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# Free-Running Eclosion Rhythm of Adult *Plodia interpunctella* Entrained by Either a Single Light Pulse or a Thermocycle

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## Abstract

Rhythms in physiological and behavioral activities of insects can be entrained by photoperiod and/or thermocycle (thermoperiod). The present study investigates the free-running eclosion rhythm of *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) by applying either a single light pulse or a thermocycle. To prevent larval diapause, all insects are maintained at 30°C and kept in constant darkness (DD) for 14 days after oviposition. Thereafter, the insects were maintained at 25°C DD. At day 27 after oviposition, the insects are subjected to a single light pulse (2-16 h). Average time from the light-off signal to the first peak of eclosion rhythm in DD is *c*. 17.5 h, indicating that the insects interpret the 'light-off' signal as a Zeitgeber to entrain the rhythm at 25°C, since the rhythm normally free-runs for a period of *c*. 23 h. A single light pulse in otherwise DD can entrain the adult eclosion rhythm in this species at 25°C. In contrast, the rhythm can be entrained by applying a thermocycle consisting of 30°C thermophase (12 h) and 20°C cryophase (12 h) regardless of background illumination (DD or constant illumination (LL)). The insects are exposed to the thermocycle in DD or LL from days 15 to 28 after oviposition and subsequently maintained at 25°C in DD or LL. The rhythm is observed to free-run for a period of *c*. 22 h. Thus, the adult eclosion rhythm in this species can be entrained by either a light pulse or a thermocycle. However, it is unclear whether the photoperiodic and thermoperiodic time-keeping mechanisms are controlled by the same physiological processes.

## Keywords

Adult Eclosion, Free-Running Rhythm, *Plodia Interpunctella,* Light Pulse, Thermoperiod

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## 1. Introduction

Circadian rhythms in most insects persist in constant darkness (DD) and/or constant light (LL) if the time-keeping system is initially entrained by the environmental time cues (Zeitgebers) such as photoperiod and/or thermocycle (see for review, 1, 2). Adult eclosion of *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) exhibits a free-running rhythm in DD at 22-30°C (3). Entraining the photoperiodic time-keeping system requires that insects are initially exposed to LD 12:12 h and subsequently subjected to DD.

Pittendrigh (4) reported that the adult eclosion rhythm of *Drosophila pseudoobscura* is entrained by a single 4-h light pulse in otherwise DD. *D. pseudoobscura* raised in LD 12: 12 h and thereafter exposed to various lengths of light pulse demonstrates a periodic adult eclosion pattern in DD (5). When this light pulse is <12 h, the light-off signal is not interpreted by the insects and their eclosion rhythm persists in DD. However, following a light pulse that lasted longer than 12 h, the oscillation is damped out and held in a fixed state

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corresponding to circadian time 12 (subjective night). When the light is discontinued, the oscillation immediately enters its steady-state motion starting from that phase point. A transfer from LL to DD at 19°C initiates free-running rhythms in a European population of *Drosophila subobscura* (6). When a wild type strain of *D. jambulina* is transferred from LL to DD at 28°C, the adult eclosion rhythm free-runs was observed (7). In the low-altitude strains of Himalayan *D. ananassae*, LL to DD transfers at 13-21°C initiates free-running rhythms (8). In the present study, to determine an effective Zeitgeber (i.e., light-on or light–off) to entrain the time-keeping system for the adult eclosion rhythm in *P. interpunctella*, a single light pulse of various lengths (2-16 h) is applied in DD at 25°C; that is, the insects experience light-on and light–off signals once during their developmental period. This experimental protocol may demonstrate an important Zeitgeber for entraining the eclosion clock.

The adult eclosion rhythm may also be phase-regulated by thermoperiodic stimuli. Previous studies demonstrate that *Anagasta kuhniella* is one such species affected by thermoperiod (9). The adult eclosion rhythm in *D. pseudoobscura* is entrained by a temperature cycle and exhibit a free-running rhythm (10). In *P. interpunctella*, peak adult eclosion occurs under thermoperiodic conditions (11). One of the aims of the present study is to investigate the free-running property of the adult eclosion rhythm that is entrained by a thermocycle. The thermocycle implemented consists of 12 h at 30°C and 12 h at 20°C and the free-running rhythm is observed at 25°C in either DD or LL. The experimental results may demonstrate the nature of the time-keeping system that is entrained by a thermoperiodic Zeitgeber.

