

原 著

温度周期下におけるカイコ蛾の羽化リズム

Thermoperiodic regulation of the adult eclosion rhythm in the commercial silk moth,
Bombyx mori

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The aim of this study was to elucidate the effects of thermoperiod on adult-eclosion timing in silk moth, *Bombyx mori*. The eclosion time of pupae maintained at various thermoperiods under continuous darkness was recorded. Under the thermoperiodic conditions, the silkworms showed unimodal or bimodal eclosion distributions: one peak occurred shortly after the cool phase began and the other in the late cool phase. Among these, the latter peak was controlled by an endogenous circadian pacemaker. The clock-mediated eclosion phase changed as a function of thermoperiod. From a 2-h warm phase/22-h cool phase to an 18-h warm phase/6-h cool phase, the phase angle maintained a constant relationship with the mid-point of the warm phase. This relationship was expected if *B. mori* used both morning (a temperature increase) and evening (a temperature decrease) signals as cues for the eclosion timing. As a similar trend in the phase relationship was also observed under photoperiodic conditions, it appeared that *B. mori* detected and interpreted the thermoperiods in the same manner as for the photoperiods.

Keywords: *Bombyx mori*, Circadian rhythm, Eclosion, Temperature cycle
カイコガ、概日リズム、羽化、温度サイクル

INTRODUCTION

The emergence rhythm of adult insects from their pupae or puparium is one of the overt population rhythms in insects. Although eclosion occurs only once in the life cycle of an insect, the timing of this event is controlled by an endogenous circadian oscillator (Saunders, 2002; Myers, 2003); thus, a clear rhythm can be observed within a mixed population of developmental ages.

The commercial silk moth, *Bombyx mori* (Linnaeus, 1758), a widely used lepidopteran model for insect physiology research, ecloses as an adult early in the morning. Photoperiod is a primary cue for the timing of eclosion (Oshiki & Watanabe, 1978; Shimizu & Matsui, 1983; Ikeda *et al.*, 2019). Under a light-dark cycle, *B. mori* shows a bimodal pattern in the timing of eclosion; the first peak occurs late in the scotophase and the second peak occurs shortly after the light phase starts (Oshiki & Watanabe, 1978; Shimizu & Miura, 1987).

In some insects, the eclosion rhythm was found to be entrained not only by the light-dark cycle but also by the

temperature cycle (Arai, 1976; Ždársek & Denlinger, 1995; Watari & Tanaka, 2010; Kikukawa *et al.*, 2016). In *B. mori*, however, whether this rhythm is entrainable to the temperature cycle remains unknown. To date, the eclosion rhythm has been examined only in the context of the light-dark cycle; however, the effect of cyclical temperature changes on the expression of several circadian clock genes has been examined in *B. mori* ovarian cells (e.g. Chu *et al.*, 2016).

The present study aimed to provide further insight into the adult eclosion rhythm of *B. mori*, particularly with respect to the role of thermoperiod in the regulation of eclosion. First, to test whether the eclosion rhythm of *B. mori* can be entrained by a temperature cycle, we kept the pupae in continuous darkness (DD), under different thermoperiods, and the distribution of adult eclosion was recorded. As the silk moth could be entrained by thermoperiod, our second experiment tested whether the silk moth synced with thermoperiod in the same manner as it does for photoperiod. We maintained the pupae at various

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thermoperiods and the obtained phase relationship between median eclosion time and thermoperiod was compared with the relationship between median eclosion time and photoperiod (Shimizu & Miura, 1987).

MATERIALS AND METHODS

Fourth instar larvae of a racial hybrid (*Syunrei* × *Shougetu*) of *B. mori* were obtained from Ehime Sanshu Co. Ltd. and maintained in continuous darkness (DD) at 25°C on an artificial diet (Silkmate2S, Nihon Nosan Kogyo Co. Ltd., Kanagawa, Japan). Pupae were removed from their cocoons two days after pupation. Only male pupae were used for the experiments.

