of activity was calculated as the mean activity for each hour in a day using all activity data except for the days on which the recording began and finished. The

rhythmicity under constant darkness (DD) was analyzed by autocorrelogram, and statistical significance of each peak value was evaluated by a test of correlation coefficient ($P < 0.05$).

RESULTS

The activity of adults raised at 20°C under constant LD 12:12 or LD 16:8 was recorded from 12 days after emergence without changing conditions. Under both conditions, adults showed a bimodal pattern with two peaks around lights on and lights off, and phases of relative inactivity in the middle of the photophase and scotophase (Fig. 1). The highest activity was observed immediately after lights off under LD 12:12 and lights on under LD 16:8, although the activity increased from a few hours before lights off and lights on. There was no marked difference in activity patterns between males and females.

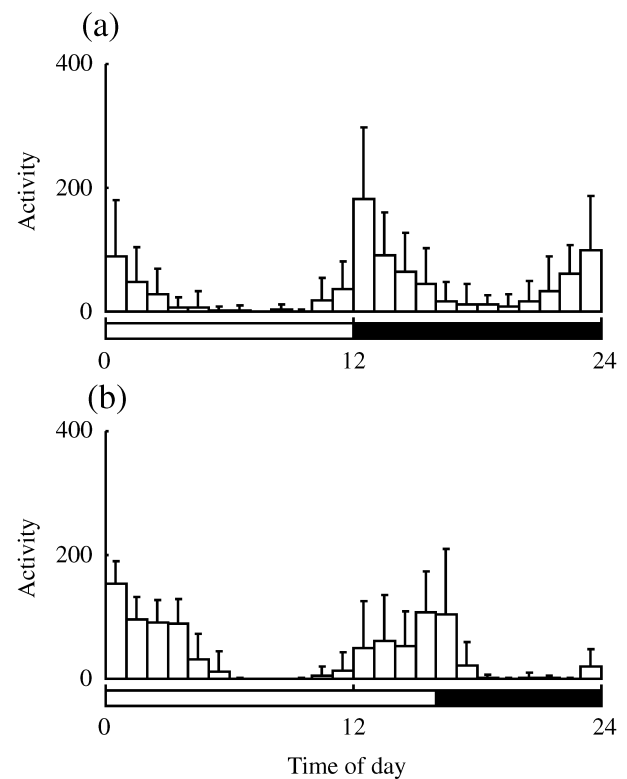


Figure 1 The hourly patterns of average activity in adults examined for 3 days in *Nicrophorus quadripunctatus*. The beetles were reared from eggs under (a) 12 h light : 12 h dark (LD 12:12; $n = 10$) or (b) LD 16:8 ($n = 6$) at 20°C , and locomotor activity was recorded from 12 days after adult emergence. □, Photophase; ■, scotophase. Error bars show SD.