## 2. Materials and Methods

A laboratory culture of *P. interpunctella* was established and maintained as described previously by Kikukawa *et al.* (3). The eggs that were deposited within 24 h were placed in transparent plastic cups (diameter 15 cm, height 9 cm) which contained commercial rice bran (1g per larva). For all experiments, larvae were maintained at 30°C DD for 14 days after oviposition, thus preventing larvae from entering diapause. In each treatment, 97-328 larvae were exposed to the experimental conditions. In the first series of experiments, larvae were transferred from 30°C to 25°C DD at day 15. At day 27, a single light pulse (2-16 h) was applied; as a control, one group was maintained in DD without a light pulse treatment. Light-on of the applied pulse was defined as Zeitgeber time zero (Zt 0). Adult eclosion occurred between days 27-31.

In the second series of experiments, larvae were exposed to a thermocycle of TC 12:12 h (30°C/20°C) under DD or LL at day 15 after oviposition. Thermophase (30°C) and cryophase (20°C) were symbolized as T and C, respectively. The temperature-rise was defined as Zt 0. At day 28, larvae were transferred to a constant temperature of 25°C at Zt 12 under DD or LL, i.e., the larvae were exposed to a T of 30°C from Zt 0 to 12 at day 28 and subsequently maintained at 25°C instead of being subjected to a C of 20°C. Eclosion occurred from days 28 to 32. The peaks of adult eclosion were defined as the mean eclosion times. Two 10-W daylight fluorescent tubes were used as a light source. Larvae were exposed to light of at least 500μW per $cm^2$ during a light pulse or light phase. The number of adults emerging from the food was counted within a few minutes at 1-h intervals. During the dark period, dim red light (610-750nm) of <100μW per $cm^2$ was used for observations.

## 3. Results

### 3.1. Free-Running Rhythm Entrained by a Single Light Pulse

Six groups of *P. interpunctella* were maintained in DD. One group was kept in DD throughout their lifetime as a control (Fig. 1A) and no adult eclosion rhythm was observed. In the remaining five groups of insects, a single light pulse ranging from 2 to 16 h was applied beginning at Zt 0 of day 27. Thus, these five groups of insects experienced light-on and light–off stimuli once during their developmental period. Thereafter the free-running eclosion rhythm was observed in adults (Fig. 1B-1F). The free-running rhythm appeared to be entrained by the light-off signal. For example, when a 2-h light pulse was applied at Zt 0-2 of day 27, the first peak of adult eclosion occurred at Zt 22.3 of day 27 followed by the second peak at Zt 21.8 of day 28 and so on (Fig. 1B). A 16-h light pulse applied at Zt 0-16 of day 27 resulted in a free-running rhythm (Fig. 1F). The first peak occurred at Zt 8.0 of day 28 and the second one at Zt 6.4 of day 29 and so on. Figure 1 clearly shows the temporal relationship between the applied light pulse and the peaks of adult eclosion rhythm. The average duration from the light-off signal to the first peak of eclosion was 17.5 h. Average duration ($\tau_1$) from the first peak to the second peak was 22.9 h; from the second to the third peak ($\tau_2$) was 22.8 h; from the third to the fourth peak ($\tau_3$) was 23.0 h. Thus, these insects eclosed as adults at 17.5 h following the light-off signal and the rhythms free-ran for *c*. 23 h.

### 3.2. Free-Running Rhythm Entrained by a Temperature Cycle of 30° C/20° C

Entraining the adult eclosion rhythm by thermocycle was determined by conducting the following experiments. Four groups of the insects were maintained at 30°C DD for 14 days after oviposition. The first group was exposed to TC 12:12 h

(30°C/20°C) in DD for 13 days. At day 28, a thermophase of 30°C was applied from Zt 0 to 12 in DD. At Zt 12 of the same day, insects were transferred to DD at 25°C, such that they were maintained in DD throughout their developmental period. Under these conditions, the larvae started adult eclosion at day 28 (Fig. 2A). The first peak was observed at Zt 5.1 on day 28, the second at Zt 2.6 on day 29 and the third at Zt 1.6 on day 30. Since the first peak was observed during the final thermophase of 30°C on day 28 as expected (see also 11) and the rhythms free-ran at 25°C DD, τ was calculated from the second to third peaks and that was 23.0 h.

