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論文：

Time-measuring mechanism controlling hatching behavior triggered by light-on signal in the katydid *Eobiana engelhardti subtropica* Bey-Bienko (Orthoptera: Tettigoniidae)

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Abstract

The mechanisms involved in triggering hatching behavior in the katydid *Eobiana engelhardti subtropica* (Orthoptera: Tettigoniidae) remain largely unknown. Therefore, in this study, I aimed to clarify the mechanism of hatching behavior triggered by a light-on (L-on) signal. Hatching under transition from continuous darkness to continuous light (i.e., L-on step, dark pulses, and photoperiods) was concentrated within 2 h after the L-on, indicating that it was triggered by the L-on signal. Furthermore, hatching was directly triggered by a single L-on signal. However, the time from L-on to hatching under dark pulses and photoperiods was affected by the duration of the dark period. This dependency on the dark period's duration before L-on indicates that it differs from the hatching trigger under the L-on step. The time from L-on to the earliest hatching time and the mean hatching time were similar under dark pulses and photoperiods. Those times were also similar at 15 and 20°C. This suggests that the time from L-on to hatching under dark pulses and photoperiods exhibits temperature compensatory properties, a characteristic of biological clocks. The time ranges from a few minutes to several tens of minutes; however, it is suggested that it is controlled by a time-measuring mechanism involving dark periods. Although it is well known that the L-on signal is involved in the time-measuring mechanism, to the best of my knowledge, this is the first study to report that the time-measuring mechanism is involved in the triggering of hatching behavior based on the L-on signal.

"Key words: dark pulse, light-on single signal, photoperiod, time-measuring mechanism, triggering hatching behavior"

INTRODUCTION

Egg hatching times have been reported to synchronize with photoperiods in several insects orders, including Phasmatodea (Kalmus, 1938), Hemiptera (White, 1968; Arai *et al*., 1994), Diptera (Nayar *et al*., 1973; Arai, 1975), Lepidoptera (Tanaka, 1961; Edwards, 1964; Minis & Pittendrigh, 1968; Riddiford & Johnson, 1971; Sasaki, 1976), Coleoptera (Arai, 1987; Arai & Matsusita, 2005), and Orthoptera (Arai, 1977, 1979ab, 1998ab, 2021a; Tomioka et al., 1991; Shimizu & Masaki, 1997; Itoh & Sumi, 2000; Nishide & Tanaka, 2016; Tanaka, 2017, 2018). However, very few reports show that an environmental change directly triggers hatching behavior. The hatching behavior of *Eobiana engelhardti subtropica* (Arai, 1977, 1979a) and *Chizuella bonneti* (Arai, 2021a) was triggered by light-on (L-on), and that of *E. engelhardti subtropica* was also triggered by oxygen exposure (Arai, 2021b). Additionally, it has been reported that the hatching behavior of grasshoppers (Nishide & Tanaka, 2016; Tanaka, 2017; Tanaka *et al*., 2018) and stick bugs (Endo *et al*., 2019) was triggered and synchronized by mechanical stimuli, such as contact and vibration.

Eggs of *E. engelhardti subtropica* hatched irregularly under continuous darkness (DD) and continuous light (LL) at 15 and 20°C, respectively. However, it was concentrated within 2 h after L-on under DD to LL transition (L-on step), dark pulses, and photoperiods, indicating that hatching behavior was triggered by L-on (Arai, 1977, 1979a). Currently, several questions regarding the mechanisms involved in egg-hatching remain unanswered. Does the hatching behavior triggered by L-on occur via the same mechanism as that under the L-on step, dark pulses, and photoperiods? Alternatively, does the mechanism that induces hatching behavior differ depending on the conditions? How does the hatching behavior change if the mechanisms are different? How do these mechanisms function under each condition? Therefore, in this study, I aimed to clarify these issues by examining the time from L-on to hatching under the L-on step, dark pulses, and photoperiods in detail.

MATERIALS AND METHODS

Adult *E. engelhardti subtropica* individuals were collected in Hirosaki City, Aomori Prefecture, Japan (40.6°N, 140.5°E), during July 1975–1977, and *E. engelhardti subtropica* eggs were collected in a laboratory at Hirosaki University. Eggs were laid on moist cotton wool, transferred to moist filter papers in glass Petri dishes of 11 cm diameter within 24 h after oviposition,

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and maintained at 25°C. Eggs of *E. engelhardti subtropica* are endogenously diapause, and eggs after diapause and awakening were used in the experiments. The eggs were maintained at 25°C for approximately 10 weeks after they were laid and then at 7.0–7.5°C for 16 weeks to awaken them from diapause. Thereafter, they were used for experiments. The temperature was controlled at 7.0–7.5, 15, 20, and 25°C using incubators (Nihonikagaku K.K., Osaka, Japan). A 10 W white fluorescent lamp producing approximately ~400 lx was used as the light source. The number of hatchings was counted manually every 5 min or 2 h. During the dark phase, the number of hatchings was counted using a red filter (Kenko Optical Filter SR 64, Kenko Tokina, Tokyo, Japan); however, the hatching time was not affected by the red filter (Arai, 2012). The number of hatchings after L-on was counted every 5 min within 8 h based on the concentration and persistence of hatching. The number of hatchings after L-on was counted every 5 min on day 25 after incubation at 15°C and day 14 after incubation at 20°C under a single L-on signal and several dark pulses. Hatching was also counted under several photoperiods from days 18–33 after incubation at 15°C and from days 9–18 after incubation at 20°C. All statistical analyses were performed using EZR version 2.7-1 (Kanda, 2013).