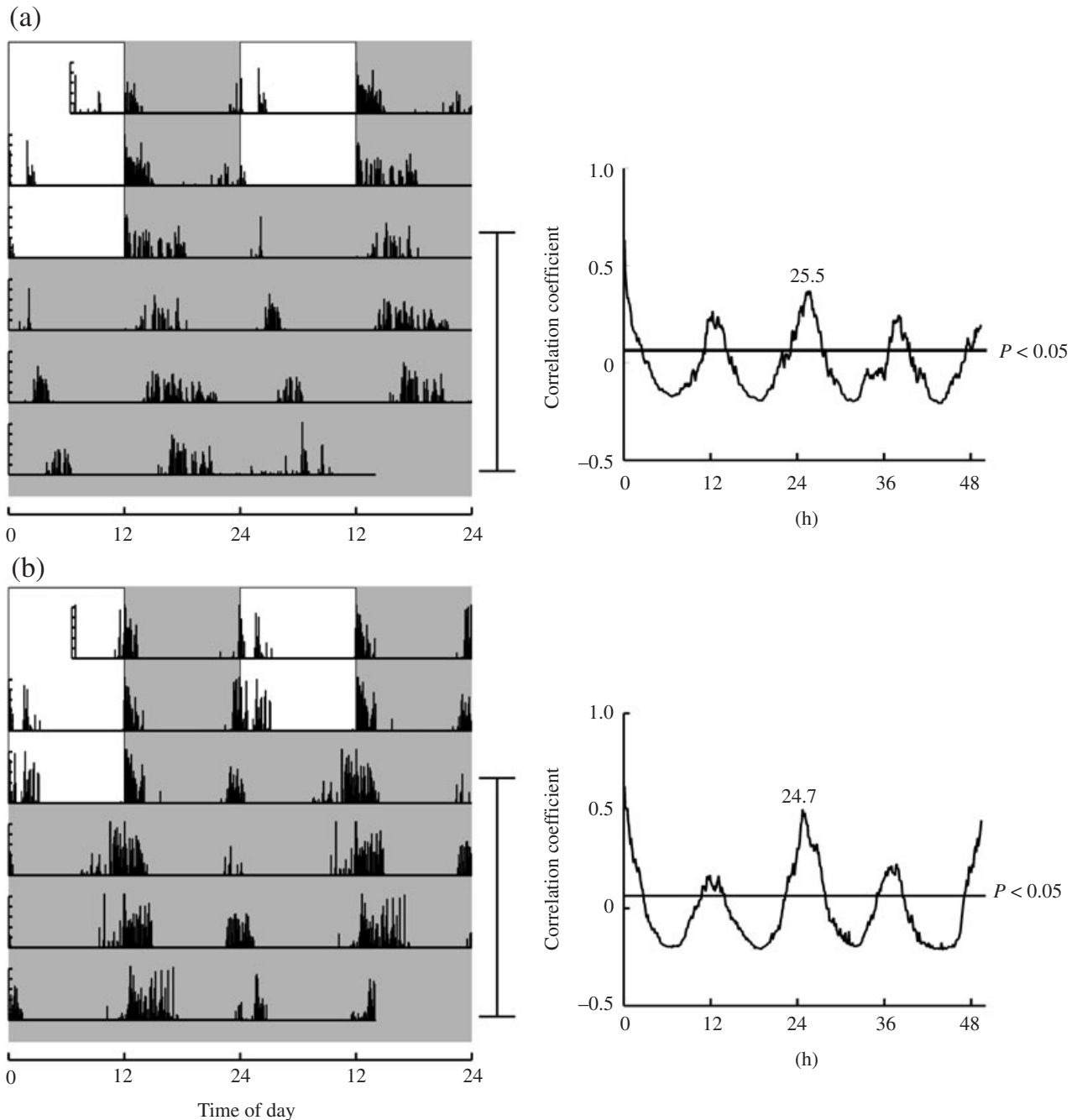


Figure 2 Two representative records (a,b) of the locomotor activity rhythm (left) under 12 h light : 12 h dark (LD 12:12) and subsequent constant darkness at 20°C, and the autocorrelogram under constant darkness (right) in adults of *Nicrophorus quadripunctatus*. Beetles were reared from eggs at 20°C under LD 12:12, and locomotor activity was recorded under LD 12:12 for 3 days and under constant darkness for the subsequent 4 days at 20°C. Shaded areas indicate the scotophase.

Twenty adults were initially kept under LD 12:12 at 20°C for 2 or 3 days and subsequently under DD for 4 or 7 days. In most of the adults, the locomotor activity was entrained to the photoperiod and free-run with a

period somewhat different from 24 h. Figure 2 shows two typical examples. Therefore, the bimodal activity was controlled by a circadian clock. The average of the free-run period was 24.6 ± 0.7 h (mean \pm SD, $n = 18$).

