Effects of photoperiod and temperature on the induction of adult diapause in *Dolycoris baccarum* (L.) (Heteroptera: Pentatomidae) from Osaka and Hokkaido, Japan

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Abstract

Effects of photoperiod and temperature on the induction of adult diapause were examined in *Dolycoris baccarum* (L.) collected in Osaka and Hokkaido, Japan. Adults started reproduction soon after adult emergence under long-day conditions, while they entered diapause under short-day conditions. The critical photoperiod for the induction of diapause was between 13 and 14 h and between 15 and 16 h in insects of the Osaka and Hokkaido populations, respectively. Under long-day conditions, most insects of the Osaka population emerged as reproductive adults at any temperature used, whereas those in the Hokkaido population tended to show lower incidence of diapause as the temperature increased. Preoviposition periods in females of the Osaka population were shorter than those of the Hokkaido population. The duration of diapause in the Osaka population was shorter at 30°C than at 25°C under short-day conditions. In Osaka, *D. baccarum* is assumed to produce three generations a year, like several other seed-sucking bugs. In Hokkaido, a greater proportion of adults produces the second generation in summer under long daylengths.

Key words: *Dolycoris baccarum*; adult diapause; photoperiod; temperature; geographical variation

INTRODUCTION

Dolycoris baccarum is a polyphagous seed-sucking bug that is distributed widely in the Palearctic region, and causes damage to a variety of crops (Panizzi et al., 2000). Also in Japan, *D. baccarum* is known as a pest on several plants such as soybeans, cotton, sesame, carrots, burdock and rice (Tomokuni et al., 1993). In Europe, the lifecycle adaptations in *D. baccarum* have been studied extensively (Kamenkova, 1958; Perepelitza, 1969; Conradi-Larsen and Sømme, 1973, 1978; Hodek, 1977; Karsavuran, 1986; Babrakzai and Hodek, 1987; Hodková et al., 1989; Hodek and Hodková, 1993). This species has a multivoltine lifecycle in the Krasnodar territory, Russia, and a univoltine lifecycle in high latitude regions such as Novosibirsk and Primorsk, Russia and southern Norway (Perepelitza, 1969; Conradi-Larsen and Sømme, 1973). Adult winter diapause is induced and averted by short- and long-day conditions, respec-

tively (Babakzai and Hodek, 1987; Hodková et al., 1989; Hodek and Hodková, 1993).

By contrast, the lifecycle of *D. baccarum* in Japan has not been studied extensively. In central Hokkaido, a northern island of Japan, *D. baccarum* is observed from May to July and in October (Hori, 1984). Kobayashi (1972) suggested that *D. baccarum* has a bivoltine lifecycle in southwestern Japan. However, no experimental study has been made to examine the lifecycle of *D. baccarum* in Japan. Our preliminary observations showed that *D. baccarum* appeared continuously from spring to late autumn in Osaka, and that many adults entered diapause in August in eastern Hokkaido. In the present study, we collected *D. baccarum* from Osaka and eastern Hokkaido, and kept insects from eggs under various combinations of photoperiod and temperature. Results in the two populations were compared, and the lifecycle adaptations in this species were inferred from these findings.

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MATERIALS AND METHODS

Adults of *D. baccarum* were collected from the fields in Tokoro, Hokkaido (44.2°N, 144.0°E) and Osaka (34.7°N, 135.5°E), Japan from June to September 1996. Insects were reared as male-female pairs in plastic cups (60 ml) on raw peanuts and water. Eggs laid by the field-collected adults were placed under experimental conditions within 24 h of oviposition. Nymphs were reared in plastic cups (200 ml). In one set of experiments, insects were kept under 18L:6D (18 h light: 6 h dark), 16L:8D, 15L:9D, 14L:10D, 13L:11D or 12L:12D at $25 \pm 1^\circ\text{C}$ to examine the effect of the photoperiod. In the other set of experiments, insects were kept under long-day (16L:8D) or short-day (12L:12D) conditions at 30 ± 1 , 27.5 ± 1 , 25 ± 1 and $20 \pm 1^\circ\text{C}$. Mortality and oviposition of adults were recorded every day. Some adults were kept continuously under 12L:12D for 120 d. The other adults were dissected 30 d after emergence, and the diapause status was judged by the criteria used for *Eurydema rugosum*, *Aelia fieberi* and *Dybowskyia reticulata* (Numata and Yamamoto, 1990; Nakamura and Numata, 1997, 1998). According to these criteria, females without mature eggs or vitellogenic oocytes in the ovarioles and males without secretory fluid in the ectodermal sac of the accessory gland were considered to be in diapause.

RESULTS

Effect of photoperiod on the induction of adult diapause

All or most females of the Osaka population reared from eggs in the laboratory under 16L:8D, 15L:9D and 14L:10D at 25°C started oviposition soon after adult emergence (Fig. 1). There was no significant difference in the pre-oviposition period among the three photoperiods (Kruskal-Wallis test, $p > 0.05$). All females including those that did not start oviposition within 30 d had mature eggs or vitellogenic oocytes (Fig. 2). Under L13:D11, no female started oviposition within 30 d (Fig. 1), but 29.2% of them had mature eggs or vitellogenic oocytes (Fig. 2). Under 12L:12D, all insects were in diapause. There was no significant difference in the incidence of diapause between sexes under each photoperiod (Fisher's exact probability test, $p > 0.05$).