RESULTS

Time from L-on to hatching under a single L-on signal

When a single L-on signal was applied on day 25 after incubation at 15°C or day 14 after incubation at 20°C, most eggs hatched within 2 h (Figure 1A) or 4 h (Figure 1B), respectively. After L-on, the hatching percentages within 2 and 4 h were 92.5% and 97.5% at 15°C and 53.3% and 93.3% at 20°C, respectively. Therefore, the time from L-on to hatching was tabulated by individuals within 8 h after L-on. The hatching pattern was not normally distributed when the number of hatchings was counted every 2 h; however, it was almost normally distributed when counted every 5 min. Therefore, the number of hatchings every 2 h is presented as both mean and median times, whereas that for every 5 min is presented as the means.

The mean and median times within 8 h from L-on to hatching were 75.0 ± 48.5 min and 42.5 min (37.5–70.0 min) at 15°C and 124.0 ± 76.8 min and 92.5 min (29.5–190.0 min) at 20°C, respectively (Figure 1A & 1B). When counting every 5 min, the earliest hatching triggered by L-on occurred 5–10 min after L-on at 15°C (Figure 2A) and 0–5 min after L-on at 20°C (Figure 2B). When hatchings after L-on were counted every 5 min at 15 and 20°C, the mean times from L-on to hatching were 61.5 ± 54.4 min and 117.2 ± 93.3 min, respectively.

Time from L-on to hatching under dark pulse

Dark pulses with various durations were applied on day 25 or 14 at 15 or 20°C, respectively, under LL. The number of hatchings was counted every 2 h. Only a few hatchings were observed under a 1/6 h-dark pulse at 15°C (row a in Figure 3A). Under a ≥ 0.5 h-dark pulse, hatching was concentrated within 4 h after L-on, especially within 2 h (rows b–m in Figure 3A). At 20°C, hatching was concentrated within 4 h after L-on under 2–48 h-dark pulses, especially within 2 h under a ≥ 12 h-dark pulse (Figure 3B). The ratio of hatchings within 2 or 4 h to the number of hatchings within 8 h after L-on at 15°C was 56 % or 78%, respectively, for a 0.5 h-dark pulse (row b in Figure 3A). When a dark pulse of more 1 h was applied, more than 80% of the eggs hatched within

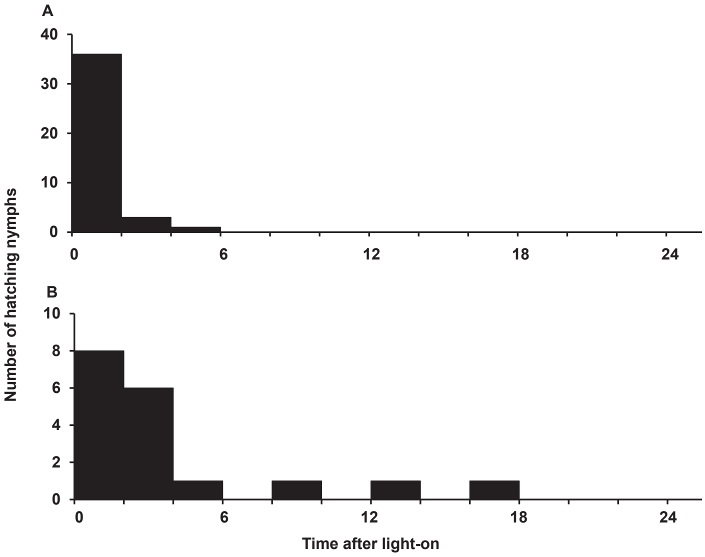


Figure 1. Number of hatching nymphs every 2 h within 24 h after a single light-on signal in *E. engelhardti subtropica.* A: 15°C. B: 20°C. n = 400 eggs.

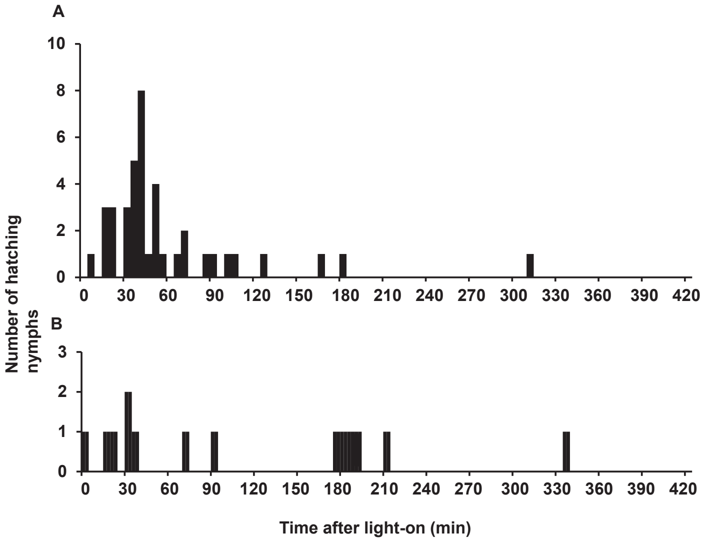
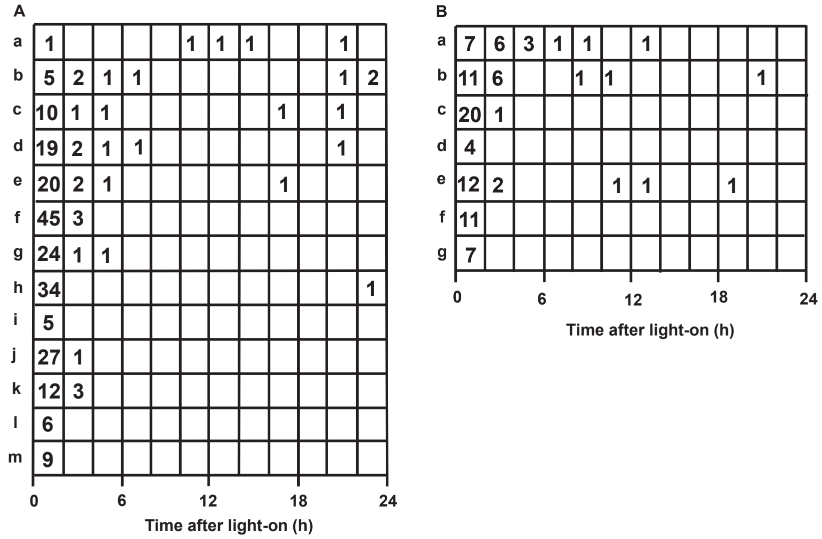


Figure 2. Number of hatching nymphs every 5 min within 8 h after a single L-on signal in *E. engelhardti subtropica.* A: 15°C. B: 20°C. n = 400 eggs.