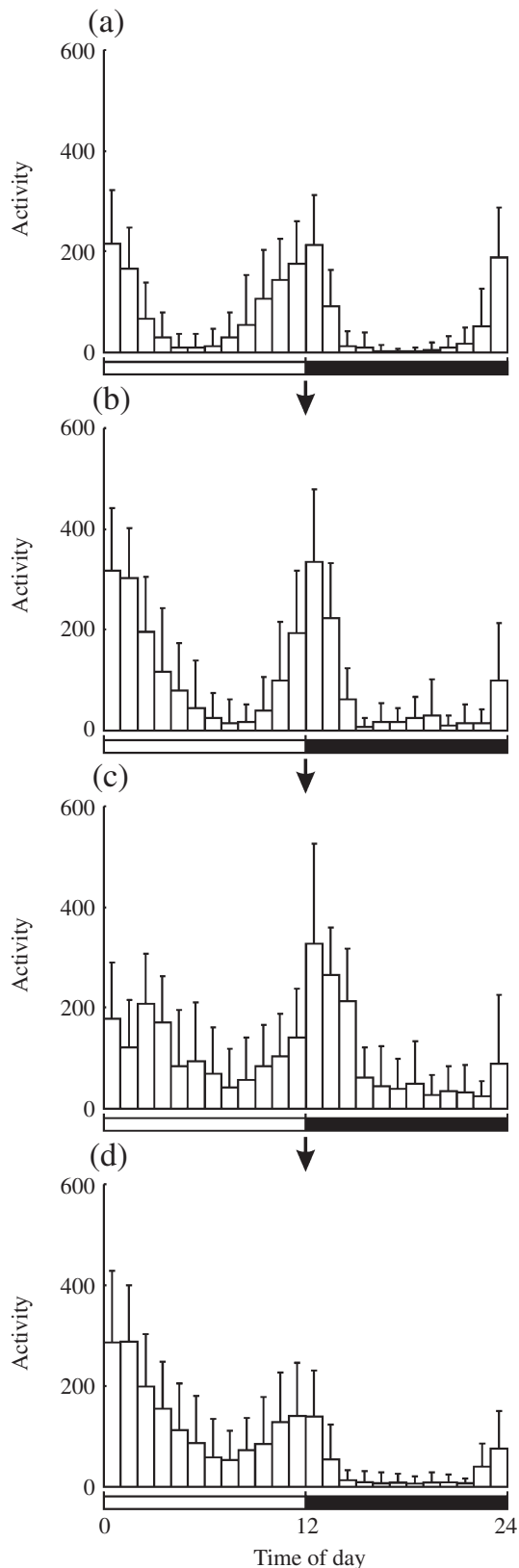


Figure 3 The hourly patterns of average activity in adults examined under 12 h light : 12 h dark (LD 12:12) at a high temperature and thermoperiods in *Nicrophorus quadripunctatus*. Beetles were reared from eggs at 20°C under LD 12:12. Locomotor activity was recorded at constant 20°C for 4 days (a; $n = 8$), at constant 25°C for 3 days (b; $n = 8$), under a thermoperiod in which high temperature coincided with darkness for 3 days (c; $n = 7$), and under a thermoperiod in which high temperature coincided with light for 4 days (d; $n = 6$). In the thermoperiods, temperature was changed between 20°C (lights on) and 25°C (lights off). □, Photophase; ■, scotophase. Error bars show SD.

Next, the effect of temperature on the activity rhythm was examined. The locomotor activity of each adult was recorded under LD 12:12 at constant 20°C, constant 25°C, and under two square-type thermoperiods, in which the temperature was changed between 20 and 25°C at lights on and lights off. Adults 36 days after emergence were used for these recordings. After a recording for 3 or 4 days under a certain temperature condition, adults were kept for 3 days at 20°C and fed five third instar fly larvae before subsequent recording. Adults showed a clear bimodal activity pattern at 20°C (Fig. 3a), as younger adults also showed (Fig. 1). At 25°C, adults also showed a bimodal activity pattern that had two peaks around lights on and lights off (Fig. 3b). The total amount of activity per day at 25°C was not significantly different from that at 20°C ($P > 0.05$, Steel-Dwass test). Under the thermoperiod in which the thermophase coincided with the scotophase, adults showed a similar bimodal activity pattern with two peaks around lights on and lights off (Fig. 3c). There was no significant difference in the total amount of activity per day between the thermoperiod and a constant 25°C ($P > 0.05$, Steel-Dwass test). Under the thermoperiod in which the thermophase coincided with the photophase, the activity in the photophase was similar to that under the thermoperiod with a different combination of temperature and light, although the total activity in the scotophase was significantly lower than under the latter conditions ($P < 0.05$, Steel-Dwass test). Nevertheless, the activity was still bimodal (Fig. 3d).

Activity was next recorded at lower temperatures. The locomotor activity of each adult was recorded under LD 12:12 at constant 15, 10 and 12.5°C. Adults 63 days after emergence were used for these recordings. After a recording for 3 or 4 days under a certain temperature condition, adults were kept for 3 days at 20°C and fed five third instar fly larvae. The total amount of activity per day was significantly less at 15°C than at

