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# Adult Eclosion Rhythm of *Plodia interpunctella* Under Non-24 h Photoperiods

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## Abstract

Adult eclosion rhythms of the Indian meal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae), were observed under a variety of non-24 h photoperiods at different temperatures - 30°C, 25°C and 20°C - to investigate the entrainability of the time-keeping system. Larvae were exposed to light-dark (LD) cycles in which the light period was fixed at 12 h and the dark period varied from 2 to 18 h, and *vice-versa*, i.e., cycles in which the dark period was fixed at 12 h and the light period varied from 2 to 18 h. At 30°C and 25°C, the temporal positions of the eclosion peak advanced as the cycle length increased. In conditions with a constant light period of 12 h, larvae seemed mainly to respond to the light-off signal, but this response was modified by the light-on signal. Under the constant dark period of 12 h, larvae seemed to measure the cycle length. The temporal position of the adult eclosion peak ($\varphi_E$) is a function of the cycle length. No statistically significant difference was noted in $\varphi_E$ between cycles observed at 30°C and 25°C. The lower and upper ends of the range of entrainability were 18-19 h and 29-30 h, respectively. At 20°C, the rhythm could be entrained only by the LD cycle durations close to 24 h. This is an uncommon phenomenon amongst insects and is a significant finding for understanding the physiological nature of the time-keeping process(es) of this species.

## Keywords

Adult Eclosion Rhythm, Indian Meal Moth, *Plodia interpunctella*, Non-24 h Photoperiod

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## 1. Introduction

Many insects exhibit circadian rhythms in their behavior and physiology (1, 2). Temporal positioning of the rhythm is known to be regulated by the time-keeping system which oscillates approximately every 24 h. One important property of this timing system is its entrainability by environmental token stimuli (known as Zeitgeber stimuli). The lower and upper limits of the entrainment of the clock system to non-24 h light-dark (LD) cycles have been investigated by Refinetti and others who have listed 25 species including three insect species (4-6). However, entrainment under a non-24 h photoperiod has not been investigated extensively. For example, Refinetti (3) and Saunders (4) determined that, in the adult eclosion rhythm of *Sarcophaga argyrostoma*, lower and upper ends of the range of entrainment were 11.8 h and 35.8 h, respectively, predicted from the phase-response curve. This is a quite wide range for entrainment to LD cycles and might be an exceptional case.

Adult eclosion rhythm of the Indian meal moth Hübner, *Plodia interpunctella* (Lepidoptera: Pyralidae), has been investigated extensively under various photoperiodic and thermoperiodic conditions (7-10). The rhythm is entrained by the light-off signal of a single light pulse occurring at 2 - 16 h,

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and free-run in constant darkness (DD) at 25°C (7). Under a normal 24-h LD cycle at 30°C-20°C, the temporal position of the eclosion peaks ($\varphi_E$) appears to be determined by the interacting effects of light-off and light–on (8). At 20°C, the rhythm occurs under the normal 24-h LD cycle, but does not free-run in DD (8), indicating that the oscillator might rapidly dump out at low temperature. It has been also reported that the eclosion rhythm of *P. interpunctella* is entrained by thermocycles (7). Under various 24-h thermocycles, the temporal position of the peak is dependent on temperature means and amplitudes (9). Under non-24 h thermocycles of 30°C /20°C, the peak appears appropriately 17 h after the temperature-fall when the thermophase is a constant 12 h (10). However, the peak occurs 5 h after the temperature-rise when the cryophase is a constant 12 h. Under the thermocycle of 25.5°C /24.5°C, $\varphi_E$ is a function of the cycle length.

Thus the time-keeping system operates in a different way under the different environmental conditions. This study investigates the adult eclosion rhythm of *P. interpunctella* under non-24 h photoperiods at 30°C, 25°C and 20°C, and focuses on the entrainability of adult eclosion clock.

## 2. Materials and Methods

A laboratory culture of *P. interpunctella* was collected and maintained as described in an earlier study (8). Since larvae do not enter diapause at 30°C, they were exposed to the experimental photo-regime immediately after oviposition. For experiments conducted at 25°C and 20°C, in order to prevent the larvae from entering diapause, eggs were kept at 30°C in DD conditions for 14 days after oviposition. The larvae were then subjected to different cycle lengths of photoperiods: non-24 h varying from 14 to 30 h at 30°C and 25°C : varying from 18 to 30 h at 20°C. The light (L) and dark (D) periods were alternately fixed at 12 h, and accordingly the duration of the opposite phase (D or L) was either shortened or prolonged. The larvae were exposed to >500μW/cm$^2$ light during the L phase. Under these conditions, the number of adults that emerged ($n$=48-351) were counted within a few minutes ( using a dim red light of <100μW/cm$^2$ during dark phases ) at 1-h intervals (see 8). The $\varphi_E$ of the adult eclosion rhythm was represented by the mean ± standard deviation (SD). The recordings of adult eclosion during the entire observation period for each non-24 h photoperiod were compiled to analyze the $\varphi_E$.