A

a	1					1	1	1			1	
b	5	2	1	1							1	2
c	10	1	1						1		1	
d	19	2	1	1							1	
e	20	2	1						1			
f	45	3										
g	24	1	1									
h	34											1
i	5											
j	27	1										
k	12	3										
l	6											
m	9											

B

a	7	6	3	1	1		1					
b	11	6			1	1					1	
c	20	1										
d	4											
e	12	2				1	1			1		
f	11											
g	7											

Figure 3. Number of hatches every 2 h within 24 h after L-on under dark pulse in *E. engelhardti subtropica.* A: 15°C. B: 20°C. A:- a: 1/6 h-dark pulse, b: 0.5 h, c: 1 h, d: 2 h, e: 6 h, f: 12 h, g: 18 h, h: 24 h, i: 30 h, j: 36 h, k: 48 h, l: 60 h, and m: 72 h. B:- a: 2 h-dark pulse, b: 6 h, c: 12 h, d: 18 h, e: 24 h, f: 36 h, and g: 48 h. n = 100 eggs for 1/6 h, 30 h, 60 h, and 72 h dark pulses. n = 300 eggs for the other treatments.

2 h after L-on, and more than 92% hatched within 4 h (rows c–m in Figure 3A). At 20°C, the ratio of hatchings within 2 or 4 h after L-on under a 2 h-dark pulse was 41% or 76%, respectively (row a in Figure 3B). With a 6 h-dark pulse, the ratio of hatchings within 2 h after L-on was 65% (row b in Figure 3B), but with 12–48 h-dark pulses, it was more than 85% (rows c–g in Figure 3B).

When the number of hatchings was counted every 5 min after L-on, the earliest hatching time was the shortest with a 12 h-dark pulse at 15 and 20°C and occurred 12.5 min after L-on (Figure 4). The earliest hatching time with dark pulses ≥ 12 h at 15 and 20°C was slightly longer, approximately 20 min after L-on, and was almost the same regardless of the dark pulse duration. The earliest hatching time for dark pulses shorter than 12 h increased with shorter dark pulses and was 92.5 min for a 1 h-dark pulse.

Hatching under dark pulses concentrated within 2 h after L-on; however, the degree of hatching concentration after L-on varied with dark pulse duration. Hatching within 8 h after L-on under dark pulse concentrated within 2 h when counted every 2 h, and the distribution was not normal. However, the distribution was normal when they were counted every 5 min. Therefore, the time from L-on to hatching within 8 h after L-on is shown as both mean and median.

The mean and median times to hatching within 8 h after L-on for various dark pulses at 15 and 20°C showed similar trends (Figure 5A & 5B). The mean and median times from L-on to hatching at 15°C were the shortest with a 12 h-dark pulse at 35.2 ± 34.6 min and 22.5 min (17.5–27.5 min), respectively. At 20°C, the 18 h-dark pulse had the shortest mean and median times, i.e., at 30.0 ± 9.6 min and 27.5 min (22.5–27.5 min), respectively. When the dark pulses were ≥ 12 or 18 h, the mean and median times were slightly longer, ~45 and 40 min, respectively, regardless of pulse duration, and similar at 15 and 20°C. For dark pulses shorter than 12 h, the shorter the dark pulse, the longer the mean and median times (Figure 5A & 5B); for example, in a 2 h- dark pulse, the mean and median times at 15°C were 125.0 ± 108.6 min and 77.5 min (53–110 min), respectively, and at 20°C, they were 157.8 ± 112.9 min and 142.5 min (78–185 min), respectively.

The earliest and mean hatching times were the shortest at 12- and 18 h-dark pulses (Figure 6A & 6B). For dark pulses longer than 18 h, these times were slightly longer or about the same, regardless of the pulse duration. These times increased for the dark pulses shorter than 12 h as the dark pulse duration became shorter. The earliest and mean hatching trends at 15 and 20°C were similar.

Time from L-on to hatching under different photoperiods

Eggs hatched daily from days 18–33 after incubation at 15°C and from days 9–18 after incubation at 20°C under various photoperiods. At 15 and 20°C, hatching occurred within 2 h after L-on when counted every 2 h; however, a longer photophase resulted in a larger variation in hatching during the photophase (Figure 7A & 7B).

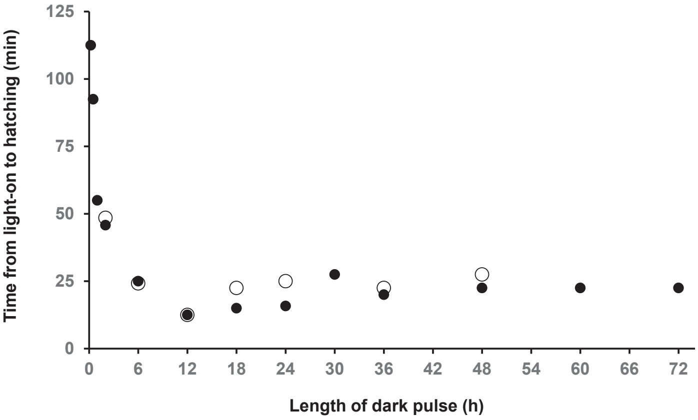


Figure 4. The earliest hatching under dark pulses in *E. engelhardti subtropica.* Black circle: 15°C. White circle: 20°C.

