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# Effects of Environmental Factors on Diapause Development and Postdiapause Oviposition in a Phytophagous Insect, *Dybowskyia reticulata*

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**ABSTRACT**—Environmental factors that regulate the induction, maintenance and termination of adult diapause and postdiapause oviposition were examined in a phytophagous insect, *Dybowskyia reticulata* (Heteroptera: Pentatomidae). When insects were reared from eggs at 25°C in the laboratory, all adults under a short-day photoperiod of 12:12 (L:D) hr and most adults under a long-day photoperiod of 16:8 (L:D) hr entered diapause. Overwintering adults started oviposition after being transferred to the long-day or short-day photoperiod at 25°C in March. Under the short-day photoperiod, however, they entered diapause again after a short oviposition period. Transferral to a long-day photoperiod after a long exposure to a short-day photoperiod synchronously terminated diapause. After exposure to a low temperature during diapause, the adults began to lay eggs under both long-day and short-day photoperiods. Low temperature in winter probably plays a dominant role in the termination of diapause in nature. In addition, the females started oviposition only after feeding when transferred from outdoor conditions to the laboratory in spring, and the onset of oviposition after starvation in the females was more synchronous than that without starvation. The regulation of the seasonal life cycle by various environmental factors in *D. reticulata* is discussed as an adaptation to the phenology of host plants.

## INTRODUCTION

Many phytophagous insects in Heteroptera show long-day type photoperiodic responses for the induction of winter adult diapause and produce three or more generations a year in southwestern Japan, e.g., *Nezara viridula* (Kiritani, 1963; Numata, unpublished), *Riptortus clavatus* (Kobayashi, 1972; Kobayashi and Numata, 1993) and *Piezodorus hybneri* (Higuchi, 1994). *Aelia fieberii* (Heteroptera: Pentatomidae) also shows a long-day photoperiodic response but with a relatively long critical daylength for the induction of diapause; this species therefore produces only two generations a year in Osaka, Japan (Nakamura and Numata, 1997). We attributed this response to the absence of suitable food in autumn (Nakamura and Numata, 1997).

*Dybowskyia reticulata* (Heteroptera: Pentatomidae) overwinters as adults and reproduces on *Torilis japonica* and *T. scabra* (Umbelliferae) plants in Osaka, Japan. Nymphs grow on the seeds of these plants, but the seeds can be found only in early summer. The question thus arises as to how *D. reticulata* adults survive the intervening long period without suitable food. The food supply is sometimes an important selection factor for the evolution of diapause (Masaki, 1980;

Danks, 1987). In the present study, we examined the environmental factors that regulate the induction, maintenance and termination of adult diapause and postdiapause oviposition in *D. reticulata* and we discuss the life cycle adaptation of this insect to its host plants.

## MATERIALS AND METHODS

Adults of *D. reticulata* were collected from the field in Osaka (34°41'N, 135°31'E), Japan in early June, 1992 and 1993. Carrot seeds and water were provided as food. Insects were reared as single male-female pairs in plastic tubes (26 mm diameter × 67 mm length) in an outdoor cage, or under a long-day photoperiod of 16:8 (L:D) hr or a short-day photoperiod of 12:12 (L:D) hr at 25 ± 1°C in the laboratory. Mortality and oviposition were recorded daily.

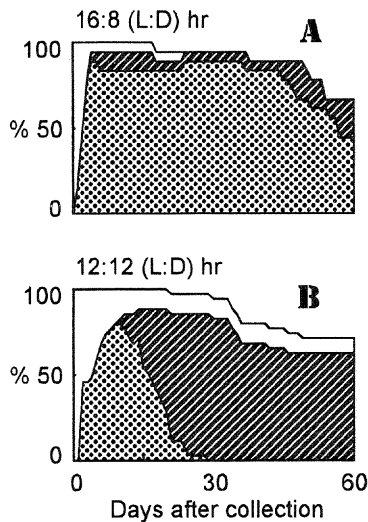
Eggs laid by the adults collected from the field were maintained under experimental conditions in the laboratory or in an outdoor cage within 24 hr after oviposition. In the laboratory, the insects were kept under a constant long-day photoperiod of 16:8 (L:D) hr, a constant short-day photoperiod of 12:12 (L:D) hr, or a shortening photoperiod at 25 ± 1°C, or a short-day photoperiod of 12:12 (L:D) hr at 10 ± 1°C. The shortening photoperiod was produced by a time-switch controlled by a personal computer; the photophase was shortened from 16 hr by 2 min every day. Nymphs were reared in plastic cups (200 ml) at an initial density of about 30 per container. Newly emerged adults were reared in the same manner as those collected from the field. Mortality and oviposition were recorded daily. In the starvation experiments, only water was supplied. The precise experimental conditions are described in the appropriate sections in the Results. The term "preoviposition period" is used for the period between the trans-

