

Adult Eclosion Rhythm of *Plodia interpunctella*: Response to Photoperiod and Thermoperiod with Different Phase Angles

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Abstract

The interacting effects of photoperiod and thermoperiod with different phase angles on the adult eclosion rhythm of the Indian meal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae), are investigated. The photoperiod is LD 12:12 h (L=light and D=dark) and the thermoperiods (TC 12:12 h where T=thermophase and C=cryophase) are 30°C/20°C, 28.5°C/21.5°C, 28°C/22°C, 27.5°C/22.5°C, 26.5°C/23.5°C and 25.5°C/24.5°C. For each temperature cycle, the average temperature is 25°C. When the photoperiod is superimposed on to the thermoperiod of 30°C/20°C, the temporal position of the adult eclosion peak (ϕ_E) is within the thermophase (30°C) regardless of the LD cycle. However, when the thermoperiod is 25.5°C/24.5°C, ϕ_E occurs at approximately Zeitgeber time (Zt) 3.3 after the light-on signal, regardless of the phase difference between photoperiod and thermoperiod. For the thermocycles of 28.5°C/21.5°C - 26.5°C/23.5°C, intermediate responses are observed. When 27.5°C/22.5°C is coupled with LD 12:12 h and the phase angle is changed, a phase jump of ϕ_E occurs at 12-16 h after light-on of the phase difference between LD cycle and TC cycle. This strongly indicates that at least two components of the time-keeping system are involved in determining the temporal position of ϕ_E . Thermocycle, therefore, appears to be a useful tool for analyzing temporal organization of the adult eclosion rhythm of this insect species.

Keywords

Adult Eclosion Rhythm, Indian Meal Moth, Photoperiod, Thermoperiod

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1. Introduction

Physiological and behavioral events governed by circadian clock have been investigated for many insect species [1, 2 for review]. The timely induction of adult eclosion is controlled by one of these time-keeping systems. For the majority of cases, photoperiod and /or thermoperiod are employed as a Zeitgeber (time cue). However, the interacting effects of photoperiod and thermoperiod superimposed at different phase angles have rarely been investigated [e.g., 3-6]. Watari and Tanaka [7] studied the interacting effects of photoperiod

and thermoperiod on the adult eclosion peak in the onion fly *Delia antiqua*. They found that under a photoperiod of LD (light-dark cycle) 12:12 h combined with a thermoperiod of TC (thermophase: cryophase) 12:12 h at 29°C/21°C, the eclosion rhythm was found to be entrained by the thermoperiod regardless of the phase angle. On the other hand, when a thermoperiod of 25.5°C/24.5°C was employed, the thermoperiod was neglected and the photoperiod combined with the thermoperiod entrained the rhythm. When the thermoperiod of 27°C/23°C was combined with the photoperiod, the temporal position (ϕ_E) of the eclosion peak was determined by the interaction of thermoperiod and

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photoperiod. A phase jump of φ_E was observed when the thermophase was delayed 15-18 h from the light-on (L-on) signal. The findings from this study confirm the existence of the two-oscillator model originally proposed by Pittendrigh and Bruce [3].

The Indian meal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) is used in the present study in order to elucidate the interacting effects of photoperiod and thermoperiod on the adult eclosion rhythm which is an intriguing aspect of the circadian clock system. *P. interpunctella* is an excellent model insect species for analyzing the adult eclosion rhythm and it is known that the rhythm is entrained by photoperiod [8, 9] and/or thermoperiod [8, 10]. Pertaining to a non-24 h cycle of photoperiod [11] or thermoperiod [12], φ_E shifts differently depending on the cycle lengths indicating there are possibly two or more processes of time-keeping systems involved in the determination of φ_E . Thus, in the present study, the effect of the phase relationship between photoperiod and thermoperiod is investigated for *P. interpunctella* to confirm the two-oscillator model [3, 7].