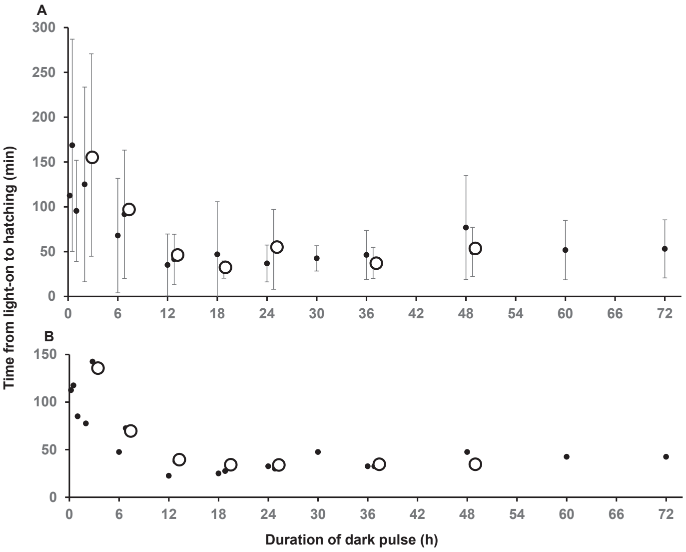


Figure 5. Mean and median hatching times within 8 h after L-on under dark pulses in *E. engelhardti subtropica.* A: mean time (±SD). B: median. Black circle: 15°C. White circle: 20°C. n = 100 eggs for 1/6 h, 30 h, 60 h, and 72 h dark pulses. n = 300 eggs for the other treatments.

Conversely, a shorter photophase increased the time from L-on to hatching and increased hatching variation during the dark phase. Hatching was concentrated within 2 h after L-on under photoperiods when the hatchings were counted every 2 h, which was not a normal distribution. However, distribution was normal when hatchings were counted every 5 min. Therefore, the time from L-on to hatching within 8 h after L-on was shown as the mean and standard deviation.

Hatching under photoperiods was repeated daily. Therefore, the time of earliest hatching triggered by L-on and the hatching time after L-on are presented as means over several days. Hatching was counted every 5 min. Under a photoperiod of 2 h dark and 22 h light (DL 2:22) at 15°C, the mean time of the earliest hatching after L-on was the longest, at 49.2 ± 15.9 min, which was significantly different from those under other photoperiods ($p < 0.05$) (Figure 8). When the dark phase of the photoperiod was ≥ 2 h, the mean time of the earliest hatching tended to be longer, but not significantly. The mean time of the earliest hatching tended to be slightly longer, whether the dark phase was shorter or longer than DL 12:12. At 20°C, the mean time of the earliest hatching after L-on was the longest at 111.8 ± 68.8 min after L-on, which was significantly different from those under other photoperiods ($p < 0.05$).

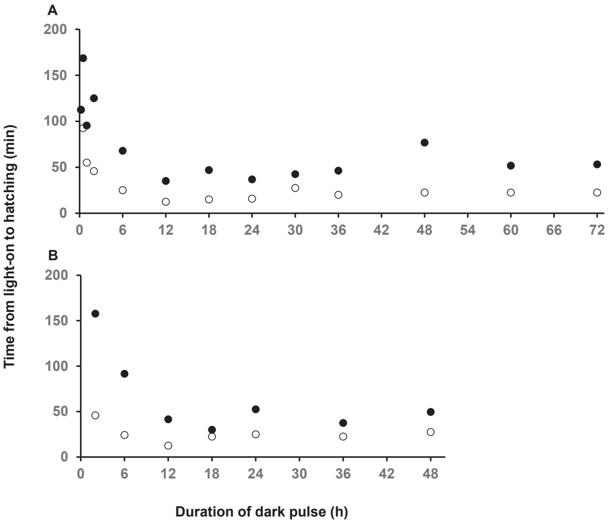
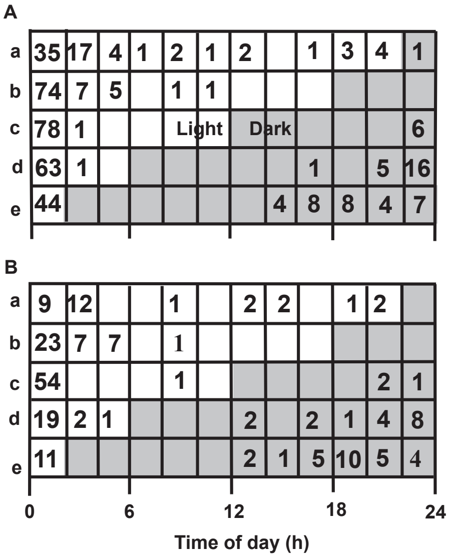


Figure 6. The earliest and mean hatching times after L-on under dark pulses in *E. engelhardti subtropica.* A: 15°C. B: 20°C. White circle: earliest hatching time. Black circle: mean hatching time.



A

a	35	17	4	1	2	1	2		1	3	4	1
b	74	7	5		1	1						
c	78	1			Light		Dark					6
d	63	1							1		5	16
e	44							4	8	8	4	7

B

a	9	12			1		2	2		1	2	
b	23	7	7		1							
c	54				1						2	1
d	19	2	1				2		2	1	4	8
e	11						2	1	5	10	5	4

Figure 7. Number of hatches every 2 h under various photoperiods in *E. engelhardti subtropica.* A: 15°C. B: 20°C. a: DL 2:22. b: DL 6:18. c: DL 12:12. d: DL 18:6. e: DL 22:2. n = 100 eggs for each treatment.