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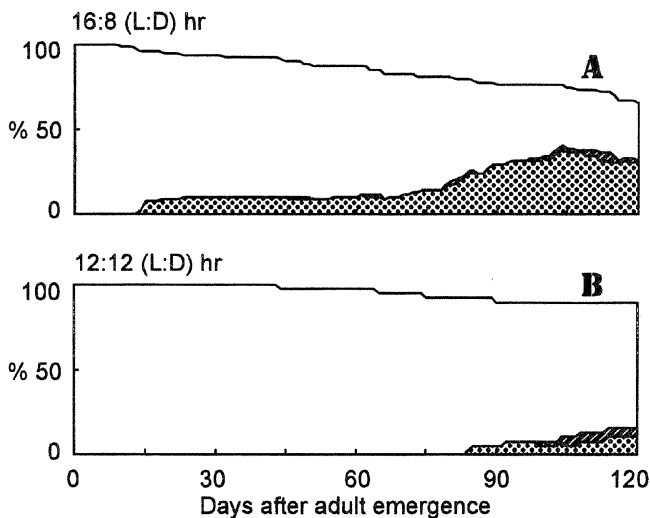
fer to the final experimental conditions and the onset of oviposition. We describe the data of only the females in the Results although the insects were always kept as male-female pairs. The Mann-Whitney *U*-test was used for the statistical analysis.

**RESULTS**

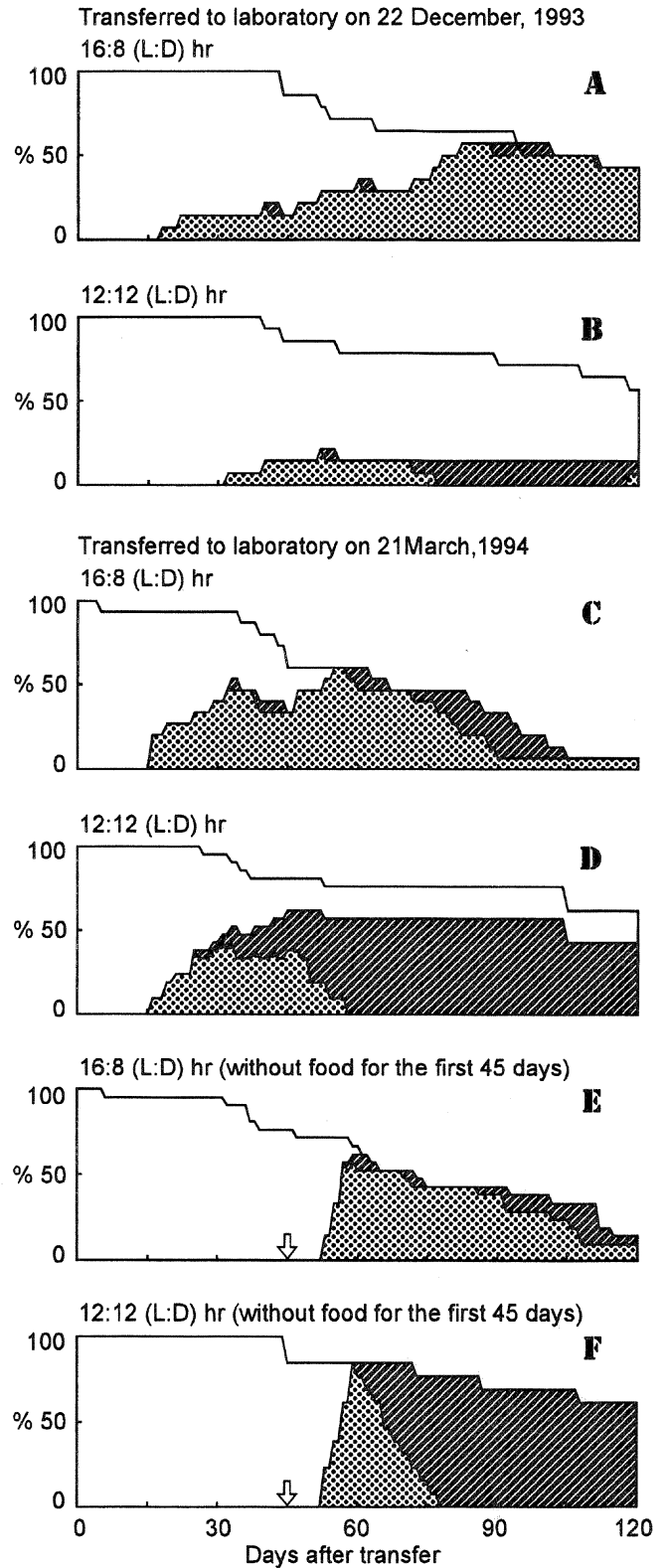
First we collected adults that had overwintered in the field and transferred them to the long-day or short-day photoperiod at 25°C on 17–21 June, 1993. The females kept in either condition started oviposition soon after the transfer (Fig. 1). These insects seemed to have already started reproduction