2. Materials and Methods

A laboratory culture of *P. interpunctella* was established from larvae collected at Toyama, Japan in 1998 and maintained as described previously [9]. These insects enter diapause at the 5th larval instar in response to environmental conditions such as short photoperiods and/or thermoperiods [13, 14]. In the present study, to prevent the insects from entering diapause, eggs deposited within 24 h in constant darkness (DD) were maintained at 30°C in DD for 14 days after oviposition. This treatment prevented diapause induction completely. The larvae were then exposed to different temperature cycles (TC 12:12 h) (T=thermophase and C=cryophase) and photoperiod (LD 12:12 h) (L=light phases and D=dark phase) with different phase angles. The temperature cycles utilized were 30°C/20°C, 28.5°C/21.5°C, 28°C/22°C, 27.5°C/22.5°C, 26.5°C/23.5°C and 25.5°C/24.5°C. For each cycle, the average temperature was 25°C. Under LD 12:12h at a constant temperature of 25°C, φ_E was at Zt 5.0±1.9 after L-on [9]. To determine whether the presence or absence of light during the initial 14-day period at 30°C affects φ_E , constant light condition (LL) was also examined under photoperiod of LD 12:12 h and thermoperiod of 30°C/20°C (see Fig. 1). Larvae were exposed to >500 μ W/cm² during the light phase. Under these conditions, the number of adults that emerged ($n=50-165$) were counted within a few minutes at 1-h intervals under a dim red light of <100 μ W/cm² during both the dark phase and light phase. The temporal position of the peak (φ_E) of the adult eclosion rhythm was represented as the mean \pm

standard deviation (SD). The recordings of adult eclosion (days 27-31 after oviposition) during the entire observation period for each treatment were compiled to analyze φ_E . The L-on time was defined as Zt (Zeitgeber time) 0. The phase difference was expressed as the duration from L-on to temperature-rise (T-rise).

3. Results

3.1. Temperature Cycle of 30°C/20°C with Photoperiod

Larvae were maintained under LL at 30°C for 14 days after oviposition to prevent from entering diapause. Larvae were then exposed to a temperature cycle of 30°C/20°C combined with a photoperiod of LD 12:12 h with different phase angles (Fig. 1A-F). When the phase difference from L-on to T-rise was zero (Fig. 1A), φ_E appeared at Zt 7.0±2.3 (mean \pm SD) (L-on = Zt 0). However, as the phase difference became larger, φ_E was delayed, showing at Zt 9.4±1.8, Zt 12.0±2.5, Zt 14.7±3.1, Zt 21.5±4.4 and Zt 3.1±1.8 in 4 h (Fig. 1B), 8 h (Fig. 1C), 12 h (Fig. 1D), 16 h (Fig. 1E) and 20 h (Fig. 1F) phase differences, respectively.

When the larvae were pre-incubated under DD at 30°C for 14 days after oviposition and transferred to the experimental conditions, φ_E was at Zt 7.4±1.8, Zt 9.4±1.4, Zt 11.9±2.0, Zt 15.0±2.8, Zt 20.4±4.9 and Zt 3.3±1.8 in the phase differences of 0 h (Fig. 1G), 4 h (Fig. 1H), 8 h (Fig. 1I), 12 h (Fig. 1J), 16 h (Fig. 1K) and 20 h (Fig. 1L), respectively. Similar to above, as the phase difference became larger, φ_E was delayed. There were no statistically significant differences in the temporal position of φ_E between the above two series of experiments with φ_E existing in thermophase regardless of the photoperiod (LD 12:12 h) (see also Fig. 4). The initial treatment (either LL or DD) at 30°C did not influence the φ_E , so that subsequent experiments were conducted using only the pre-treatment of DD at 30°C.

3.2. Temperature Cycles of 28.5°C/21.5°C, 28°C/22°C and 27.5°C/22.5°C with Photoperiod

Under 28.5°C/21.5°C (TC 12:12 h) with photoperiod of LD 12:12 h, slight phase advances of φ_E were observed for the phase difference 0-12 h compared to that under the 30°C/20°C regime. φ_E was observed at Zt 5.9±2.4, Zt 8.8±2.4, Zt 10.1±2.1 and Zt 11.6±3.8 in the phase differences of 0 h (Fig. 2A), 4 h (Fig. 2B), 8 h (Fig. 2C) and 12 h (Fig. 2D), respectively. At 12 h phase difference, φ_E occurred just before T-rise and then moved to Zt 23.1±4.1 and Zt 2.7±2.6 in the phase differences of 16 h (Fig. 2E) and 20 h (Fig. 2F), respectively (see also Fig. 4). The temporal position of φ_E appeared to be modified by the combination of temperature cycle and photoperiod.