When the dark phase of the photoperiod was longer than 2 h, the mean time of the earliest hatching tended to be longer, but not significantly. The mean time of the earliest hatching tended to be slightly longer when the dark phase was shorter or longer than DL 12:12. The earliest hatching time under DL 2:22 at 15°C was earlier than that at 20°C ($p < 0.05$); however, there was no significant difference in the hatching times between 15 and 20°C during the other photoperiods.

The mean hatching time after L-on under DL 2:22 at 15°C was the longest at 122.6 ± 89.4 min, and it was significantly different from that under other photoperiods ($p < 0.05$) (Figure 9). Additionally, this value was 71.9 ± 71.8 min under DL 6:18, which was significantly longer than that under DL 12:12 and 18:6 ($p < 0.05$); otherwise, there were no significant differences. At 20°C, the mean hatching times after L-on under DL 2:22 and 6:18 were 143.0 ± 66.5 and 127.7 ± 98.7 min, respectively, significantly longer than those under DL 12:12, 18:6, and 22:2 ($p < 0.05$); otherwise, there were no significant differences. The mean hatching time under DL 6:18 at 15°C was shorter than that at 20°C ($p < 0.05$); however, there was no significant difference between 15 and 20°C in other photoperiods.

The mean time of the earliest hatching time and the mean hatching time from L-on under different photoperiods were shortest at DL 2:12, which were slightly longer with photophases ≥ 12 h and longer with photophases ≤ 12 h, with similar trends at 15 and 20°C (Figure 10A & 10B).

Hatching under photoperiods continued for several days, and the correlation between the egg period and the time from L-on to hatching was investigated. At 15°C under DL 2:22, a longer egg stage resulted in a longer time from L-on to hatching. The regression line was positive, and the correlation coefficient was 0.81469, showing a strong correlation (Figure 11). The regression line at 15°C was positive under DL 6:18, and negative

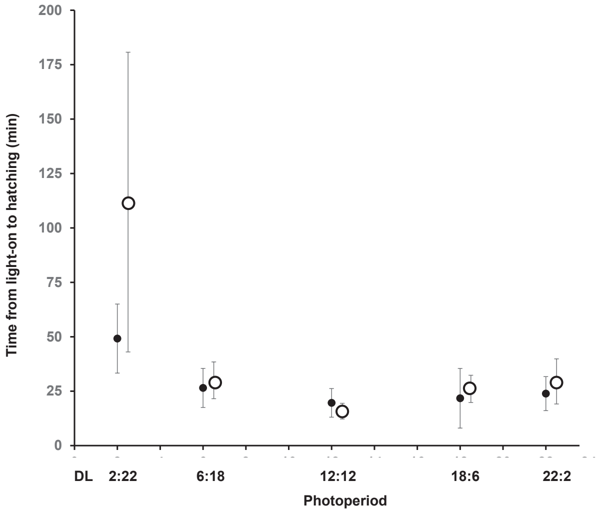


Figure 8. Mean (± SD) of the earliest hatching time under photoperiods in *E. engelhardti subtropica.* Black circle: 15°C. White circle: 20°C.

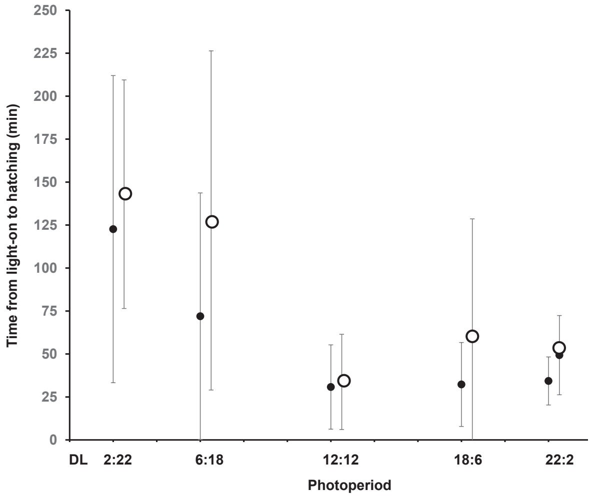


Figure 9. Mean (± SD) hatching time within 8 h after L-on under photoperiods in *E. engelhardti subtropica.* Black circle: 15°C. White circle: 20°C

under DL 12:12, 18:6, and 22:2, with very weak or no correlation. The regression line at 20°C was positive, but a very weak or absent correlation was found for all photoperiods (Figure 12).

The time from L-on to the earliest hatching was shortest under dark pulses and photoperiods with a 12-h dark period. This period was slightly longer under darkness ≥ 12 h, and longer under darkness ≤ 12 h (Figure 13). The dark pulse and photoperiod treatments with the same duration of the dark period were similar; the same trend was observed at 15 and 20°C.

The mean time after L-on was shortest under dark pulse and photoperiods with dark periods of 12 and 18 h, which was slightly longer when the dark period was longer. It was the longest when the dark period was shorter than those periods (Figure 14). The mean time in the dark pulse and photoperiod of the same duration were similar and not significantly different; the same trend was observed at 15 and 20°C.

