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Larval diapause induction and termination in a phytophagous lady beetle, *Epilachna admirabilis* Crotch (Coleoptera: Coccinellidae)

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Abstract

Diapause induction and termination in larvae of a phytophagous lady beetle, *Epilachna admirabilis* Crotch, were studied in the laboratory. The critical photoperiod was approximately 14.7 h. In photoperiodic transfer experiments between long, and short-daylengths at 24°C to estimate the beginning and end of diapause sensitivity, we estimated the time required for 50% diapause by probit analysis to be 2.1 days. The sensitive period began 6.3 days and ended 12.0 days after molting of 4th-stadium larvae. As their pupation starts mostly from 12 days after the 4th-stadium molt under conditions averting diapause caused by long daylength, larvae of this species are sensitive, and diapause ensues during a later stage of the 4th stadium. In relation to diapause termination, pupation was accelerated with increasing duration of cold storage (5°C). Larval diapause terminated without cold exposure in 33.2 days under long daylength and 90.6 days under short daylength. These results suggest that diapause termination and post diapause development are related to temperature, duration of cold storage, and photoperiod as a token stimulus.

Key words: Critical photoperiod, diapause induction, diapause termination, *Epilachna admirabilis*, stage sensitive to diapause

INTRODUCTION

Most Coccinellidae species overwinter at the adult stage (Hodek, 1973; Hodek and Honěk, 1996). Phytophagous lady beetle species of the genus *Epilachna* have remarkably different mechanisms of diapause induction. For example, induction in *E. vigintioctopunctata* and *E. vigintioctomaculata* is accelerated by short daylength (Maki et al., 1964; Maki and Kurihara, 1965; Kono, 1980, 1986). In contrast, all adults of *E. yasutomii* that emerged in June entered diapause 2 weeks after emergence regardless of the conditions (Katakura, personal observation).

E. admirabilis depends on wild cucurbit plants, such as *Gynostemma pentaphyllum, Trichosanthes cucumeroides, T. kirilowii*, and *Schizopepon bryoniaefolius*, and has 1 generation a year. Hibernation occurs during the 4th-larval stadium (Takahashi, 1932; Katakura, 1976). We previously reported the life cycle, in which 4th-stadium larvae enter diapause from November to April in Tokyo and Kanagawa (Takeuchi and Tamura, 1994), but we did not deal specifically with diapause.

Hoshikawa (personal observation) found that short daylength (10L and 12L) induced diapause in 4th-stadium larvae of *E. admirabilis* and that long daylength (16L) partially induced pupation without diapause. To clarify this in detail, we studied the critical photoperiods for larvae, stages sensitive to diapause-inducing photoperiod, and effects of low-temperature exposure on diapause termination.

MATERIALS AND METHODS

All our experiments used 1st-stadium larvae of *E. admirabilis* from Hadano City, Kanagawa, Japan. Eggs collected from the field were maintained in Petri dishes with water-soaked absorbent paper at 24°C and a photoperiod of 16L : 8D. Four or 5 hatched larvae were reared in each of 6 Petri dishes (9 cm diameter, 1.5 cm depth) kept in climate chambers. The larvae were supplied with leaves of *T. kirilowii* var. *Japonica*. Food was replenished daily.

In experiments to investigate the critical photoperiod, newly hatched 1st-stadium larvae were reared under a range of constant photoperiods from 12L : 12D to 16L : 8D and 24L : 0D at 24°C. In preliminary examinations, 4th-stadium larvae reared under long daylength (16L) pupated within 20 days after they stopped feeding. Thus, we regarded 4th-stadium larvae that had stopped feeding for more than 20 days as diapause-induced. We counted the number of diapause larvae and pupae daily.