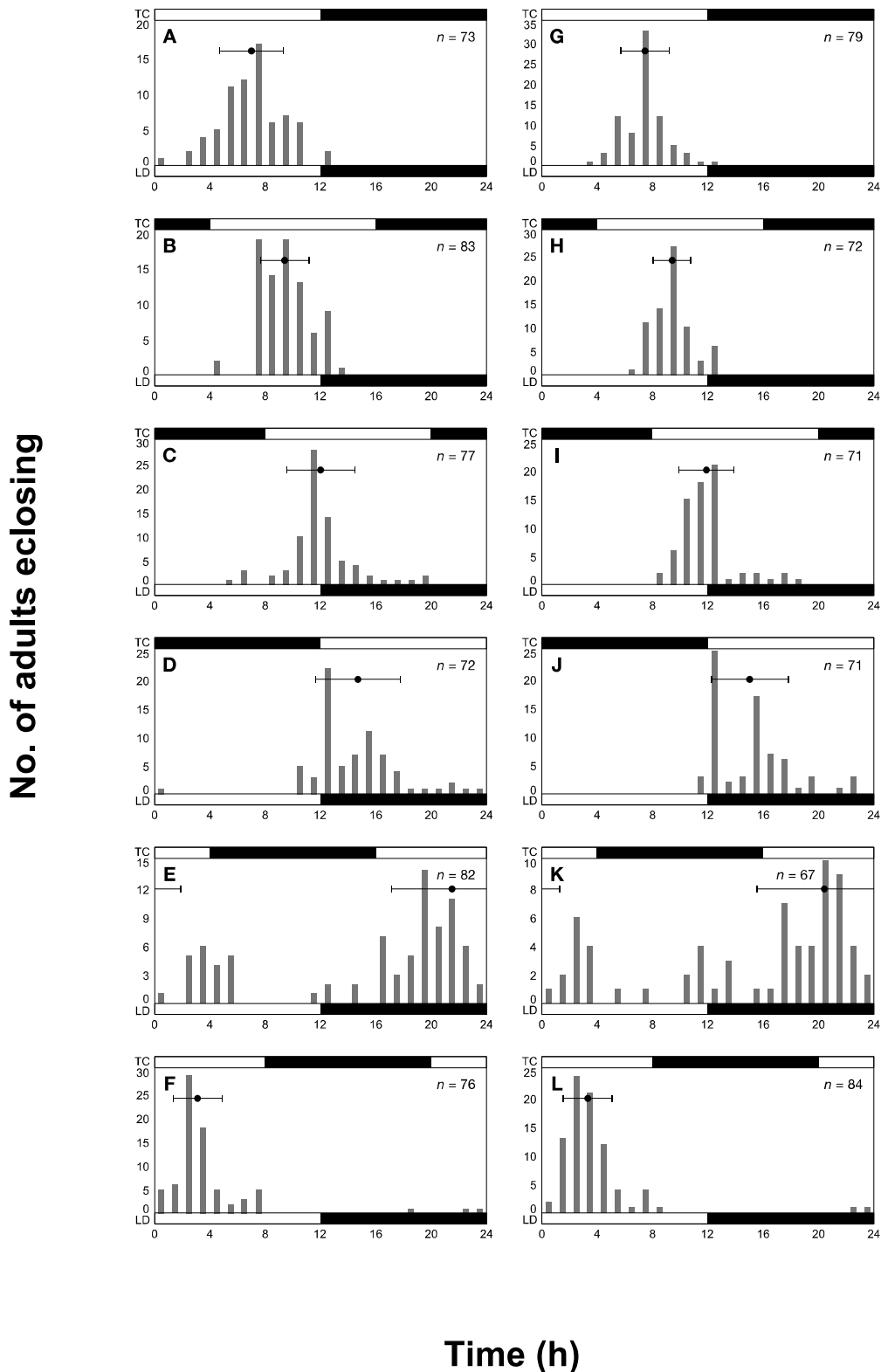


Figure 1. Adult eclosion of the Indian meal moth *Plodia interpunctella* under the various phase differences between photoperiod of LD 12:12 h (L=light and D=dark) and thermoperiod of TC 12:12 h (T=thermophase and C=cryophase). A pre-incubation (30°C) period of 14 days to prevent entering diapause was applied in constant light (LL) (A-F) or constant dark (DD) (G-L) conditions. In each panel, LD (L=white and D=black) and TC (T=white and C=black) cycles are shown as the lower and upper bars, respectively. Temperature cycle was 30°C/20°C. Phase differences (from light-on to temperature-rise) were 0 h (A and G), 4 h (B and H), 8 h (C and I), 12 h (D and J), 16 h (E and K) and 20 h (F and L). Light-on was defined as Zeigter time (Zt) 0. The number (*n*) of adults that demonstrate eclose was observed at 1-h intervals. • indicates the mean eclosion time with standard deviation (bar).