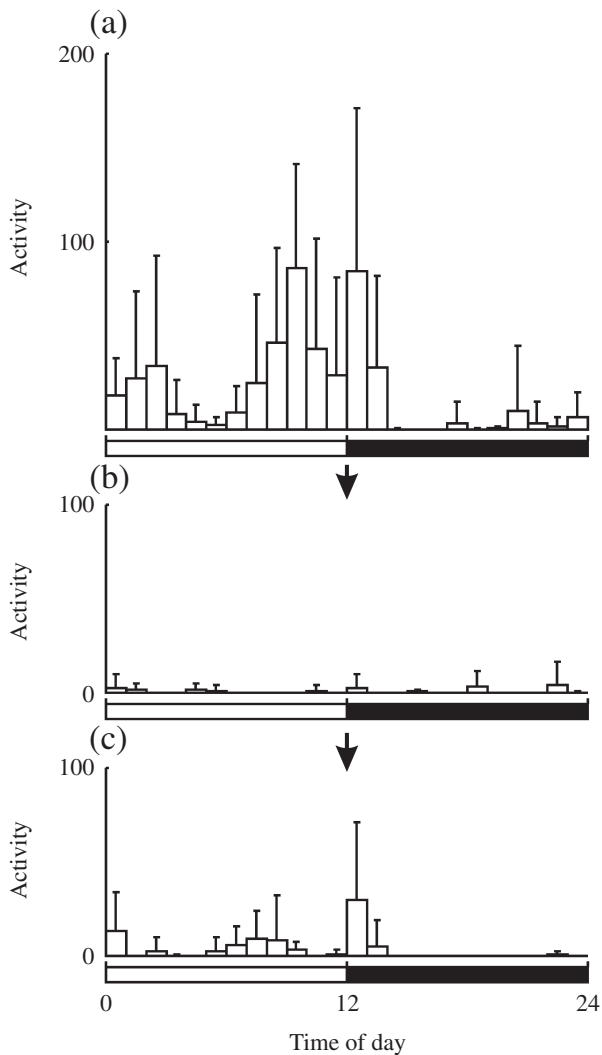


Figure 4 The hourly patterns of average activity at low temperatures in adults of *Nicrophorus quadripunctatus*. Beetles were reared from eggs at 20°C under 12 h light : 12 h dark (LD 12:12). Locomotor activities were examined under LD 12:12 at 15°C for 4 days (a; $n = 4$), 10°C for 3 days (b; $n = 4$), and 12.5°C for 4 days (c; $n = 4$). □, Photophase; ■, scotophase. Error bars show SD.

20°C ($P < 0.05$, Steel-Dwass test), although the activity of the adults was bimodal at 15°C (Fig. 4a). The peak around lights on was delayed but that around lights off advanced. The activity pattern was not clear at 10 and 12.5°C, probably because locomotion was suppressed by the low temperatures (Fig. 4b,c).

Figure 5 shows the morning and the evening peaks at various temperature conditions, based on the results shown in Figures 3 and 4. A peak in a day was defined

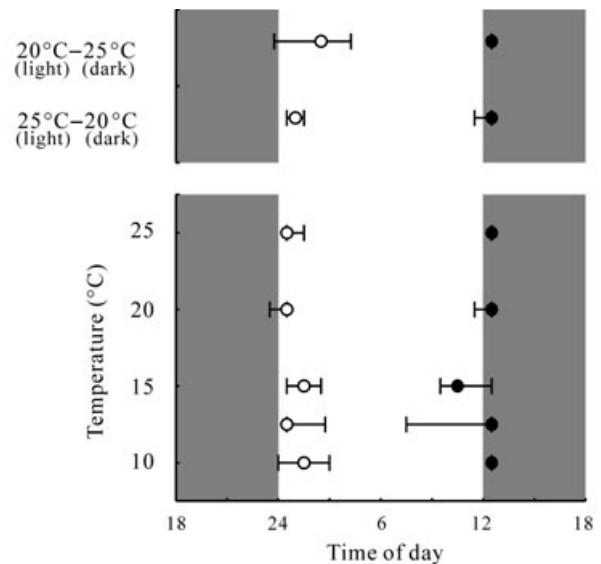


Figure 5 The effect of temperature on the morning (○) and evening peaks (●) in activity of *Nicrophorus quadripunctatus*. Original data are from Figures 3 and 4. Each peak was defined as the median value for hourly times at which adults showed the highest activity within 12 h around lights on or lights off. Horizontal lines indicate interquartiles.

as a median value for hourly times at which the beetle showed the highest activity within 12 h around lights on or lights off; that is, 18.00–06.00 or 06.00–18.00 hours. At 20 and 25°C, the morning peak was 0.5 h after lights on and the evening peak was 12.5 h after lights on. The evening peaks under the thermoperiods were not different from those at 20 and 25°C. However, under the thermoperiod in which the thermophase coincided with the scotophase, the morning peak was 2.5 h after lights on and the interquartile range was from 0.25 h before to 4.25 h after lights on. The bimodal activity was kept at constant temperatures and thermoperiod. The fact that the morning peaks were delayed under the thermoperiod in which the thermophase coincided with the scotophase suggests that temperature affected not only the amount of activity but also the time of the peaks.