The second group of insects was maintained under the thermocycle in DD from day 13 to 27. At Zt 0 of day 28, insects were transferred to LL. After thermophase (30°C) from Zt 0 to 12 on day 28, they were maintained at 25°C. The first peak of adult eclosion was observed at Zt 7.1 during this thermophase on day 28. The rhythm free-run under LL was detected at 25°C. The second peak occurred at Zt 7.3 of day 29 and the third at Zt 1.3 of day 30. Thus, τ from the second to third peaks was determined to be 18.0 h (Fig. 2B).

The third group was maintained under the thermocycle in LL. At Zt 0 of day 28, they were transferred to DD. After thermophase from Zt 0 to 12 on day 28, they were maintained at 25°C. The first peak was observed at Zt 6.3 of day 28, the second at Zt 2.9 of day 29, the third at Zt 4.0 of day 30 and the fourth at Zt 1.6 of day 31. Thus, τ from the second to third peaks was calculated to be 25.1 h and the τ from the third to fourth peaks was shown to be 21.6 h (Fig. 2C).

The fourth group was maintained under the thermocycle in LL. The insects were kept at 25°C LL after the thermophase (Zt 0-12) of day 28, so that they were maintained in LL from day 15 after oviposition. The first peak was found at Zt 7.7 on day 28, the second at Zt 2.9 on day 29 and the third at Zt 23.5 on day 29. The τ between the second and third peaks was calculated to be 20.6 h (Fig. 2D).

Thus, the τs obtained in DD were slightly longer in duration than those in LL. However, the statistical analysis (StatMate III for Macintosh, Atomusu Co. Ltd., Japan) showed that these differences were non-significant among these groups.

## 4. Discussion

The present study demonstrates that the adult eclosion rhythm of *P. interpunctella* can be entrained by either a single light pulse (2-16 h) at 25°C or a thermocycle of TC 12:12 h (30°C/20°C).