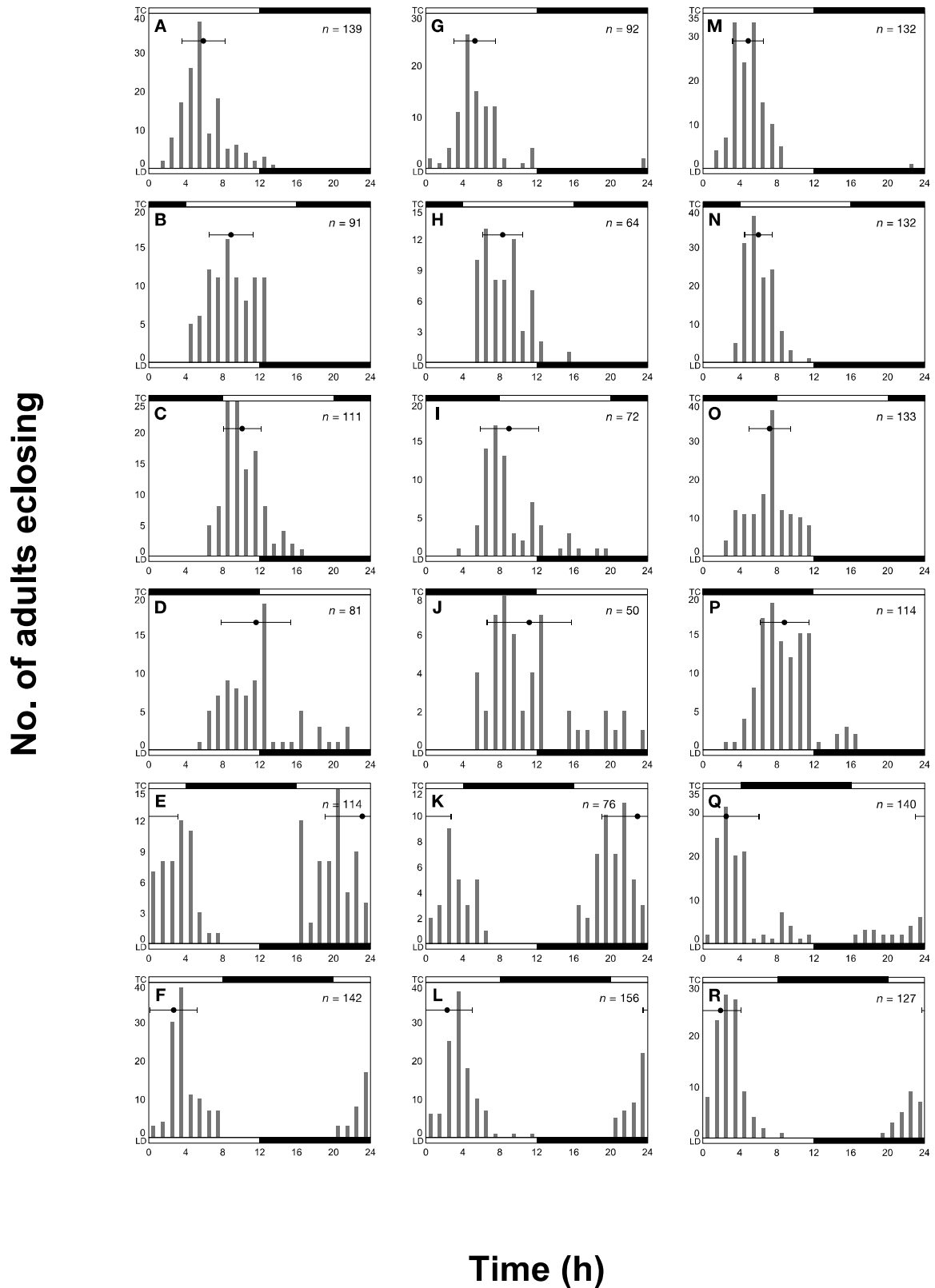


Figure 2. Adult eclosion of *Plodia interpunctella* under various phase differences between LD and TC. TC was 28.5°C/21.5°C (A-F), 28°C/22°C (G-L) and 27.5°C/22.5°C (M-R). Phase differences from light-on to temperature-rise were 0 h (A, G and M), 4 h (B, H and N), 8 h (C, I and O), 12 h (D, J and P), 16 h (E, K and Q) and 20 h (F, L and R) (refer Fig. 1).