## 3. Results

### 3.1. Adult Eclosion at 30°C

Under LD 12:12 h at 30°C, the $\varphi_E$ occurred at Zt 5.9±1.7 (mean ± SD) (light-on = Zt 0) (8). Light period of 12 h was fixed and dark period was varied. When the photoperiod was LD 12:2 h - LD 12:5 h, >60% of the larvae eclosed as adults within 2 h after light-off (data not shown). Under these LD cycles, adult eclosion seemed to be triggered simply by the light-off stimuli. Eclosion peaks in the <24 h cycles were found at Zt 15.1±3.7, Zt 11.4±2.5, Zt 12.1±2.3, Zt 9.9±3.0, Zt 8.7±2.2 and Zt 5.9±2.5 under LD 12:6 h (Fig. 1A), LD 12:7 h (Fig. 1B), LD 12:8 h (Fig. 1C), LD 12:9 h (Fig. 1D), LD 12:10 h (Fig. 1E) and LD 12:11 h (Fig. 1F), respectively: $\varphi_E$ advanced as dark period was prolonged. Eclosion peaks in the >24 h cycles were Zt 1.8±3.4, Zt 26.0±3.1, Zt 22.7±4.1, Zt 24.4±4.2, Zt 23.6±5.4 and Zt 20.4±5.1 for LD 12:13 h (Fig. 1G), LD 12:14 h (Fig. 1H), LD 12:15 h (Fig. 1I), LD 12:16 h (Fig. 1J), LD 12:17 h (Fig. 1K) and LD 12:18 h (Fig. 1L), respectively: $\varphi_E$ advance was observed as the dark period was prolonged (see also Fig. 6). It should be noted that the standard deviation under LD 12:17 h and LD 12:18 h was >5.0 indicating that adult eclosion occurred across a comparatively wide time scale. Therefore, the rhythm was entrained in the range of 18-30 h LD cycles.

In the second series of experiments, the dark period was fixed at 12 h and the light period was varied at a temperature of 30°C. Under LD 2:12 h - LD 5:12 h, approximately 50% of the larvae emerged as adults in the 3 h immediately following light-off (data not shown), likely because of a direct response to light-off stimuli. The lower limit of entrainability seemed to be a cycle length of 18 h. For LD 6:12 h (Fig. 2A), LD 7:12 h (Fig. 2B), LD 8:12 h (Fig. 2C), LD 9:12 h (Fig. 2D), LD 10:12 h (Fig. 2E) and LD 11:12 h (Fig. 2F), the eclosion peak of the rhythm was at Zt 12.5±3.7, Zt 11.9±3.0, Zt 9.9±3.0, Zt 8.9±1.8, Zt 6.0±2.7 and Zt 5.2±2.4, respectively (light-on = Zt 0). The eclosion peak advanced as the total length of the LD cycle was prolonged. In LD 13:12 h (Fig. 2G) and LD 14:12 h (Fig. 2H), the peak was found at Zt 4.4±3.6 and Zt 3.3±5.0, respectively (see also Fig. 7). For LD 15:12 h - LD 18:12 h, adult eclosion pattern was arrhythmic (data not shown). Thus, the upper end of entrainability was cycle length of 26 h.

### 3.2. Adult Eclosion at 25°C

At 25°C, eclosion peak was at Zt 5.0±1.9 (light-on = Zt 0) under LD 12:12 h (8). Light period of 12 h was fixed and dark period was varied systematically. Under LD 12:2 h - LD 12:5 h, adult eclosion was arrhythmic (data not shown). Under LD 12:6 h (Fig. 3A), LD 12:7 h (Fig. 3B), LD 12:8 h (Fig. 3C), LD 12:9 h (Fig. 3D), LD 12:10 h (Fig. 3E) and LD 12:11 h (Fig. 3F), eclosion peaks occurred at Zt 11.9±3.3, Zt 11.9±2.4, Zt 10.1±2.8, Zt 11.4±1.3, Zt 8.5±1.5 and Zt 4.8±2.9, respectively. The lower end of entrainability appeared to be with the cycle length of 18-19 h. The eclosion peak advanced as the cycle length was prolonged. For LD 12:13 h (Fig. 3G), LD 12:14 h (Fig. 3H), LD 12:15 h (Fig. 3I), LD 12:16 h (Fig. 3J), LD

12:17 h (Fig. 3K) and LD 12:18 h (Fig. 3L), $\varphi_E$ fell at Zt 3.3±3.2, Zt 1.8±3.4, Zt 25.5±3.9, Zt 23.6±3.9, Zt 23.1±3.7 and Zt 20.9±5.3, respectively. The peak advanced as the cycle length was prolonged (see also Fig. 6). In LD 12:18, SD was notably large (>5.0). Thus, upper end of entrainability was a cycle length of approximately 30 h.