DISCUSSION

Egg hatching under the L-on step, dark pulses, and photoperiods was concentrated within 2 h after L-on. However, the earlieast hatching time and mean hatching

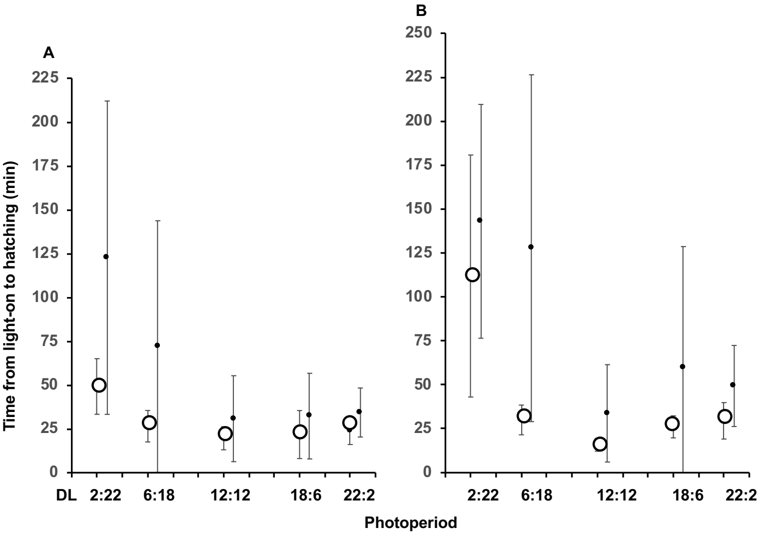


Figure 10. The earliest and mean hatching times from L-on under different photoperiods in *E. engelhardti subtropica.* A: 15°C. B: 20°C. White circle: earliest hatching time. Black circle: mean hatching time.

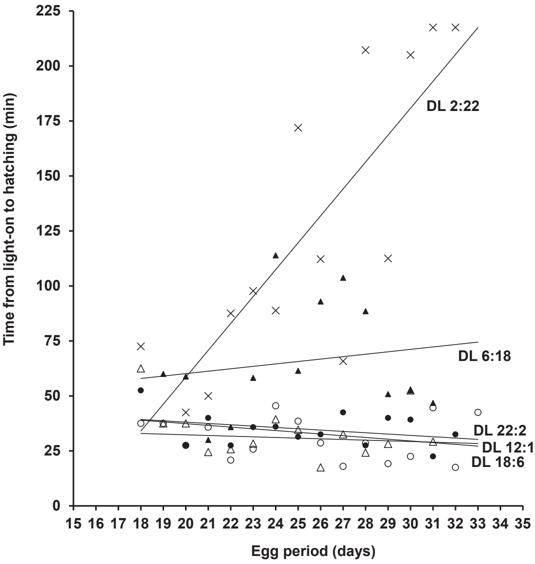


Figure 11. Correlation between the mean hatching time within 8 h after L-on and egg stage period under different photoperiods at 15°C in *E. engelhardti subtropica.* DL 2:22: cross. DL 6:18: black triangle. DL 12:12: white circle. DL 18: 6: white triangle. DL 22: 2: black circle.

time after the L-on signal under the L-on step differed from those under dark pulses and photoperiods. The earliest hatching time was shorter for the L-on step than for dark pulses and photoperiods, and the mean hatching time was the opposite; shorter for dark pulses and photoperiods than for the L-on step. The times also varied with the duration of dark periods under dark pulses and photoperiods; that is, the time from L-on to the earliest hatching time and the mean hatching time were slightly longer with dark periods > 12 h, and these times increased with dark periods < 12 h. These results suggest that triggering the hatching behavior under the L-on step is a mechanical response to the L-on signal and that it is related to a time-measuring mechanism under dark pulses and photoperiods. The earliest hatching time and mean hatching time changed corresponding to

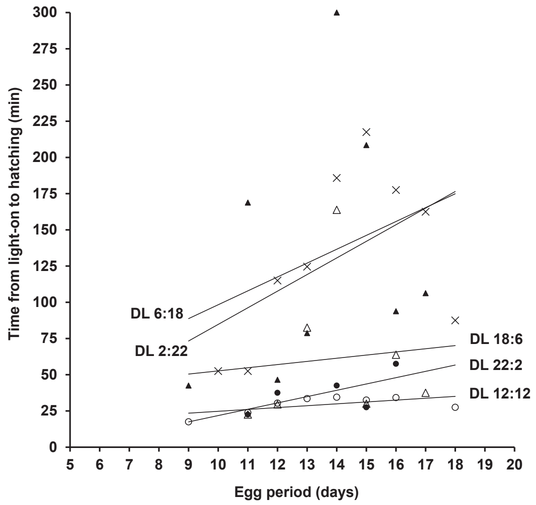


Figure 12. Correlation between the mean hatching time within 8 h after L-on and egg stage period under different photoperiods at 20°C in *E. engelhardti subtropica.* DL 2:22: cross. DL 6:18: black triangle. DL 12:12: white circle. DL 18: 6: white triangle. DL 22: 2: black circle.

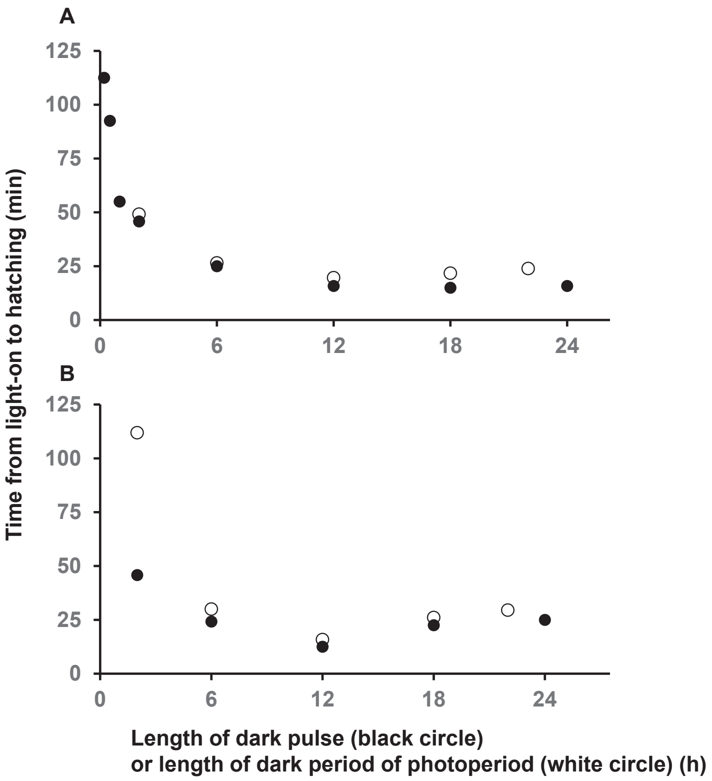


Figure 13. The earliest hatching time from L-on under dark pulses and photoperiods in *E. engelhardti subtropica.* A: 15°C. B: 20°C. Black circle: dark pulse. White circle: photoperiod.