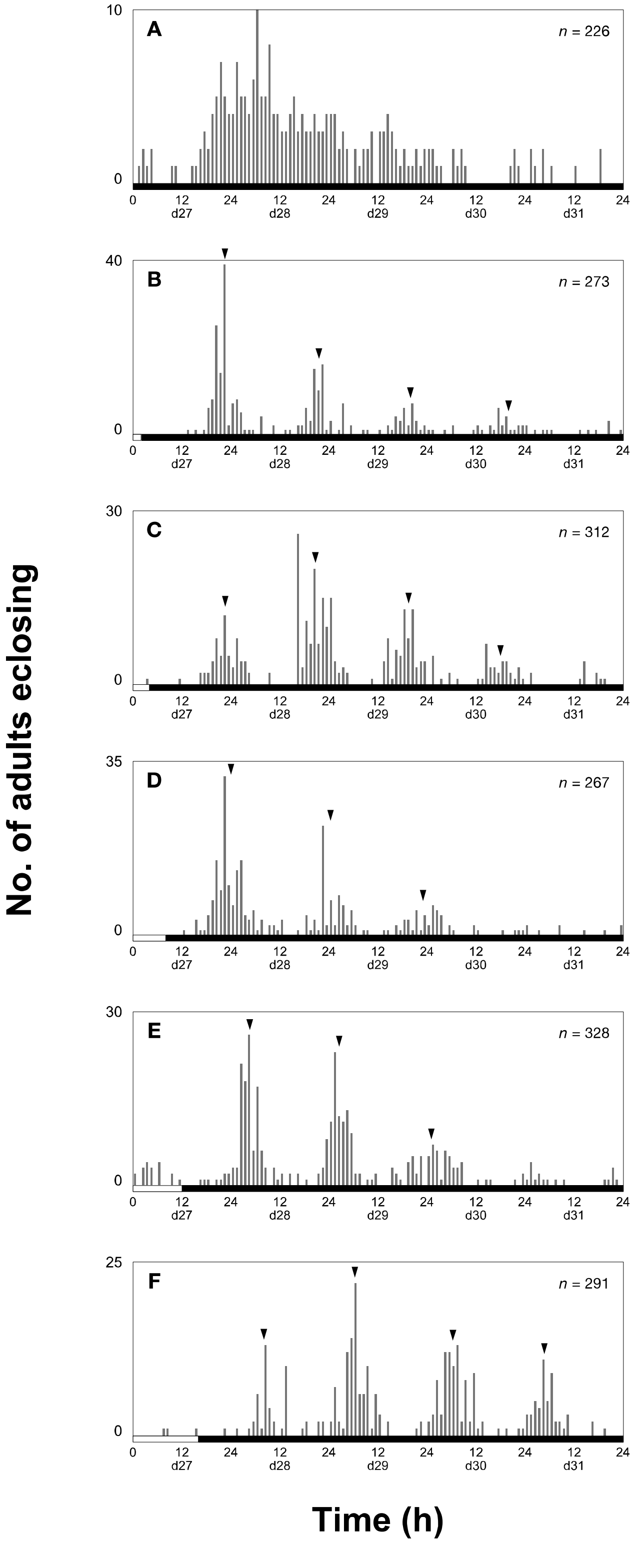

**Figure 1.** The free-running adult eclosion rhythm in *Plodia interpunctella* entrained by light pulses under constant darkness (DD) at 25°C. The horizontal axis represents Zeitgeber time (Zt) and days since oviposition (d). Light-on is defined as Zt 0. The insects were maintained in DD at 30°C for 14 days following oviposition and subsequently transferred to 25°C in DD (A). Five groups of insects were exposed to light pulses on day 27 at 25°C. The length of light pulses tested was 2 h (B), 4 h (C), 8 h (D), 12 h (E) and 16 h (F). Arrowheads indicate peaks of adult eclosion. The horizontal white bar on the X-axis represents the duration of applied light pulse; the black bar represents the duration of DD. The number of individuals tested (*n*) are indicated in each panel.