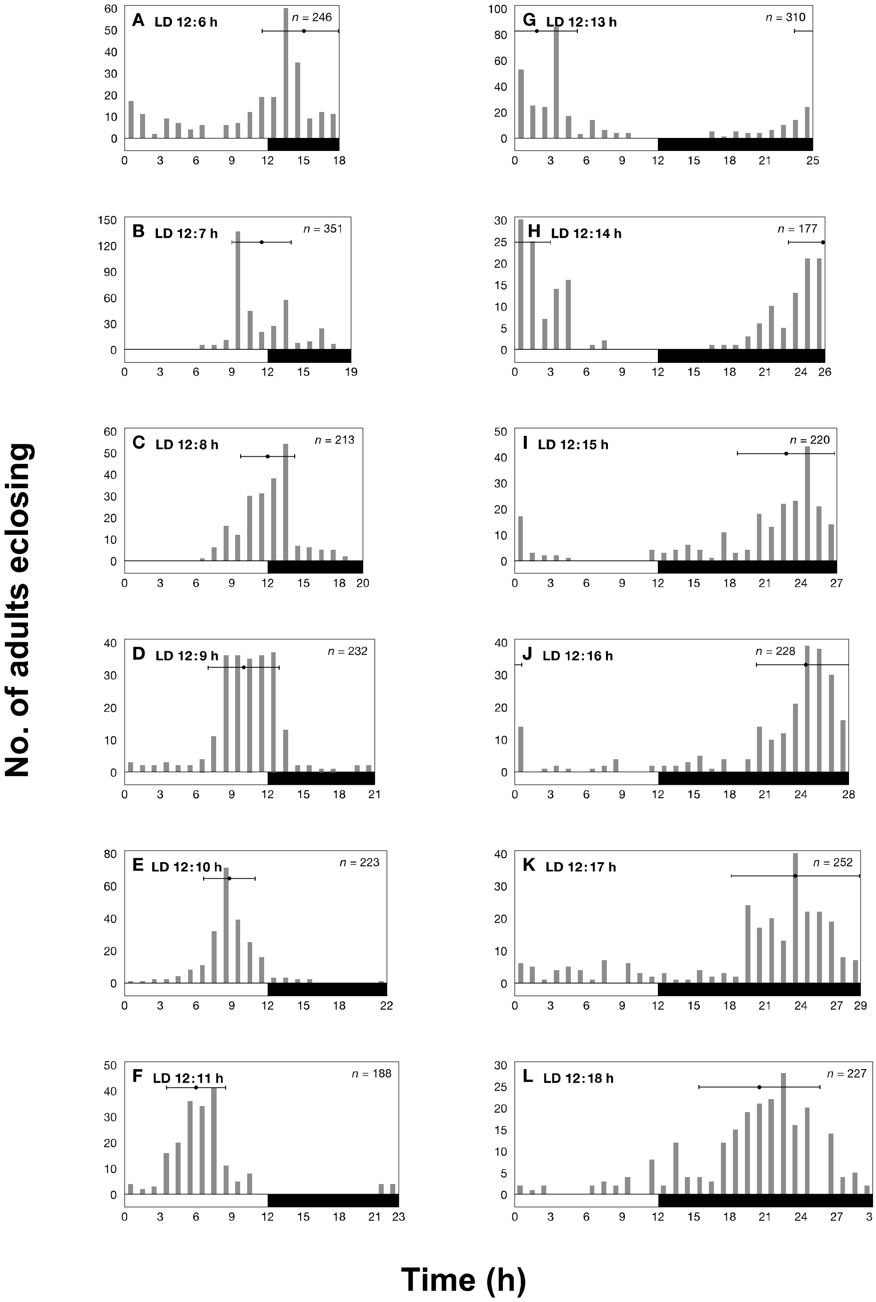

**Figure 1.** Adult eclosion of the Indian meal moth *P. interpunctella* under various non-24 h photoperiods at 30°C. (A) LD 12:7 h, (B) LD 12:8 h, (C) LD 12:9 h, (D) LD 12:10 h, (E) LD 12:11 h, (F) LD 12:13 h, (G) LD 12:14 h and (H) LD 12:15 h where L represents light phase and D represents dark phase. The number (*n*) of adults that demonstrated eclosion was observed at 1-h intervals. Horizontal white and black bars show the L and D phase of each photoperiod, respectively. ● indicates the mean eclosion time with standard deviation (bar). Note that the L phase was held constant at 12 h.