We next investigated the sensitivity of stages to diapause induction. We denoted the beginning and end of the sensitive periods as Sb and Se. Between these 2 stages, the developing insect appears to count the days and begins diapause if a threshold number of days is reached. The threshold number is termed the required day number (RDN) (Saunders, 1981). It is defined in terms of the number of diapause-inducing days during the sensitive period required to increase the incidence of diapause to 50%. To estimate the sensitive period, Taylor (1985) devised a series of 3 experiments that allow Sb, Se, and RDN to be determined. The 1st experiment involves transfer of individuals from diapause-inducing to diapause-averting conditions at various stages. The stage at which 50% diapause is reached is termed Xb. Sb is calculated as Xb−RDN. The 2nd experiment involves transfer of individuals from diapause-averting to diapause-inducing conditions at various stages. The stage at which 50% diapause is reached is termed Xe. Se is calculated as Xe+RDN−1. RDN is determined from a transfer experiment in which individuals are reared in diapause-inducing and diapause-averting conditions for various periods. The number of days under diapause-inducing conditions required to raise the incidence of diapause to 50% is termed the required day number.

To investigate stages of *E. admirabilis* sensitive to photoperiodism stimuli, we transferred larvae from 16L : 8D diapause-averting conditions to 12L : 12D diapause-inducing conditions at 24°C and vice-versa to allow Sb and Se to be estimated. Insects were transferred when they had just molted to 2nd-, 3rd-, and 4th-stadium larvae and at 3, 6, 9, and 12 days after molting to the 4th stadium. There were 6 replicates for each stage.

Next, we determined the duration of exposure (RDN) needed for diapause induction. Larvae were housed in 6 dishes under diapause-averting conditions (16L) for varying lengths of time, transferred for subsequent rearing in diapause-inducing conditions (12L) until 12 days after molting to the 4th stadium (Fig. 3), and then transferred back to diapause-averting conditions. After diapause-induction, larvae were reared under long daylength conditions to pupation.

Finally, we investigated the effects of low temperature on diapause termination. Before exposure to low temperature, diapausing 4th-stadium larvae were progressively acclimatized by exposure to temperatures of 21°C (16L), 18°C (16L), 15°C (16L), and 10°C (12L) for 5 days at each temperature. The larvae were then exposed to 5°C (24D) for 5, 30, 60, 90, and 120 days. Afterwards, the larvae were progressively acclimatized back up to 24°C (16L) diapause-averting conditions. Six replicates were carried out.

RESULTS AND DISCUSSION

Critical photoperiod

Studies conducted at a constant 24°C showed photoperiodism (Fig. 1). Diapause was induced in 4th-stadium larvae reared under photoperiods shorter than 15 h and at 24 h. The mean critical photoperiod that caused 50% of insects to enter diapause was 14.7 h.

Stages sensitive to diapause induction

When larvae were transferred from a diapause-averting long daylength (16L) to a dia-

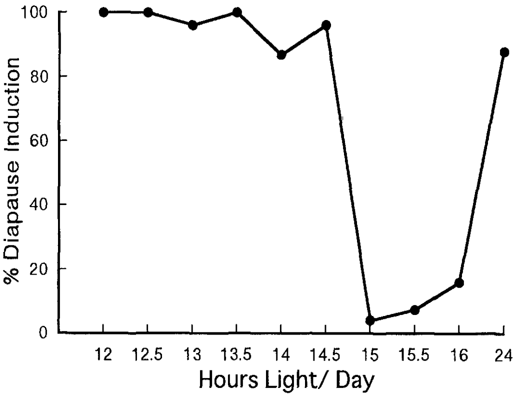

Fig. 1. Photoperiodic response curve for diapause induction in *E. admirabilis* at 24°C.

pause-inducing short daylength (12L), diapause was induced in all insects transferred between the 2nd-stadium molt and 6 days after the 4th-stadium molt (Fig. 2 upper), in 81% of insects transferred at 9 days after the 4th-stadium molt, and in 29% transferred at 12 days after the 4th-stadium molt.

When larvae were transferred from short to long daylength (Fig. 2 lower), diapause was induced in no insects transferred by 3 days after the 4th-stadium molt, in 54% of insects transferred at 9 days after the 4th-stadium molt, and in 90% transferred at 12 days after the 4th-stadium molt. In *E. admirabilis*, the duration of the photosensitive period for induction of diapause in 4th-stadium larvae extended over 10 days (from day 3 to day 12 after molting) for all of a population, and over 6 days (day 7 to day 12) for approximate 50% of a population. In addition, a probit analysis estimated that Xb and Xe were 8.93 and 10.92, respectively.