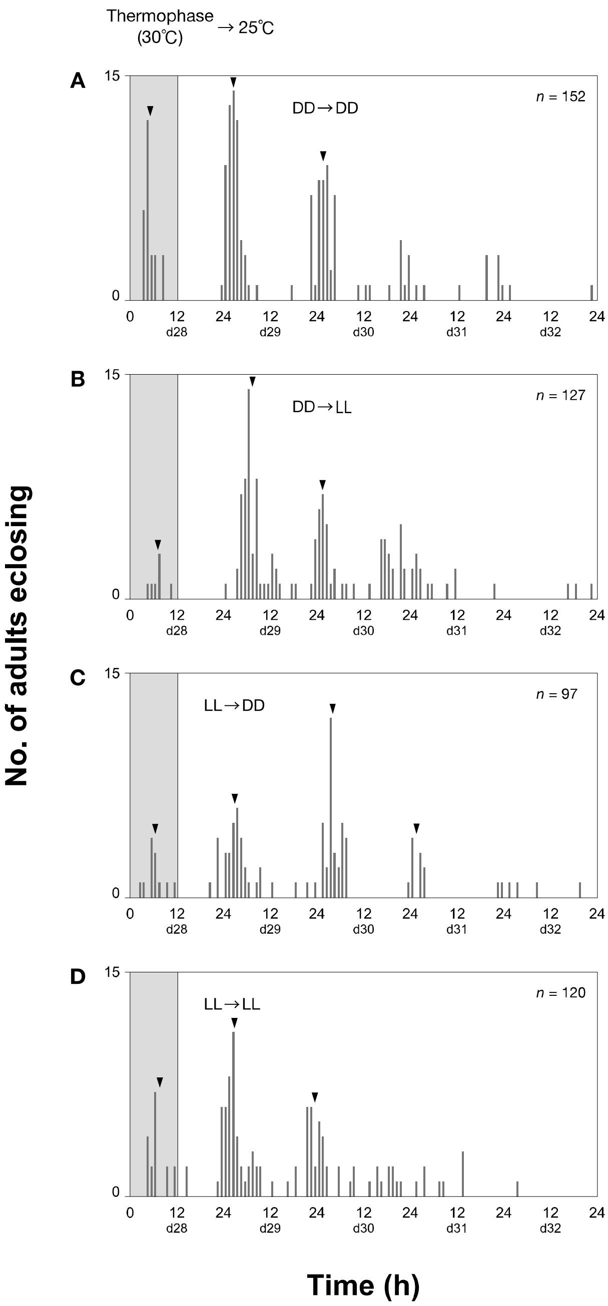

**Figure 2.** The free-running adult eclosion rhythm in *Plodia interpunctella* entrained by a temperature cycle (30°C/20°C) containing a thermophase (T) and cryophase (C) of TC 12:12 h. The horizontal axis represents Zeitgeber time (Zt) and days since oviposition (d). The start of the temperature-rise is defined as Zt 0. All insects were maintained in DD at 30°C for 14 days following oviposition. Thereafter, insects were exposed to TC 12:12 h in DD and subsequently transferred to DD (A) or LL (B) at 25°C at Zt 12 on day 28. Insects were also exposed to TC 12:12 h in LL and transferred to DD (C) or LL (D) at 25°C at Zt 12 on day 28. The final thermophase is represented by the gray section spanning Zt 0-12 on day 28 and temperature is maintained at constant 25°C. Arrowheads indicate peaks of adult eclosion. The number of individuals tested (*n*) are indicated in each panel.

Kikukawa *et al*. (3) have reported the interacting effects of light-on and light–off signals on timing of the adult eclosion rhythm in *P. interpunctella* under 24-h photoperiods of 20-30°C. At 25°C, for example, under LD 2: 22 h, the peak of adult eclosion occurs at *c*. 19 h after the light-off signal. Under LD 20:4 h, eclosion occurs at *c*. 15.5 h after the light-off signal. The present study indicates that the insects respond primarily to the light-off signal to entrain their eclosion clock at 25°C. The insects eclose as adults at 17.5 h after the light-off signal of a simple light pulse and the rhythms free-run for a period of *c*. 23 h. The light-on signal appears to modify slightly the process of time measurement in the normal 24-h photoperiods (3).

The results demonstrate that the thermocycle is among the Zeitgebers that can entrain the adult eclosion rhythm of *P. interpunctella* as observed in other insects (see 1, 2) and that thermoperiodic entrainment is independent of background illumination (i.e., DD or LL). In *D. jambulina*, following thermoperiodic entrainment (TC 12:12 h (20°C/28°C)), the rhythm free-runs occur in DD but not in LL at 28°C (7). Similarly, after exposure to TC 12:12 h (13°C/21°C) in *D. ananassae*, the rhythm free-runs occur in DD at 13°C and 21°C but not in LL (8). In these species, therefore, LL may have suppressed time-keeping process (es).

In *P. interpunctella,* the average τ obtained from the experiments of temperature-cycle entrainment is *c*. 22 h which is shorter than that (*c*. 23 h) of the single light-pulse experiment. In the 24-h thermoperiod (*e.g*., TC 12:12), peaks of adult eclosion are advanced in DD as compared with LL (11). Under TC 12:12 h in DD or LL after 30°C in LL, peaks are observed at Zt 3.9±2.0 and Zt 4.8±2.8, respectively. The difference is statistically significant (t-test, $P<0.05$). Therefore, DD or LL has a marginal influence on the temporal organization of the oscillator operating under temperature cycles of 30°C/20°C. Adult eclosion rhythms of *Delia antiqua* entrained by thermoperiods are known to persist both in DD and LL (12). The eclosion peak occurs a few hours earlier in DD than LL. In the present study, the τs in DD are slightly longer than those in LL; however, the difference is not statistically significant.

## 5. Conclusion

The present study showed that the adult eclosion rhythm of *P. interpunctella* could be entrained by either a single light pulse or a thermocycle. Free-running eclosion rhythm of adult *D. antiqua* was examined and the temperature cycles with different amplitudes acted as Zeitgeber somewhat differently (12). In *P. interpunctella*, observation was made only under a thermoperiod of 30°C/20°C. Understanding the mechanisms underlying the photoperiodic and thermoperiodic time-keeping system(s) warrants further study.

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