In other experiments, the dark period was fixed in 12 h and the light period was varied. In LD 2:12 h - LD 5:12 h, adult eclosion rhythm was not observed (data not shown). Under LD 6:12 h (Fig. 4A), LD 7:12 h (Fig. 4B), LD 8:12 h (Fig. 4C), LD 9:12 h (Fig. 4D), LD 10:12 h (Fig. 4E), LD 11:12 h (Fig. 4F), LD 13:12h (Fig. 4G), LD 14:12 h (Fig. 4H), LD 15:12 h (Fig. 4I), LD 16:12 h (Fig. 4J) and LD 17:12 h (Fig. 4K), $\varphi_E$ was Zt 11.6±3.5, Zt 9.6±2.9, Zt 8.8±1.8, Zt 8.6±1.8, Zt 8.2±2.5, Zt 6.4±1.3, Zt 4.4±2.1, Zt 3.6±2.3, Zt 2.3±2.7, Zt 0.1±3.9 and Zt 27.7±4.5, respectively. For LD 18:12 h, no rhythm was found. Thus, lower and upper ends of the entrainability were the cycle lengths of 18 h and 29 h, respectively. It should be noted that the $\varphi_E$ did advance as the LD cycle became longer (see also Fig. 7).

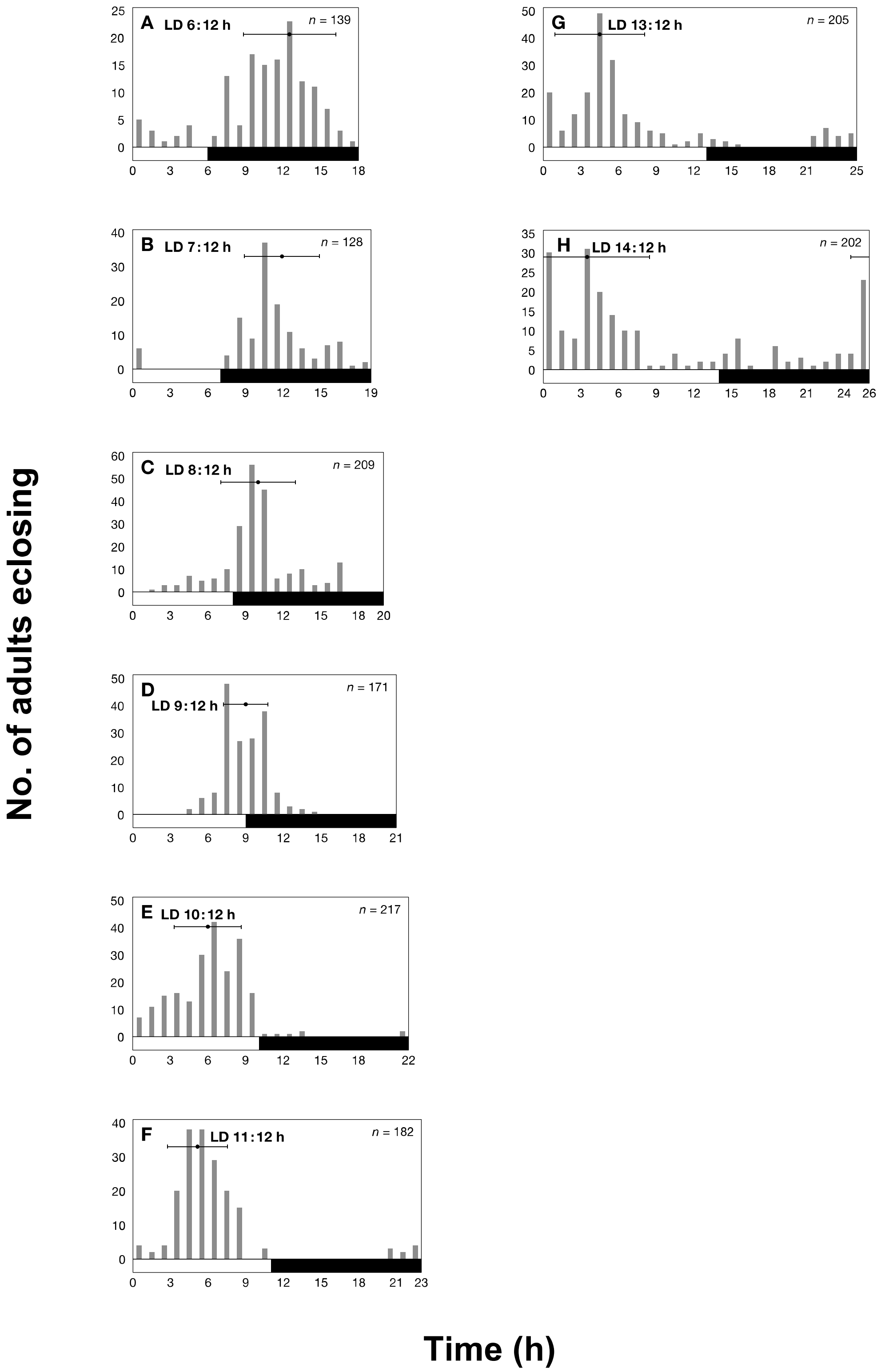

**Figure 2.** Adult eclosion of *P. interpunctella* under various non-24 h photoperiods at 30°C. (A) LD 6:12 h, (B) LD 7:12 h, (C) LD 8:12 h, (D) LD 9:12 h, (E) LD 10:12 h, (F) LD 11:12 h, (G) LD 13:12 h and (H) LD 14:12 h (refer to Fig. 1). Note that the D phase was held constant at 12 h.