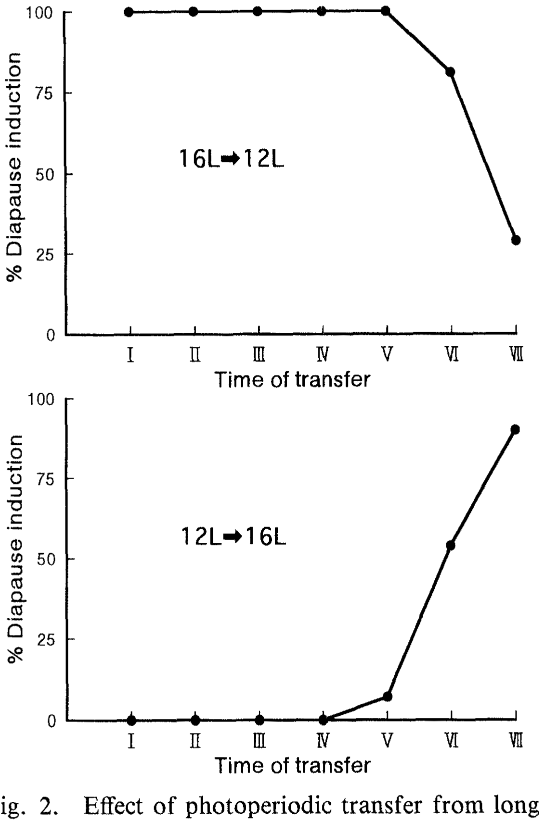

Fig. 2. Effect of photoperiodic transfer from long to short days (upper) and vice versa (lower) on diapause induction in *E. admirabilis*. Roman figures indicate the time of photoperiodic transfer: I, just molted to 2nd stadium; II, just molted to 3rd stadium; III, just molted to 4th stadium; IV, 3 days after 4th molt; V, 6 days after 4th molt; VI, 9 days after 4th molt; VII, 12 days after 4th molt.

Percentage diapause induction increased with the length of short days experienced (Fig. 3). A probit analysis estimated that 2.11 diapause-inducing short days were required to reach RDN. Thus, by Taylor's methods (Taylor, 1985), the sensitive period of *E. admirabilis* begins 6.28 days and ends 12.03 days after the 4th-stadium molt at 24°C (7 days).

The effects of low-temperature periods on diapause termination

There were significant differences in the mean pupation periods of 4th-stadium larvae at different low temperatures (ANOVA; $F=50.12$, df=4, 71; $p<0.05$ with Tukey's HSD test; Fig. 4). The differences tended to increase until 60 days' storage. At 120 days of cold storage, larvae pupated before introduction to 24°C. The percentage pupation also tended to increase with the length of low-temperature storage (Fig. 4).

The critical photoperiod for *E. vigintioctopunctata* adults is 13 h 50 min; ovaries develop in all females in photoperiods longer than 15 h (Kono, 1986). They lay an egg mass about 10 days after emergence under a long-day photoperiod but enter diapause without oviposition under a short-day photoperiod (Kono, 1980). In newly emerged adults of *E. vigintioctomaculata*, ovarian development is reversibly changed by the length of the photoperiod. Ovaries develop under longer photoperiods and degenerate under photoperiods shorter than 14–15 h (Maki et al., 1964). However, when temperatures are higher than 28°C, the ovaries do not develop, even under 16 h of light. When the

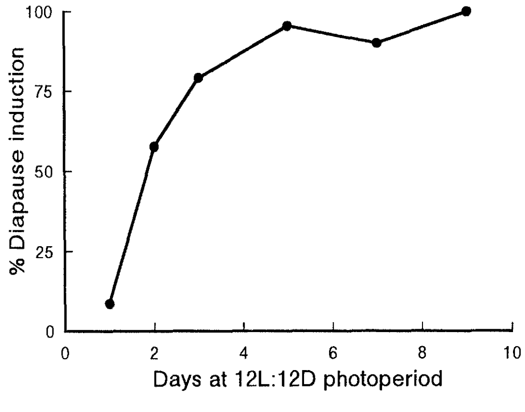

Fig. 3. Relationship between diapause induction and duration of exposure to short photoperiod.