The morning peak at 15°C was 1.5 h after lights on, which was later than those at 20 and 25°C. The evening peak was 10.5 h after lights on, which was earlier than that at 20 and 25°C (Fig. 5). The activity in the photophase increased compared with that in the scotophase. Although the activity pattern was not clear at 12.5°C, a peak was observed in the middle of photophase, if peaks immediately after lights off could be neglected as artifacts due to an abrupt change of light intensity

(Fig. 4c). Under all of these conditions between 20 and 12.5°C, the time of the evening peaks advanced as temperatures decreased (Fig. 5). Therefore, the burying beetles probably would be active in photophase under lower temperatures.

DISCUSSION

Nicrophorus quadripunctatus showed bimodal patterns of daily activity with two peaks: one around lights on and the other around lights off under various photoperiodic conditions. In the burying beetles, this bimodal activity pattern has been reported in *N. vespilloides* (Herbst) (Ohkawara *et al.* 1998) and *N. nigrita* Mannerheim (Sikes 1996), both of which show crepuscular daily activities with an activity peak at sunset and sunrise in the field. In *Ptomascopus morito* Keatz, a species of the subfamily Nicrophorinae (*Nicrophorus* and *Ptomascopus* spp.), pheromone emission by male adults shows crepuscular daily activity (Trumbo *et al.* 2001). In the present study, the bimodal daily activity of *N. quadripunctatus* appeared to be controlled by an endogenous circadian mechanism. However, it is not known whether the daily activities of other *Nicrophorus* species are controlled by circadian mechanisms. Bimodal activity rhythms are underlain by separate oscillators in the mosquito *Aedes aegypti* (Linnaeus) (Taylor & Jones 1969) and the moth *Hyalophora cecropia* (Linnaeus) (Truman 1974) and by a single oscillator in the cockroach *Periplaneta americana* (Linnaeus) (Wiedenmann 1980). In *N. quadripunctatus*, because the bimodal activity rhythm persisted under DD ($\tau = 24.6$ h), each peak was not an exogenous effect of lights on or lights off, and two peaks in one cycle were not caused by the direct inhibiting (masking) effect by the above threshold light intensity on the daytime activity. This suggests that these peaks would be controlled by a single oscillator with two active phases or by separate oscillators. If two separate oscillators control the bimodal active phase, different phase relationships between the active peaks that are established during entrainment to different photoperiods should be retained during subsequent DD free-run (Saunders 2002). In the present study, when the beetles under LD 12:12 were transferred to DD, the phase relationship between two active peaks appeared to be stable during DD free-run. To determine whether the peaks are controlled by a single oscillator with two active phases or by separate oscillators, it is necessary to transfer beetles from different photoperiods to DD.

In many insects, thermoperiod as well as photoperiod can act as a Zeitgeber to entrain circadian rhythms

(Saunders 2002). In the present study, activity peaks under the thermoperiod in which the thermophase coincided with the photophase were similar to those at constant 25 and 20°C. However, morning peaks were delayed under the thermoperiod in which the thermophase coincided with the scotophase. The phase angle relationship between light and temperature cycles under this thermoperiod was remarkably different from that under the natural environment, in which the air temperature rises after dawn and falls late in the light period. Therefore, the phase delay of morning peaks may be caused by a temperature step-down at lights on or a step-up at lights off. The phase of the oscillator may be affected by temperature.

In *N. quadripunctatus*, the evening peaks advanced as temperatures decreased, while the timing of morning peaks was little influenced by temperature. Temperatures during recording modified the phase-angle relationship between the endogenous rhythm and the photoperiod. In the field, this mechanism probably activates adults at the time of more preferable temperature. The adults in the field are reproductively active in May and from September to October in Kyoto (Nisimura *et al.* 2002), and monthly averages of daily lowest temperatures in May and October are 13.7 and 13.2°C, respectively (National Astronomical Observatory 2004). When temperatures at night in the field are cooler than those preferable to search for a carcass, adults should be active early in the evening because the present study showed that their activity is suppressed at less than 15°C (Fig. 4). Thus, the temperatures at which beetles were active in the present study were similar to those at which they are active in the field. In Nakagawa, Hokkaido (44°45'N, 142°15'E), adults of *N. quadripunctatus* are active in the field when air temperatures are between 19.5 and 25.5°C (Ohkawara *et al.* 1998). It has been reported for some *Nicrophorus* species that the ability to search for carcasses is temperature dependent. In *N. orbicollis* Say, for example, discovery of carcasses is positively correlated with temperature (Wilson *et al.* 1984).