The apparatus employed for recording the eclosion time was similar to that described by Tanaka et al. (2013), and consisted of a plastic box equipped with an infrared light emitter and a detector (GT2; Takenaka Electronic Industrial Co., Kyoto, Japan). The mechanism was based on the “falling ball” principle (Saunders, 1976; Truman, 1972); where a stainless-steel ball was impacted by an eclosing moth so that it crosses an infrared beam, a signal was fed to a computer and the number of eclosions was counted. The recording apparatus was filled with pupae and placed in an incubator (Nippon Medical and Chemical Instruments Co., Osaka, Japan, or MIR-253, SANYO Co., Osaka, Japan) where thermoperiods that consisted of a square-wave cycle of a warm phase (W) and a cool phase (C) could be programmed. In the present study, the pupae were kept at various thermoperiods in which the warm phase lasted for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 21, and 22 h per 24 h. The warm phase and cool phase temperatures were fixed at 29.5°C and 20.5°C, respectively. The temperature inside the incubator was monitored using a portable data logger (Ondotori, TR-71S, TandD Co., Nagano, Japan), and fluctuated within a range of ±0.5°C of the desired setting. At 25°C, adult eclosion occurred between day 12 and day 16 after pupation (unpublished observation). As the moths emerged over several days, daily data for eclosion times during the entire emerging period were pooled. The phase of the eclosion peak ($\bar{\theta}_E$) was represented by the median time of eclosion. The silkworm showed a bimodal eclosion distribution; one peak occurred shortly after the cool phase began, and the other in the late cool phase (Fig. 1). Because the former peak seemed to be evoked directly by an exogenous temperature-drop stimulus (masking), the data just after onset of cool phase was deleted to calculate it. The effect of thermoperiod on adult eclosion timing was analyzed using the Steel-Dwass test. Statistical analysis was performed using JMP (SAS Institute Inc., NC, USA).

RESULTS

Figure 1 shows the eclosion rhythm of *B. mori* at 6-h warm:18-h cool (WC 6:18) in DD. The silkworm showed a bimodal eclosion distribution; one peak occurred shortly after the cool phase began, and the other in the late cool phase. We called them temperature-down peak (T-down peak) and cool phase peak (CP peak), respectively.

Figure 2 shows the eclosion rhythm of *B. mori* transferred from WC 6:18 to a constant temperature (20.5°C) in DD. Under the thermoperiodic conditions, both T-down and CP peaks appeared. When pupae were transferred from the temperature cycle to a constant temperature, the CP peak showed a free-running rhythm with a period of approximately 22 h under constant temperature at least in the first free-run cycle. The eclosion median ($\bar{\theta}_E$) on the day of transfer and on the first circadian cycle were 16.5 h and 14.5 h, respectively. For the second circadian cycle and later, however, the eclosion rhythm rapidly dampened and became arrhythmic. In contrast to the CP peak, the T-down peak disappeared after transfer to a constant temperature. Thus, it appeared that the CP peak was controlled

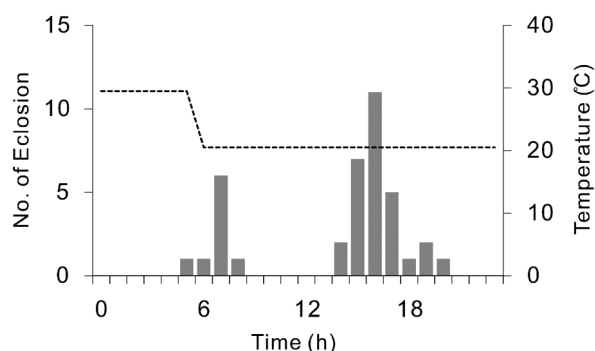


Figure 1 Distribution of adult eclosions at 1 h intervals from pupae of *Bombyx mori* at WC 6:18 in continuous darkness. The warm phase and cool phase temperature were fixed as 29.5°C and 20.5°C, respectively. Sample size was 38.