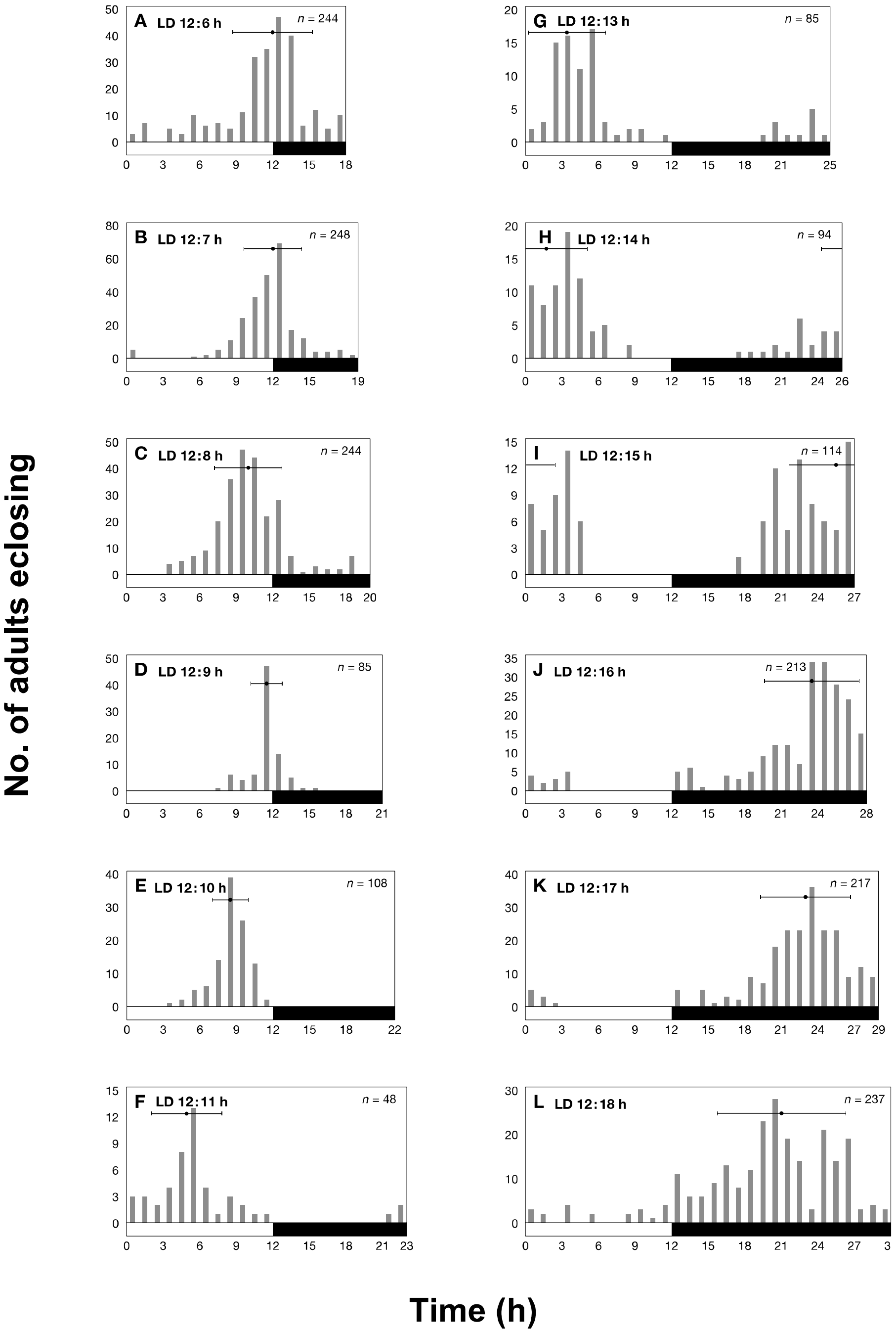

**Figure 3.** Adult eclosion of *P. interpunctella* under various non-24 h photoperiods at 25°C. (A) LD 12:6 h, (B) 12:7 h, (C) LD 12:8 h, (D) LD 12:9 h, (E) LD 12:10 h, (F) LD 12:11 h, (G) LD 12:13 h, (H) LD 12:14 h, (I) LD 12:15 h. (J) LD 12:16 h, (K) LD 12:17 h and (L) LD 12:18 h (refer to Fig.1). Note that the L phase was held constant at 12 h.