The present results suggest that in the field in Kyoto, adults of *N. quadripunctatus* show crepuscular activity. The ecological significance of the bimodal activity rhythm of this species is unclear, although the time of activity in some insects is possibly related to mating opportunities. In 16 species of the *Drosophila melanogaster* Meigen group, differences in the diurnal pattern of courtship activity may function as an isolating mechanism between sibling species (Hardeland 1972). The function of the circadian rhythm, which modulates

swarming in *Chironomus yoshimatsui* Martin et Sublette, might be to increase the possibility of the encounter between a sexually active male and a receptive female by restricting the time and place of this activity (Kon 1984, 1985). In the burying beetles *N. vespilloides* and *N. humator* (Gleditsch), adult males in the field emit pheromones during species-specific periods of the day in order to attract females for mating, even in the absence of a carcass (Müller & Eggert 1987). However, it is not known whether the pheromone emissions of these species have a rhythm. We assumed that the adults of *N. quadripunctatus* used in the present study were reproductively active, because they were reared under conditions inducing reproduction; that is, LD 12:12 at 20°C (Nisimura *et al.* 2002). Therefore, the adults were searching for not only carcasses but also for mating partners. It is possible that restriction of the active periods to a certain time of day contributes to increasing mating opportunities and avoidance of interspecific competition.

In interspecific and intraspecific competition of burying beetles for carcasses, larger individuals win against smaller individuals (Wilson & Fudge 1984; Otronen 1988). Therefore, smaller species may be active during the period in which the larger ones are inactive. In Michigan, USA, both the seasonal pattern of reproductive activities and the habitat of *N. defodiens* Mannerheim and *N. orbicollis* overlap, although the former is active from evening to midnight and the latter is active from midnight (Wilson *et al.* 1984). However, results did not support the hypothesis that the temporal head start of the smaller congener, *N. defodiens*, provided extra time to conceal a carcass from *N. orbicollis* (Trumbo & Bloch 2002). In Kyoto, the seasonal activity of *N. quadripunctatus* overlaps with that of *N. concolor* Kraatz (Trumbo *et al.* 2001), which is a larger species than *N. quadripunctatus*. However, it is unknown whether the daily activity pattern of *N. quadripunctatus* is affected by the presence of this sympatric larger species.

Daily activity patterns could be differentiated among conspecific populations of *Nicrophorus*. For example, the species *N. vespilloides* in Hokkaido, Japan, shows a daily activity pattern consisting of a morning and a night peak (Ohkawara *et al.* 1998). However, Müller and Eggert (1987) reported that this species in Europe is active mainly during the afternoon and evening. In the adults of *N. quadripunctatus*, there are differences not only in the daily activity pattern but also in the seasonal pattern between Kyoto and Hokkaido. In Hokkaido, adults are reproductively active in summer; that

is, July and August, so the seasonal pattern of these adults is unimodal (Katakura & Ueno 1985; Ohkawara *et al.* 1998). However, adults in Kyoto suppress reproductive behaviors from June to August because of high temperatures (Nisimura *et al.* 2002). The monthly average temperatures at which the adults are reproductively active are 18.8°C (May), 23.6°C (September) and 17.5°C (October) in Kyoto, and 19.3°C (July) and 20.8°C (August) in Rumoi, Hokkaido (National Astronomical Observatory 2004). Therefore, the preferred temperature of this species in Kyoto may be similar to that of the populations in Hokkaido (located 09°44' north from Kyoto). The daily activity pattern of *N. quadripunctatus* in Hokkaido is unimodal: the adults are active at night when air temperatures are between 19.5 and 22.5°C in August in the field (Ohkawara *et al.* 1998). According to Ohkawara *et al.* (1998), air temperatures around noon are more than 30°C in August, when these daily activities were recorded. Adults are active at night only when air temperatures are lower than daytime. We suggest therefore that the daily activity patterns of this species depend on population-specific seasonal differences in temperature.

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