No. of adults eclosing

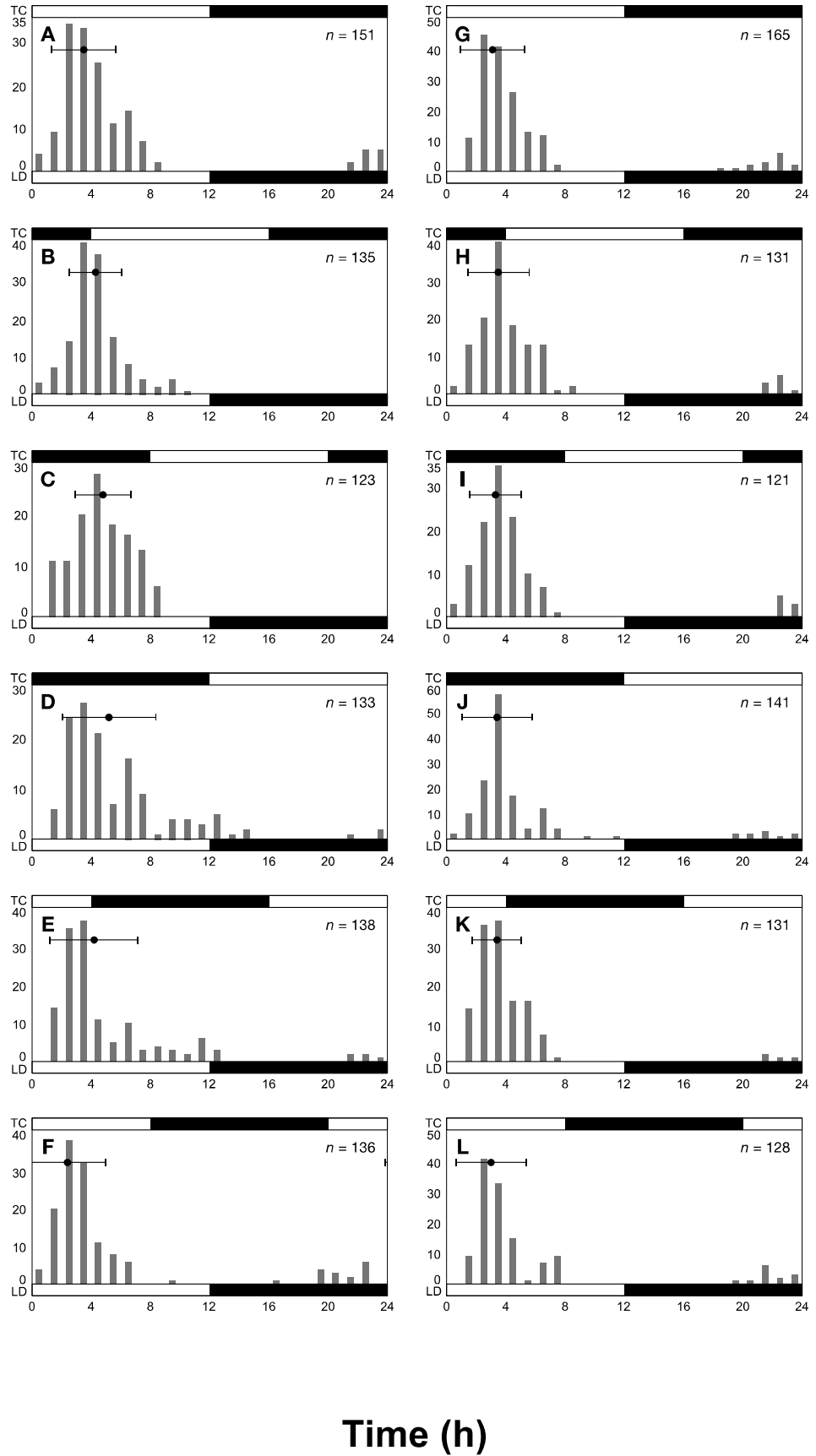


Figure 3. Adult eclosion of *Plodia interpunctella* under the various phase differences between LD and TC. TC was 26.5°C/23.5°C (A-F) and 25.5°C/24.5°C (G-L) (refer Fig. 1).

Similar results were obtained for the temperature cycle of 28°C/22°C. φ_E was at Zt 5.3±2.3, Zt 8.3±2.2, Zt 9.0±3.2, Zt 11.2±4.6, Zt 22.9±3.9 and Zt 2.3±2.8 in the phase differences of 0 h (Fig. 2G), 4 h (Fig. 2H), 8 h (Fig. 2I), 12 h (Fig. 2J), 16 h (Fig. 2K) and 20 h (Fig. 2L) (see also Fig. 4).

However, when the temperature cycle of 27.5°C/22.5°C was utilized, quite different responses were obtained. φ_E was at Zt 4.9±1.7, Zt 6.0±1.5, Zt 7.2±2.3 and Zt 8.8±2.7 under the phase differences (from L-on to T-rise) of 0 h (Fig. 2M), 