## 3.3. Adult Eclosion at 20°C

Under LD 12:12 h at 20°C, the $\varphi_E$ was observed at Zt 3.7±3.9 (light-on = Zt 0) (8). At 20°C, the rhythm did not free-run in DD, indicating that the time-keeping system involved did rapidly dump at this temperature (8). When the larvae were exposed to LD 12:6 h - LD 12:10 h and LD 12:14 h - LD 12:18 h, adult eclosion was arrhythmic (data not shown). Under LD 12:11 h (Fig. 5A) and LD 12:13 h (Fig. 5B), $\varphi_E$ was at Zt 6.7±5.2 and Zt 5.8±5.1, respectively. In both cases, SD was quite large (>5.0). Under LD 6:12 h - LD 10:12 h and LD 14:12 h - LD 18:12 h, eclosion was again arrhythmic (data not shown). For LD 11:12 h (Fig. 5C), the $\varphi_E$ was at Zt 5.1±4.1. For LD 13:12 h (Fig. 5D), the $\varphi_E$ was not identified. Thus, at 20°C, the adult eclosion rhythm was entrained by a photoperiod close to 24 h.

At 30°C-20°C, growth rate and mortality of *P. interpunctella* were not influenced by the cycle length of the photoperiod, unlike in some other insect species (*eg*., see 11).

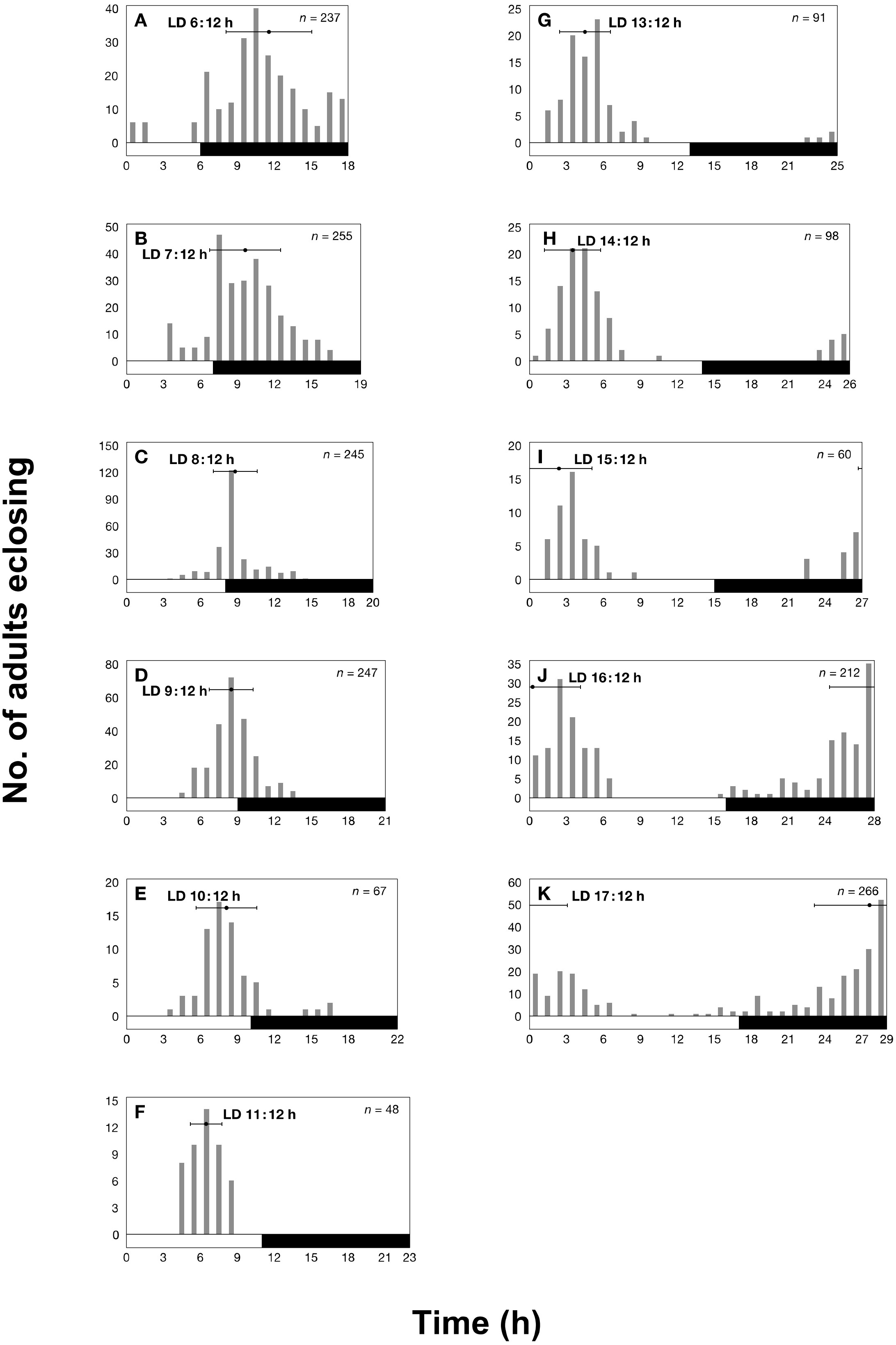

**Figure 4.** Adult eclosion of *P. interpunctella* under various non-24 h photoperiods at 25°C. (A) LD 6:12 h, (B) LD 7:12 h, (C) LD 8:12 h, (D) LD 9:12 h, (E) LD 10:12 h, (F) LD 11:12 h, (G) LD 13:12 h, (H) LD 14:12 h, (I) LD 15:12 h, (J) LD 16:12 h and (K) LD 17:12 h (refer to Fig. 1). Note that the D phase was held constant at 12 h.