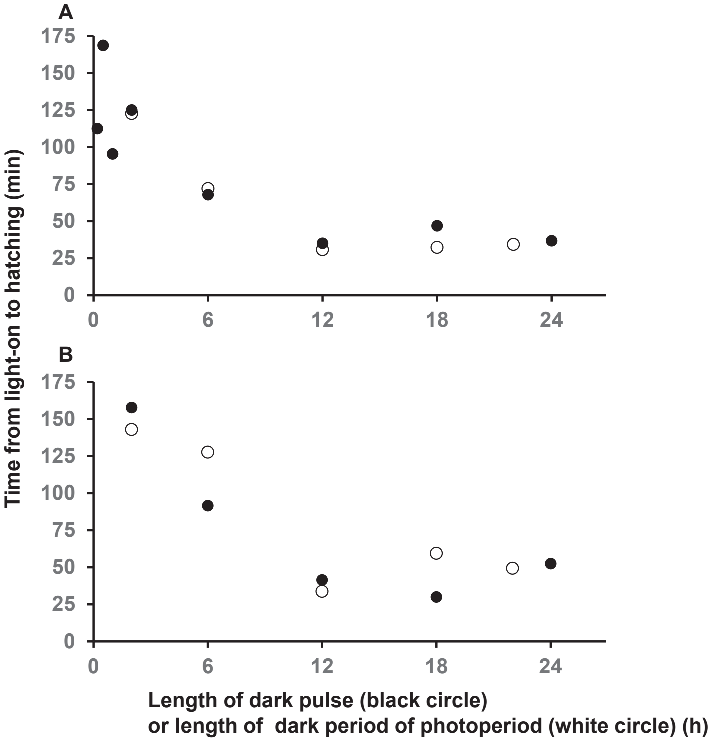


Figure 14. Mean hatching time from L-on under dark pulses and photoperiods in *E. engelhardti subtropica.* A: 15°C. B: 20°C. Black circle: dark pulse. White circle: photoperiod.

the dark periods. These changes showed similar trends under dark pulses and photoperiods, suggesting that the same time-measuring mechanism controls these periods and that this mechanism is involved in the dark period. Additionally, these periods show a similar trend at 15 and 20°C, suggesting a time determination mechanism. This is strongly corroborated by the temperature compensatory, a characteristic of biological clocks.

Several pioneering studies have demonstrated that circadian rhythms govern biological clocks (Bünning, 1936, 1960; Pittendrigh, 1954, 1966; Danilevsky et al., 1970; Truman, 1971), and results indicating an hourglass time-measuring mechanism have been reported (Lees, 1970). The hatching rhythm of *E. engelhardti subtropica* did not continue when the eggs were transferred from the periodic conditions to the constant conditions. Even when these cycles changed 180 degrees in the middle of the cycles, hatching quickly synchronized with the new cycles (Arai, 1977, 1979a, c, 2012). These results suggested that the hourglass-type timing mechanism determined the hatching time of *E. engelhardti subtropica*.

One time-measuring mechanism involved in determining the hatching time of *E. engelhardti subtropica* was initiated by a single L-off signal, and hatching was concentrated ~13 h after L-off, after which the hatching rhythm did not persist. After the hatching peak, eggs did not hatch for approximately 24 h, after which eggs hatched irregularly (Figure 15A). It is suggested that the time-measuring mechanism started by L-off is completed by inducing hatching ~13 h after L-off. When the eggs were transferred from LL to DD, and then back to LL after a 2 h-dark period, they never hatched after L-on or they did only in small numbers (Figure 15B). However, when dark periods were longer, hatchings were concentrated after L-on. This indicates that the response to L-on in the timing system started by L-off changes over time.

Hatching occurred within 2 h after L-on under photoperiods (Figure 7); however, hatchings after L-on were less under photoperiods with extremely short photophases or dark phases, e.g., LD 1:13 and 23:1 (Arai, 1979a). It was concentrated around L-on under LD 12:12. Furthermore, the shorter the dark phase, the greater the variation in hatching during the photophase, and the longer the dark phase, the greater the variation during the dark phase (Figure 16) (Arai, 1979a). The concentration of hatching after L-on was similar to that after the dark pulses; however, there was a variation in hatching during the dark or photophases when the duration of dark periods varied (Figures 15 B & 16). This suggested that only L-off is involved in determining hatching time under dark pulses; however, both L-on and L-off are involved under photoperiods. Conversely, the correspondence between the earliest hatching time and mean hatching time under different dark periods was similar for dark pulses and photoperiods, suggesting that the time-measuring system started by L-off is the same process in the dark pulses and photoperiods

The time from L-on to hatching was affected by the

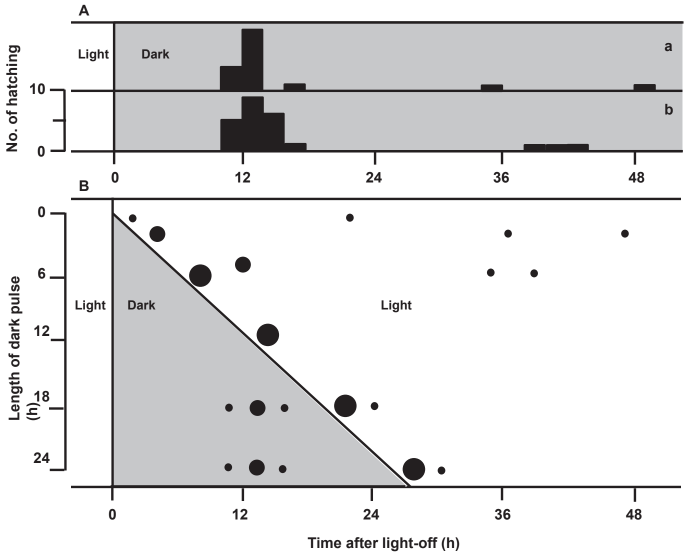


Figure 15. Hatching under L-on step and dark pulses at 15°C in *E. engelhardti subtropica.* A: L-on step. a: adapted from Arai (1997a). b: adapted from Arai (2012). B: dark pulses (adapted from Arai, 1979a). Large circle: ≥ 6. Medium circle: 3–7. Small circle: ≤ 2.