4 h (Fig. 2N), 8 h (Fig. 2O) and 12 h (Fig. 2P), respectively, which indicated that the peak of the rhythm occurred quicker than that for the 28.5°C/21.5°C and 28°C/22°C regimes. φ_E then jumped to Zt 2.5±3.6 and Zt 1.9±2.3 in the phase differences of 16 h (Fig. 2Q) and 20 h (Fig. 2R), respectively (see also Fig. 4).

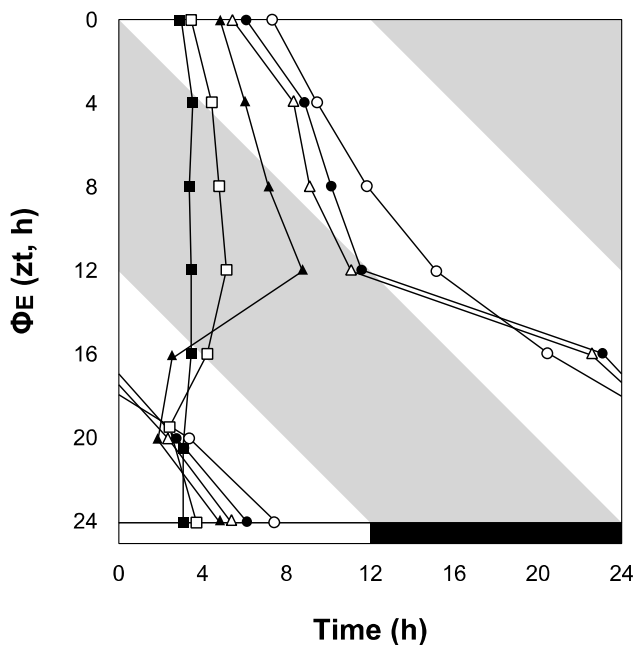


Figure 4. Temporal relationship between adult eclosion peak (φ_E) of *Plodia interpunctella* and LD combined with TC at different phase angles. LD 12:12 h is shown at the bottom of the figure (L=white and D=black). The white portion of the figure is T and the gray portion is C. ○: 30°C/20°C, ●: 28.5°C/21.5°C, ▲: 28°C/22°C, ▲: 27.5°C/22.5°C, □: 26.5°C/23.5°C and ■: 25.5°C/24.5°C. Data were obtained from Figs. 1-3.