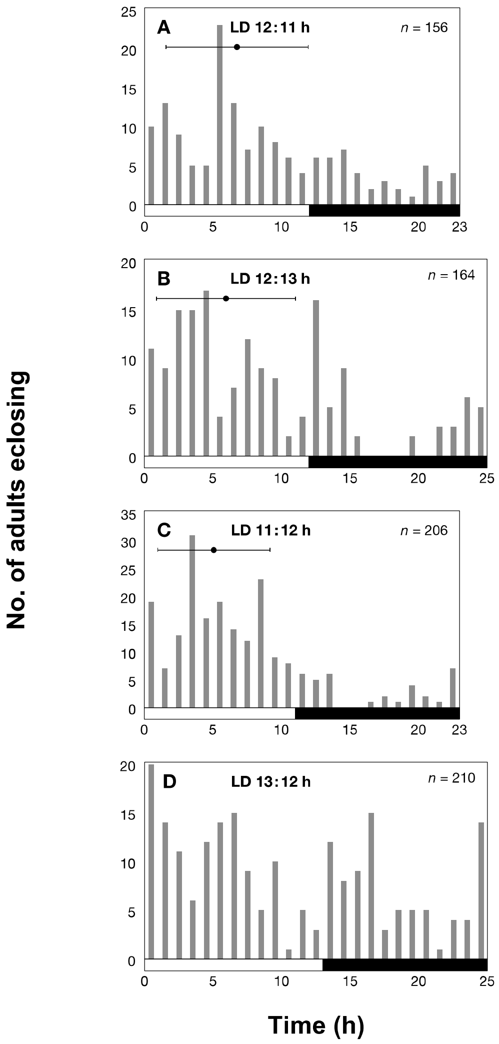

**Figure 5.** Adult eclosion of *P. interpunctella* under different non-24 h photoperiods at 20°C. (A) LD 12:11 h, (B) LD 12:13 h, (C) LD 11:12 h and (D) LD 13:12 h (refer to Fig. 1).

## 3.4. Linear Regression Analysis

Figure 6 is a summary of Figures 1 and 3 in which the light period was fixed at 12 h and dark period was varied at both 30°C and 25°C, respectively. There was no statistically significant difference between the temporal positions of the adult eclosion peaks at 30°C and 25°C. Thus, data obtained were pooled for the regression analysis: $y=-1.88x+48.90$ ($r=-0.98$, $P<0.001$). The longer the cycle length, the earlier $\varphi_E$ occurred. The time-keeping system might be entrained by the light-off signal, but the regression line was not statistically parallel to the light-off line in Figure 6. Apparently, $\varphi_E$ may also be affected by the light-on signal.

Figure 7 illustrates the regression line based on the $\varphi_E$ obtained from Figures 2 and 4 in which the dark period was fixed in 12 h and the light period was varied at 30°C and 25°C, respectively. There was no statistically significant difference between $\varphi_E$ at 30°C and 25°C. The equation of the regression line was $y=-1.12x+32.15$ ($r=-0.98$, $P<0.001$). As the cycle length became longer, $\varphi_E$ advanced. Unexpectedly, however, the temporal position of $\varphi_E$ was not related to the light-off and/or light–on signals. Thus, $\varphi_E$ was determined as a function of the cycle length.

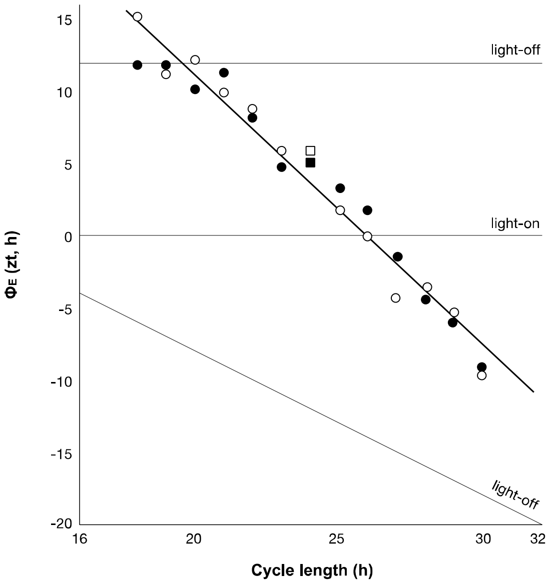

**Figure 6.** Temporal relationship between adult eclosion peak ($\varphi_E$) of *P. interpunctella* and cycle lengths of non-24 h photoperiods. The light period was fixed at 12 h (light-on = Zt 0). ○: 30°C and ●: 25°C. □ and ■ (LD 12:12 h) show data from Kikukawa *et al.* (8) at 30°C and 25°C, respectively. The regression line was defined as $y=-1.88x+48.90$ ($r=-0.98$, $P<0.001$). Data obtained from Fig. 1 (30°C) and Fig. 3 (25°C ) were pooled as the difference between these data was not statistically significant.