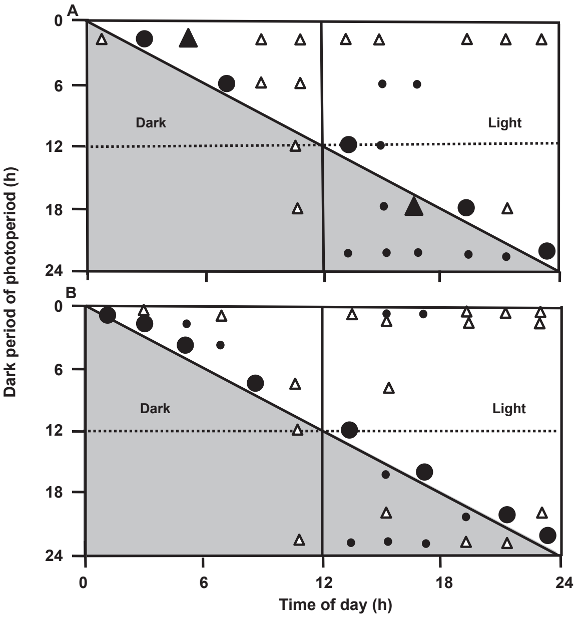


Figure 16. Hatching under various photoperiods at 15°C in *E. engelhardti subtropica.* A: adapted from Figure 7. B: adapted from Arai (1979a). Large black circle: ≥ 50%. Large black triangle: 15–49%. Small black circle: 4–14%. Small white triangle: ≤ 3%.

length of the dark period under D-pulse and photoperiod, and it was approximately 150 min after L-on with a 10-min dark period, approximately 75 min after L-on with a 360 min dark period, and approximately 25 min after L-on with a 720 min dark period (Figure 17). When the dark period was longer than 12 h, the time from L-on to hatching became almost constant. The relationship between the time-measuring system of determination of hatching time started by L-off and hatching behavior triggered by L-on was inferred as follows. Under a 12 h-dark pulse, the time from L-on to hatching behavior was the shortest, corresponding to the completion of the time-measuring system that determines the hatching time started by L-off. Under dark pulses of less than 12 h, the time from L-on to triggering of hatching behavior gradually increased as the time after L-off became shorter. When the dark pulse was ≥ 12 h, the time from L-on to hatching was almost the same as the 12 h dark pulse, regardless of the difference in dark pulse duration. This suggests that the eggs that developed up to 1–2 days before the hatching progressed over time and hatched in response to L-on. As described above, it is suggested that the time-measuring mechanism that determines the time of hatching by L-off and that involved in the trigger of hatching behavior by L-on are closely related.

A time-measuring mechanism is considered to be

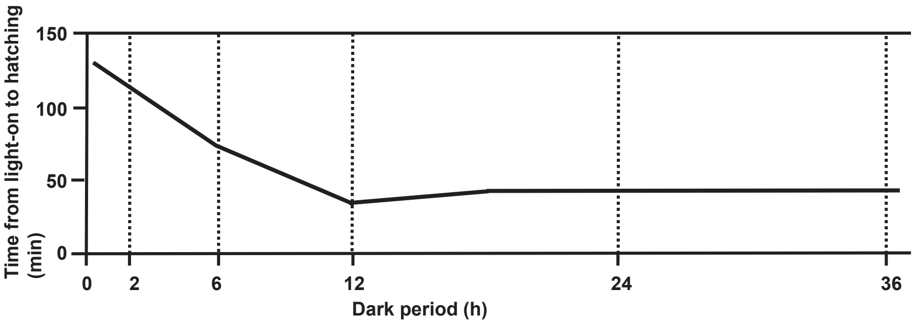


Figure 17. Time from L-on to hatching after the dark period under the dark-pulse and photoperiod.

involved in the triggering of hatching behavior by the L-on signal under dark pulses and photoperiods, unlike the mechanical response to the L-on signal in the L-on step. It suggests that the system was interlocked with the L-off start timing mechanism that determined the hatching time. Presumably, the time-measuring mechanism involved in the trigger of hatching behavior was considered to depend on the L-off-started hatching timing mechanism. Previous studies on the time-measuring mechanisms have aimed at modeling the time-measuring mechanism for one phenomenon and analyzing the process of system progression. However, the results in this paper suggest that other timing systems operate in connection with the basic timing system. To the best of my knowledge, no study has reported results similar to this study. However, I believe that detailed observation of various behaviors in the future may lead to the discovery of new phenomena that have been overlooked. In addition, the time from L-on signal to hatching is extremely short (in minutes), and the ecological significance of this behavior and the significance of the timing mechanism are currently unknown. Many of these factors remain unexplained. The phenomenon elucidating the linkage between the timing mechanisms that determine both the behavior time and time until the behavior trigger may contribute to the analysis and illumination of a previously unknown aspect of the biological clock mechanism. In addition, even if the time to trigger the behavior is short, it is thought that it would present important processes for elucidating the timing mechanism.

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