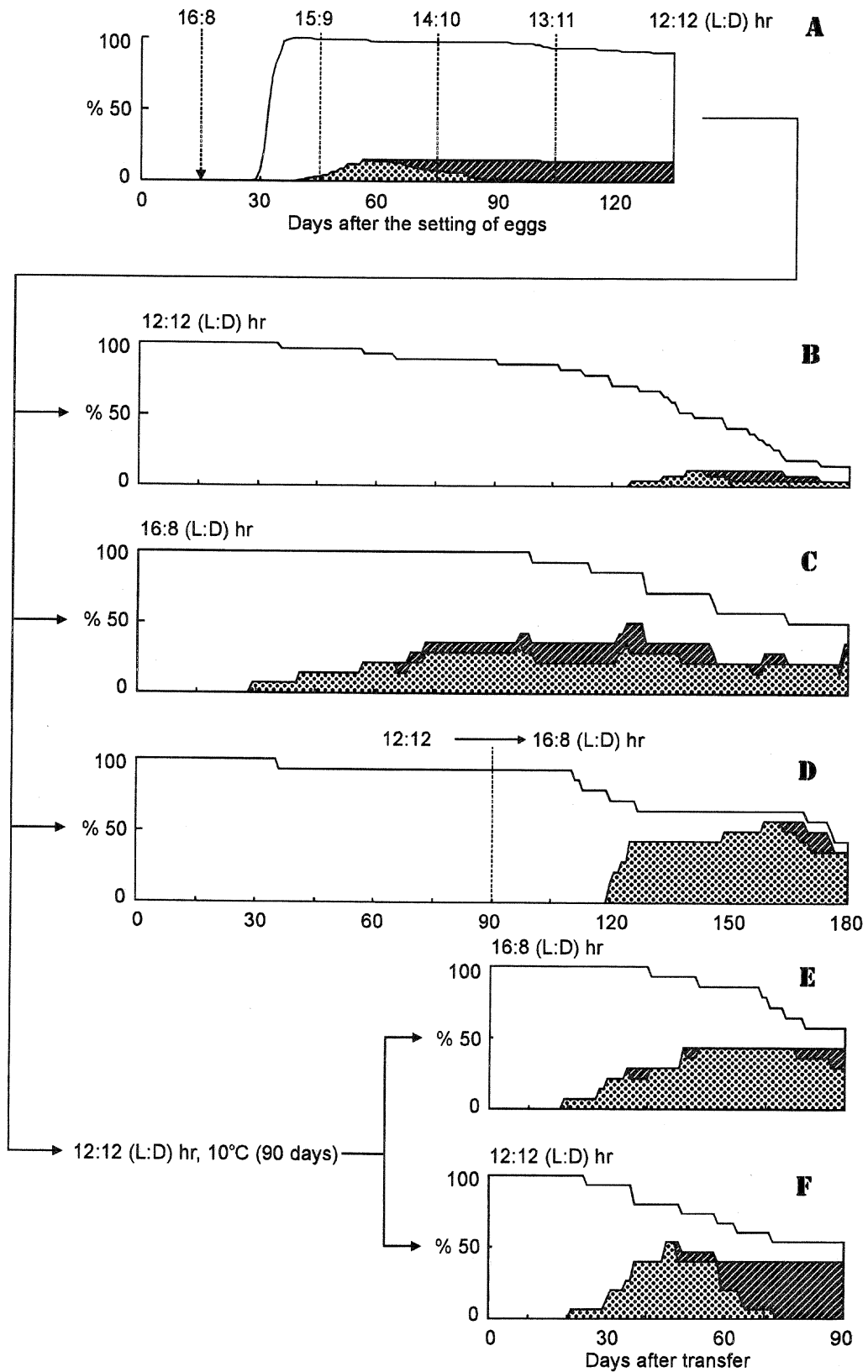
**Fig. 1.** Survival and oviposition at 25°C in female adults of *Dybowskyia reticulata* collected from the field in June, 1993. Open areas, females before oviposition; dotted areas, ovipositing females; shaded areas, females after oviposition. n = 18 (A) and 35 (B).