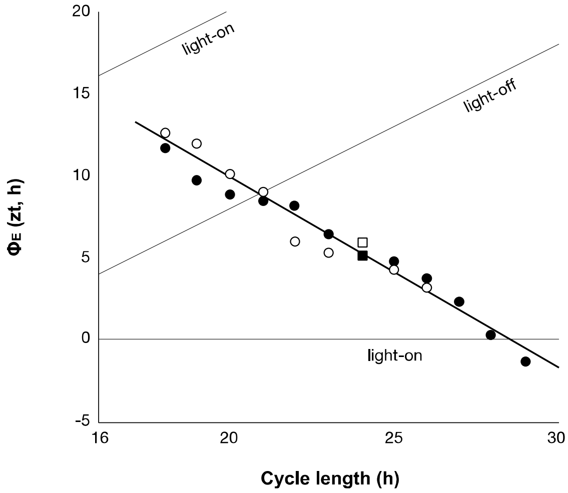

**Figure 7.** Temporal relationship between adult eclosion peak ($\varphi_E$) of *P. interpunctella* and cycle length of non-24 h photoperiods. The dark phase was fixed at 12 h (light-on = Zt 0). ○: 30°C and ●: 25°C. □ and ■ (LD 12:12 h) show data from Kikukawa *et al.* (8), at 30°C and 25°C, respectively. The regression line was defined as $y=-1.12x+32.15$ ($r=-0.98$, $P<0.001$). Data obtained from Fig. 2 (30°C ) and Fig. 4 (25°C ) were pooled as the difference between these data was not statistically significant.

## 4. Discussion

At 25°C, a single light pulse (2-16 h) in otherwise DD conditions entrains the adult eclosion rhythm of *P. interpunctella* (7). The average time from the light-off signal of the light pulse to the first peak is *c.* 17.5 h. This rhythm free run for a period of *c.* 23 h, indicated that the light-off signal is a major time cue for the time-keeping system.

This study further investigated the entrainability of this adult eclosion clock under non-24 h photoperiods at 30°C-20°C. The adult eclosion rhythm under non-24 h LD cycles has not been observed extensively among insect species (3). At 30°C and 25°C, when the light period is fixed at 12 h and the dark period is varied, $\varphi_E$ is advanced as cycle length increases. No significant difference of the time-keeping process was detected between 30°C and 25°C conditions. Light-off appears to be the major Zeitgeber stimulus to entrain the eclosion rhythm in which the $\varphi_E$ is modified by light-on signal slightly and this has been suggested by Kikukawa *et al.* (8). When the dark period is fixed at 12 h and the light period is varied at 30°C and 25°C, we find an unexpected and curious phenomenon. The eclosion peak advances with cycle length but it does not seem to be entrained simply by the light-off or light–on signal: the $\varphi_E$ is a function of cycle length. This means that the time-keeping system must read the time interval from the light-off signal of a given cycle to that of the subsequent cycle, or the time interval from the light-on signal of a given cycle to that of the next cycle. It is proved that both the light-off and light–on signals can act as time cues, otherwise larvae do not sense the length of the LD cycle. As has been stated, the dark period is a constant 12 h: is not an abnormal duration (1, 2). The $\varphi_E$ determined by the clock system should be further investigated by using a different photo-regime.

At 20°C, the adult eclosion rhythm of *P. interpunctella* does not free-run in DD conditions (8). Eclosion peak occurs at Zt 3.7 ± 3.9 (light-on = Zt 0) under LD 12:12 h, so that the eclosion rhythm could be entrained by a 24 h photoperiod. The present study clearly shows that the clock system could not operate under non-24 h (<23 h or >25 h) photoperiods at a low temperature of 20°C. This is a significant finding and is not commonly found among insect species.

## 5. Conclusion

We determined the entrainability of the time-keeping system for the adult eclosion rhythm of *P. interpunctella*. Under non-24 h photoperiods at 30°C and 25°C, the $\varphi_E$ was regulated mainly by the light-off signal but was affected by the light-on signal, when the light period was fixed at 12 h. However, when the dark period was fixed at 12 h, $\varphi_E$ was a function of the cycle length of the photoperiod. A significant finding was that the time-keeping system ‘knew’ the length of the LD cycle. At 20°C, the eclosion clock operated only in the LD cycles that were very close to 24 h. It is recommended that the experimental protocol used in this study should be employed against other insect species in order to better understand the nature of their time-measuring system.

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