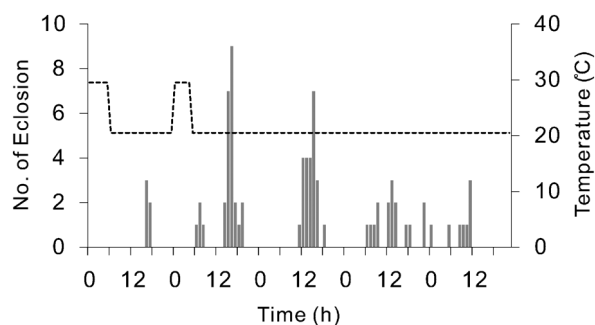


Figure 2 Distribution of adult eclosions from pupae of *Bombyx mori* at 1 h intervals after transferred from WC 6:18 to constant temperature (20.5°C) in DD. Sample size was 80.

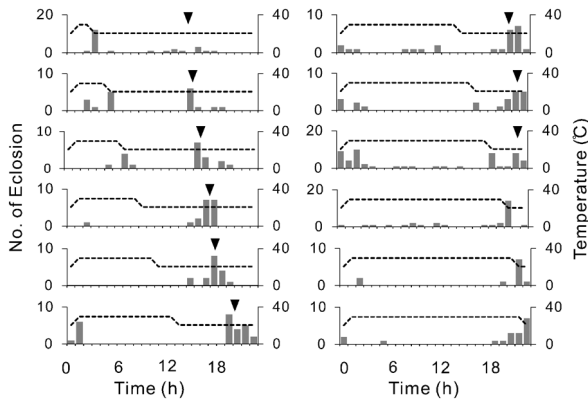


Figure 3 Distribution of adult eclosions at 1 h intervals from *Bombyx mori* pupae under various thermoperiod conditions. Triangles represent the eclosion median (Θ_E) of the CP peak and the dotted line represents the temperature. Θ_E was 15.2, 15.9, 16.9, 17.7, 18.6, 20.3, 21.3, 22.4, and 22.9 from 2W22C to 18W6C. Sample sizes were 24, 18, 19, 18, 17, 26, 25, 22, 54, 30, 11 and 18 from 2W22C to 22W2C, respectively.

by an endogenous circadian pacemaker, but the T-down peak was evoked directly by an exogenous temperature-drop stimulus (masking).

Figure 3 shows the distribution and peak time (Θ_E) of adult eclosion under thermoperiods of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 21, and 22 hours of warm phase (W) per 24 hours and a complementary cool phase (C) in DD. In all thermoperiodic regimes, a clear eclosion rhythm occurred, but the eclosion patterns varied somewhat with the thermoperiod. A CP peak was observed from WC 2:22 to WC 18:6, while T-down peaks were observed from WC 2:22 to WC 6:18 and from WC 16:8 to WC 22:2. As a result, the eclosion pattern was bimodal from WC 2:22 to WC 6:18 and from WC 16:8 to WC 18:6, but unimodal from WC 8:16 to WC 14:10, and from WC 20:4 to WC 22:2. Among the thermoperiodic regimes, the CP peak became dominant from WC 4:20 to WC 18:6, while the T-down peak was dominant at WC 2:22 and from WC 20:4 to WC 22:2.

In order to elucidate the phase relationship between the eclosion time and the thermoperiods, the Θ_E -value of the CP peak was plotted against the onset of the warm phase in Figure 4a. Θ_E changed as a function of the length of the warm phase. From WC 2:22 to WC 16:8, Θ_E tended to be later as the length of the warm phase increased (Steel-Dwass test, $P < 0.05$), while no difference was found in Θ_E between WC 16:8 and WC 18:6 ($P > 0.05$). Figure 4b plots Θ_E -values against the mid-point of the warm phase. From WC 2:22 to WC 18:6, Θ_E did not differ among the regimes ($P > 0.05$), and occurred at ca. 14 hours after the mid-