3.3. Temperature Cycles of 26.5°C/23.5°C and 25.5°C/24.5°C with Photoperiod

Temperature cycles of 26.5°C/23.5°C and 25.5°C/24.5°C did not exert a considerable effect on φ_E when these cycles were combined with the photoperiod of LD 12:12 h. For the temperature cycle of 26.5°C/23.5°C, φ_E was at Zt 3.5±2.2, Zt 4.3±1.8, Zt 4.8±1.9, Zt 5.2±3.2, Zt 4.2±3.0 and Zt 2.4±2.6 in the phase differences of 0 h (Fig. 3A), 4 h (Fig. 3B), 8 h (Fig. 3C), 12 h (Fig. 3D), 16 h (Fig. 3E) and 20 h (Fig. 3F), respectively (see also Fig. 4). For the 25.5°C/24.5°C, φ_E was found at Zt 3.1±2.2, Zt 3.5±2.1, Zt 3.3±1.8, Zt 3.4±2.4, Zt

3.4±1.7 and Zt 3.0±2.4 in the phase differences of 0 h (Fig. 3G), 4 h (Fig. 3H), 8 h (Fig. 3I), 12 h (Fig. 3J), 16 h (Fig. 3K) and 20 h (Fig. 3L), respectively (see also Fig. 4). This implies that thermocycles with small amplitudes appeared not to be the major time cue for this insect species.

4. Discussion

The interacting effects of thermoperiod and photoperiod on φ_E are summarized in Figure 4, where it can be seen that as the amplitude of the temperature cycle decreases, the response curve shifts from right to left. Under LD 12:12 h at a constant temperature of 25°C, φ_E is located at Zt 5.0±1.9 after L-on [9]. When the amplitude of the temperature cycle is large, φ_E is delayed and when it is small, φ_E is advanced to some extent. Since the average temperature in the present study was 25°C for all the thermocycles tested, this shift of φ_E occurred according to the amplitude of the temperature cycle. For the thermocycles of 30°C/20°C, 28.5°C/21.5°C and 28°C/22°C, φ_E was determined regardless of the photoperiod of LD 12:12 h. Under the temperature cycles of 26.5°C/23.5°C and 25.5°C/24.5°C, φ_E was for the most part defined by the photoperiod. Under the temperature cycle of 27.5°C/22.5°C, φ_E jumped between the phase differences of 12 and 16 h. Therefore, at least two components of the time-keeping system are involved in *P. interpunctella* adult eclosion rhythms similar to what has been proposed for *Drosophila pseudoobscura* [3, 4] and *Delia antiqua* [7]: i.e., the two-oscillator model. However, it should be noted that the φ_E under temperature cycles of 26.5°C/23.5°C and 25.5°C/24.5°C DD is at Zt 1.5±2.4 and Zt 21.9±3.2 (T-rise = Zt 0), respectively [10]. The average φ_E under the photoperiod of LD 12:12 h combined with the thermocycle of 26.5°C/23.5°C and 25.5°C/24.5°C (TC 12:12 h) is 4.1 h and 3.3 h after L-on, respectively. Thus, the photoperiod of LD 12:12 h is not simply ignored: i.e., there is the interaction between photoperiod and thermoperiod even when the amplitude of the temperature cycle is very small.

5. Conclusions

The aim of this study was to elucidate whether the two-oscillator model [3, 4, 7] is involved in the time-keeping system of *P. interpunctella*. The interacting effects of photoperiod of LD 12:12 h and thermoperiod of TC 12:12 h (30°C/20°C-25.5°C/24.5°C) on the temporal position of φ_E were investigated by changing the phase angle. Since the amplitude of the temperature cycle could be controlled to create the different intensities of the time cue (Zeitgeber) (temperature-rise or -fall), a change of φ_E was systematically observed. A phase jump was found in the phase differences of

12 and 16 h under LD 12:12 h with a thermocycle of 27.5°C/22.5°C. This finding strongly suggests the involvement of at least two or more components of the time-keeping system in this species. Similar experiments for other insect species should be conducted in the future to generalize the circadian rhythm model.

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