**Fig. 2.** Survival and oviposition in female adults of *Dybowskyia reticulata* reared from eggs at 25°C. Open areas, females before oviposition; dotted areas, ovipositing females; shaded areas, females after oviposition. n = 79 (A) and 40 (B).



**Fig. 3.** Survival and oviposition in female adults of *Dybowskyia reticulata* transferred from outdoor conditions to the laboratory at 25°C in December, 1993 (A, B) and March, 1994 (C–F). Open areas, females before oviposition; dotted areas, ovipositing females; shaded areas, females after oviposition. Open arrows indicate the onset of food supply. n = 13–21.



**Fig. 4.** Survival and oviposition in female adults of *Dybowskia reticulata* under shortening photoperiod at 25°C (A) and subsequent various conditions (B–F). Open areas, adult females before oviposition; dotted areas, ovipositing females; shaded areas: females after oviposition. Broken arrow shows start of shortening photoperiod. n = 94 (A), 14–27 (B–F).

in the field. They continued oviposition throughout the experimental period (60 days) under the long-day photoperiod but stopped it after about 20 days under short-day photoperiod.

Next we reared the insects from eggs under short-day or long-day conditions at 25°C to examine whether *D. reticulata* shows a photoperiodic response. Under the long-day photoperiod, only 9 of 79 females started oviposition about 15 days after adult emergence. The others did not lay any eggs for about 70 days after emergence, and some of them sporadically started oviposition (Fig. 2A). Under the short-day photoperiod, no females started oviposition until about 80 days after adult emergence (Fig. 2B).

We placed eggs in the outdoor cage on 23 June 1993, when adults were laying eggs in the field. The adults that emerged on 27–31 July did not lay any eggs until 22 December; we judged them to be in diapause. We transferred some of them to 25°C in the laboratory on 22 December. Under the long-day photoperiod, 11 of 14 females started oviposition (Fig. 3A). The preoviposition period of these females was 59 [17–93] days (hereafter preoviposition periods are shown as a median value with a range in brackets). Under the short-day photoperiod, however, only four females oviposited during the observation period of 120 days (Fig. 3B). Thus, in December, the long-day photoperiod could terminate diapause in some individuals, although the oviposition onset was not synchronized. We transferred some adults from the outdoor cage to 25°C in the laboratory on 21 March. Under the long-day photoperiod, 12 of 15 females began to lay eggs (Fig. 3C), and the preoviposition period (29.5 [15–54] days) was significantly shorter than that of the females transferred to the same condition in December ( $p < 0.05$ ). Under the short-day photoperiod, 15 of 21 adults began to lay eggs 24 [15–41] days after the transfer (Fig. 3D). There was no significant difference in the preoviposition period between the two photoperiodic conditions ( $p > 0.05$ ). However, under the short-day photoperiod the females stopped oviposition and the duration of oviposition period was 11.5 [1–34] days.