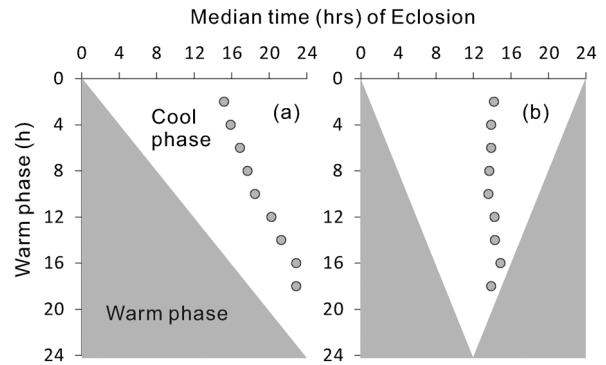


Figure 4 The change in Θ_E of *B. mori* in thermoperiods. Θ_E is plotted as a function of (a) an onset or (b) the mid-point of the warm phase.

point. Thus, these peaks maintained a constant-phase relationship with the mid-point of the warm phase.

DISCUSSION

The results presented here demonstrate that, in DD, the adult-eclosion rhythm of *B. mori* was effectively entrained by the temperature cycle (Fig. 1). Thus, it appears that not only photoperiods (Oshiki & Watanabe, 1978; Shimizu & Matsui, 1983; Shimizu & Miura, 1987), but also thermoperiods serve as a time cue for adult-eclosion timing in the silk moth.

Under thermoperiodic conditions, a bimodal pattern appeared in the timing of eclosion (Fig. 1), and included the CP peak and T-down peak. Among them, the CP peak was controlled by an endogenous circadian pacemaker, while the T-down peak was evoked directly by an exogenous temperature decrease. A bimodal pattern also appeared under photoperiodic conditions in the timing of eclosion; the first peak occurred in the scotophase and the second peak shortly after the light phase began (Oshiki & Watanabe, 1978; Shimizu & Miura, 1987). Among them, only the first peak continued to appear after transfer from the light-dark cycle to DD, suggesting that it was controlled by an endogenous circadian pacemaker (Shimizu & Miura, 1987).

When pupae were transferred from a temperature cycle to constant temperature conditions, the CP peak did occur in the first circadian cycle, but rapidly dampened after the second cycle (Fig. 2). In contrast, the eclosion rhythm of the pupae transferred from a light-dark cycle to constant darkness persisted for at least the first three circadian cycles under continuous darkness (Shimizu & Matsui, 1983). The observed difference in the robustness of free-running rhythms strongly suggests that the temperature cycle is a weaker time cue than the light-dark cycle for the eclosion timing of *B. mori*.

Under thermoperiodic conditions, the CP peak, which is controlled by an endogenous circadian pacemaker, maintained a specific relationship with the thermoperiods; from WC 2:22 to WC 18:6, the phase angle maintained a constant relationship with the mid-point of the warm phase. Specifically, the time from the mid-point in the warm phase to eclosion was constant (ca. 14 hours), irrespective of the length of the warm phase (Fig. 4b). As inferred previously (Aschoff, 1981), such a relationship can be expected if *B. mori* uses both morning (a temperature increase) and evening (a temperature decrease) signals as cues for eclosion timing.

A steady-state phase relationship of Θ_E with exogenous environmental cycles has also been observed under photoperiodic conditions (Shimizu & Miura, 1987). From 4-h light:20-h dark (LD 4:20) to LD 16:8, eclosion occurred at ca. 16 hours after the mid-point of the photophase irrespective of the length of photophase, again suggesting that both morning (light-on) and evening (light-off) signals are used for this timing. Moreover, as was the case with longer thermoperiods, in longer photoperiods, this phase relationship was lost; in LD 20:4 and LD 22:2 conditions, mass eclosion occurred just before light off and just after light on. The phase relationship of Θ_E with the exogenous environmental photoperiod cycle was thus nearly the same as that with thermoperiod cycles. This finding suggests that *B. mori* detects and interprets thermoperiods and photoperiods in the same manner.

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