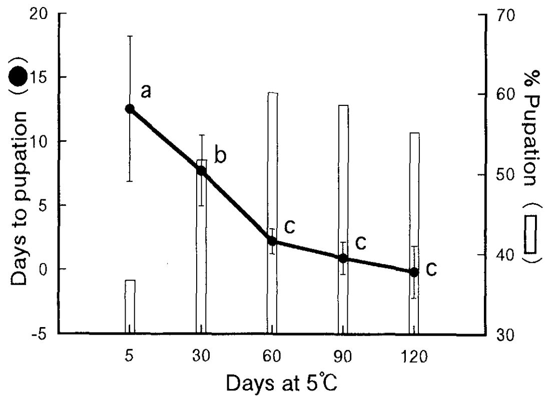

Fig. 4. Diapause termination as indicated by pupation at various durations of low-temperature (5°C) storage. Days to pupation from transfer to long-daylength (24°C, 16L : 8D). Vertical bars show standard deviations. Values with the same letter are not significantly different ($p < 0.05$; multiple comparison Tukey's HSD test).

temperature is lowered to below 28°C, the ovaries develop again under a long photoperiod (Maki and Kurihara, 1965). Induction of the overwintering state frequently results from the combined effects of several cues rather than from the single cue of photoperiod (Leather et al., 1993). Although diapause induction in *E. admirabilis* is caused at least in part by a short photoperiod, further experiments are needed to understand the diapause-inducing mechanism.

The extent of the period during which insects are sensitive to diapause-inducing stimuli varies considerably among insect species, ranging from a few days to almost an entire year. A phytophagous lady beetle, the Mexican bean beetle *E. varivestis*, has a diapause-sensitive period that at 25°C begins approximately on day 9 for males and on day 10 for females, corresponding to the 2nd-stadium, and that ends on day 21 for both sexes, corresponding to the start of the pupal stage (Taylor, 1985). For *E. admirabilis*, we estimated that 2.1 days is required to induce diapause and that the sensitive period at 24°C begins 6.28 days and ends 12.0 days after the 4th-stadium molt. Thus the 4th stadium larva is sensitive, and diapause ensues during a later stage of larval development. Takeuchi and Tamura (1994) reported that different availabilities of host plants cause variations in the life cycle of *E. admirabilis*. The larval period of the Setagaya population feeding on *T. kirilowii* was shorter than that of the Hadano population feeding on *G. pentaphyllum* and *T. cucumeroides*. A small part of the Setagaya population emerged in autumn and overwintered. These adults may have been deposited earlier in the season than the others, and may have responded to changes in daylength from long photoperiod to short through the critical photoperiod (14.7 h) during the 4th stadium.

Temperature is one of the major environmental stimuli controlling the maintenance and termination of diapause. The duration of cold storage has been shown to affect the responses of many species of insects (Tauber et al., 1986; Leather et al., 1993). In *E. vigintioctomaculata*, newly emerged adults exposed to 5°C for 40 days lost their light sensitivity and showed no significant difference in their ovarian development under any photoperiod (Maki and Kurihara, 1965). Similarly, the duration of cold storage affected diapause termination or post-diapause development of *E. admirabilis*, for which longer periods of exposure to low temperature shortened the period over which pupation took place.

Additionally, we analyzed our data on larval diapause termination. The percentage pupation and period from cessation of feeding to pupation (prepupation period) of nondiapausing 4th-stadium larvae ($n=70$) reared under long daylength at 24°C were 91.4% and 12.4±3.4 days (mean±SD). When diapause-induced larvae ($n=44$) that had been exposed for 5, 7, and 9 days to short daylength at 24°C were moved to long daylength (16L), their percentage pupation and prepupation period were 74.6% and 33.2±8.5 days. The percentage pupation and prepupation period of diapause-induced larvae ($n=40$) reared under short daylength at 24°C were 44.0% and 90.6±29.6 days.

These results suggest that: (1) under long daylength, larvae pupate without diapause about 12 days from cessation of feeding; (2) diapausing larvae and/or larvae in post diapause development are light sensitive; (3) diapause frequently stops without low-temperature exposure; and (4) too long a period of diapause at 24°C increases the risk of mortality. Because under long daylength, diapause-induced larvae show a higher percentage pupation and a shorter pupation period, daylength may play an important role in diapause maintenance. Thus, in *E. admirabilis*, diapause termination may be influenced by interaction of the temperature, length of low-temperature storage, and daylength conditions.

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