Based on these results, we speculated that the insects transferred from natural conditions in March have lost the sensitivity to photoperiod but regain it after a short oviposition period. However, another interpretation is possible: These insects are continuously sensitive to photoperiod, and an abrupt increase in temperature induces a temporary oviposition under short-day conditions. To test this hypothesis, we kept some adults that were transferred from the outdoor cage to the laboratory in March without food for 45 days at 25°C. If the above hypothesis is valid, the insects would not lay any eggs under short-day conditions after the food supply was reinstated. However, not only under long-day conditions but also under short-day conditions, females began to lay eggs soon after food was supplied (Fig. 3E, F). The preoviposition period was 10 [7–11] and 11 [7–13] days under the long-day and short-day conditions, respectively; there was no significant difference between these two conditions ( $p > 0.05$ ). A significant difference in the preoviposition period was detected between the females that had been deprived of food and those

without starvation under the same conditions ( $p < 0.005$ ). The insects continued oviposition under the long-day photoperiod, whereas under the short-day photoperiod they stopped laying eggs after a short oviposition period. The oviposition period of the latter (13 [3–23] days) was not significantly different from that of the females without starvation under the short-day photoperiod ( $p > 0.05$ ). Therefore, the onset of temporary oviposition under the short-day photoperiod was not induced by an increase in temperature, and the photoperiodic sensitivity was once lost and regained after a short period of oviposition.

The results presented above show a change in photoperiodic sensitivity under natural conditions. However, it is not clear which factor is responsible for this change among various seasonal cues, e.g., temperature, daylength or humidity. We examined the effects of a gradual change of photoperiod and subsequent low temperature on the sensitivity to photoperiod in the laboratory. Insects were reared from eggs for 15 days under a long-day photoperiod of 16:8 (L:D) hr at 25°C, and then the photoperiod was shortened by 2 min every day until it became 12:12 (L:D) hr to simulate the natural daylength change from early summer to autumn in Osaka. Fourteen of 93 females laid eggs once but stopped oviposition until the photophase was shortened to 13 hr (Fig. 4A). When the photoperiod became 12:12 (L:D) hr no insect was laying eggs.

Then we divided the surviving 84 pairs into 5 groups: 1) Twenty-seven females including 13 that had once oviposited were kept under the short-day photoperiod at 25°C. Most of them did not start oviposition for more than 120 days (Fig. 4B). 2) Under the long-day photoperiod, 11 of 14 females started oviposition and the preoviposition period was 105 [28–178] days (Fig. 4C). 3) When insects were kept under the short-day photoperiod for 90 days, and transferred to the long-day photoperiod at 25°C, 9 of 13 females started oviposition (Fig. 4D). The preoviposition period of these females (34 [29–114] days) was significantly shorter than that of females transferred directly to the long-day photoperiod ( $p < 0.05$ ). Therefore, the sequence of the short-day photoperiod followed by the long-day photoperiod had a stimulating effect on oviposition. To examine the effect of low temperature, we kept adults for 90 days under the short-day photoperiod at 10°C, and then transferred them to 4) the long-day photoperiod or 5) the short-day photoperiod at 25°C. These insects started oviposition under both conditions (Fig. 4E, F). The preoviposition period was 37 [18–52] and 35 [20–44] days under the long-day and short-day conditions, respectively, and there was no significant difference between the two conditions ( $p > 0.05$ ). Therefore, the exposure to a low temperature induces reproduction regardless of photoperiod. Under the short-day photoperiod, females stopped laying eggs after a short oviposition period (22 [11–44] days).

## DISCUSSION

Long diapause induced before the height of summer and terminated in the next spring is often found in insects whose

food supply is restricted to spring or early summer (Masaki, 1980; Danks, 1987). We found here that adults of *D. reticulata* did not lay any eggs when reared from eggs under long-day conditions in both laboratory and natural conditions. They therefore entered diapause in summer, and the time of diapause induction was early compared with several other phytophagous heteropterans living in southwestern Japan (Kiritani, 1963; Kobayashi, 1972; Higuchi, 1994). Adults of another pentatomid, *Eurydema rugosum*, enter long diapause when they grow on the seeds of crucifers under long-day conditions (Numata and Yamamoto, 1990; Ikeda-Kikue and Numata, 1994). Ikeda-Kikue (1991) pointed out that this response is an adaptation to host plants producing seeds in early summer. The major host plants of *D. reticulata*, i.e., *T. japonica* and *T. scabra*, also produce seeds only in early summer. The induction of diapause of *D. reticulata* in summer and its persistence throughout autumn are probably adaptations to seasonally limited food supply.

Masaki (1980) divided long diapause induced before the height of summer and terminated in the next spring into two categories: One is long winter diapause, which is terminated by stimuli characteristic of winter such as low temperatures, and the other is summer-winter diapause consisting of two phases, each of which requires a different environmental stimulus to be terminated. Short-day-long-day photoperiodic responses are common in the species with the latter type of diapause; a sequential exposure to short-day and long-day photoperiod is required to terminate the diapause (Zaslavski, 1988; Danks, 1987). *D. reticulata* showed a photoperiodic response similar to short-day-long-day responses. A short-day or long-day photoperiod alone did not terminate diapause in all insects (Fig. 2), but a prolonged exposure to a short-day photoperiod followed by a transfer to a long-day photoperiod synchronously terminated diapause (Fig. 4D). It is therefore likely that adult diapause in *D. reticulata* is summer-winter diapause, and that the diapause development proceeds under short-day and long-day conditions in the first and second phases, respectively.

A few adults started oviposition 80–120 days after emergence under a constant short-day photoperiod (Fig. 2B). Under a decreasing photoperiod, however, a small portion of females that had once started oviposition entered diapause 30–70 days after emergence (59–103 days after the start of the experiment), and the others remained in diapause (Fig. 4A). Diapause was maintained in these insects for more than 120 days under the short-day photoperiod (Fig. 4B). In addition, adults kept under outdoor conditions remained in diapause until the next spring. Decreasing photoperiods in autumn therefore play a role in the maintenance of diapause in nature.

Short-day-long-day photoperiodic responses enable insects to discriminate autumn and spring photoperiodic conditions (Zaslavski, 1988). Many species with this type of response terminate diapause in response to the long-day photoperiod in spring, e.g., *Agonum assimile*, *Chrysopa downesi*, *Pterostichus coerulescens*, *P. cupreus*, *P. nigrita* and *Rhynchaenus fagi* (Krehan, 1970; Neudecker and Thiele,

1974; Tauber and Tauber, 1976; Ferenz, 1977; Bale, 1979). We do not regard the photoperiodic response in *D. reticulata* as a typical short-day-long-day response, because diapause was terminated without the insects experiencing long-day photoperiod when they were exposed to a low temperature (Fig. 4F); low temperatures can therefore complete diapause development in the second phase of diapause. In addition, adults that had overwintered in outdoor conditions started oviposition similarly under long-day and short-day conditions (Fig. 3C, D). The long-day photoperiod in spring plays no role in the onset of oviposition in the field. Low temperature in winter probably plays a dominant role in the termination of diapause in nature, as reported in many other species (Danks, 1987). We have not examined the effect of low temperature without the intervention of a short-day photoperiod at high temperature; it is therefore unknown whether low temperatures also accelerate diapause development in the first phase of diapause.

In *D. reticulata*, the food supply plays a substantial role in starting oviposition following diapause termination. After a period of starvation in spring, females soon started oviposition synchronously (Fig. 3E, F). Because *T. japonica* and *T. scabra* produce seeds in early summer, adults of *D. reticulata* after overwintering must survive a certain period without suitable food. They begin to reproduce synchronously when their food is available. The predominant role of food in the onset of oviposition after the termination of adult diapause has been shown in two phytophagous heteropterans, *Pyrrhocoris apterus* and *Riptortus clavatus* (Hodková, 1982; Numata and Hidaka, 1984).

Several heteropterans have been reported to respond to the diapause-promoting photoperiod again after starting oviposition in spring (Hodek, 1971, 1977; Numata, 1987; Muraji *et al.*, 1989; Ikeda-Kikue and Numata, 1992; Nakamura *et al.*, 1996). *D. reticulata* also responded to a short-day photoperiod and entered diapause in spring (Fig. 2B). The photoperiodic response after overwintering enables an insect to enter diapause more than once during its life span (Hodek and Hodková, 1992; Nakamura *et al.*, 1996). In *D. reticulata*, however, it is yet to be determined whether adults after overwintering enter a second diapause and reproduce in the following year under natural conditions.

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