Fig. 1. Effects of photoperiod on survival and oviposition in females of Osaka and Hokkaido populations of *Dolycoris baccarum* at 25°C . Dotted areas, percentage of cumulative ovipositing females; open areas, percentage of non-ovipositing females. $N=9-33$.

Fig. 2. Photoperiodic response curves for the induction of adult diapause in *Dolycoris baccarum* of Osaka and Hokkaido populations at 25°C . Open circles, female adults of the Osaka population; closed circles, males of the Osaka population; open triangles, female adults of the Hokkaido population; closed triangles, males of the Hokkaido population. $N=7-48$. Asterisks indicate significant differences in the incidence of diapause between sexes. ** $p < 0.01$ (Fisher's exact probability test).

Most females of the Hokkaido population kept continuously from eggs under 18L:6D at 25°C started oviposition (Fig. 1). Their preoviposition period was significantly longer than that in females of the Osaka population under 16L:8D (Mann-Whitney's *U*-test, $p<0.01$). Many females did not start oviposition within 30 d under 16L:8D (Fig. 1), although most of them had mature eggs or vitellogenic oocytes (Fig. 2). The incidence of diapause in females was significantly higher than that in males under 16L:8D and 18L:6D (Fisher's exact probability test, $p<0.01$). Under 15L:9D, 14L:10D or 12L:12D, all insects were in diapause.

Effect of temperature on the induction of adult diapause

We examined the effects of temperature and

Fig. 3. Effects of temperature on the induction of adult diapause under long-day and short-day conditions in Osaka and Hokkaido populations of *Dolycoris baccarum*. Open circles, female adults of the Osaka population; closed circles, males of the Osaka population; open triangles, female adults of the Hokkaido population; closed triangles, males of the Hokkaido population. $N=8-33$. Asterisks indicate significant differences in the incidence of diapause between sexes. * $p<0.05$, ** $p<0.01$ (Fisher's exact probability test).

Fig. 4. Effects of temperature on survival and oviposition in adults of Osaka and Hokkaido populations of *Dolycoris baccarum* under short-day conditions (12L:12D). Dotted areas, cumulative percentage of ovipositing females; open areas, percentage of non-ovipositing females. $N=14-26$.

photoperiod on the induction of diapause. Under 16L:8D, insects of the Osaka population became reproductive regardless of temperature except for males at 20°C (Fig. 3). The incidence of diapause in females was significantly higher than that in males at 20°C (Fisher's exact probability test, $p<0.05$). In both males and females of the Hokkaido population, the incidence of diapause under the long-day conditions was significantly different among temperatures (chi-square test, $p<0.01$). There was a tendency for higher temperatures to lower the incidence of diapause. The incidence of diapause was significantly higher than that in males at 25°C (Fisher's exact probability test, $p<0.01$) and at 20°C ($p<0.05$). Under 12L:12D, diapause was induced regardless of temperature in both populations.

Effect of temperature on the termination of adult diapause

When insects from eggs were continuously kept under 12L:12D at 30°C, all females of the Osaka population started oviposition by 70 d after adult emergence (Fig. 4). At 25°C, the preoviposition period was significantly longer than that at 30°C (Mann-Whitney's *U*-test, $p<0.01$). In the Hokkaido population, more than half of the females at 30°C died within 120 d, and about half of the surviving females started oviposition. The preoviposition period was significantly longer than that in the Osaka population under the same conditions (Mann-Whitney's *U*-test, $p<0.01$). At 25°C, only one of 21 females started oviposition within the experimental period.

DISCUSSION

Adults of *D. baccarum* reproduced under long-day conditions but entered diapause under short-day conditions. European populations of *D. baccarum* have also been reported to show this type of response (Babrzai and Hodek, 1987; Hodková et al., 1989; Hodek and Hodková, 1993). In the Osaka population, the critical photoperiod for the induction of diapause was between 13 and 14 h at 25°C (Fig. 2). Daylengths including 0.5 h twilight periods in Osaka are about 13 h in early September. In Osaka, therefore, *D. baccarum* probably reproduces in spring and summer, and adults emerging in September or later enter winter diapause. Several other heteropterans of central Japan have critical daylengths of 13 to 14 h for the induction of adult diapause, e.g., *Riptortus clavatus* (Kobayashi and Numata, 1993) and *Plautia crossota stali* (Numata and Kobayashi, 1994). These bugs were assumed to produce three generations a year (Numata and Nakamura, 2002). From the present results, we suggest that *D. baccarum* has a similar lifecycle in Osaka. However, Kobayashi (1972) reported that *D. baccarum* might have a bivoltine lifecycle in southwestern Japan because insects were found twice in the spring and autumn. Many adults of *D. baccarum* were parasitized by tachinid flies in the summer, although no parasitism was recorded before overwintering in Morioka, Iwate Prefecture, Japan (Honda, 1985). It is possible that the mortality of *D. baccarum* during the summer is also high in Osaka because of parasitism, and the high mortality makes it difficult to find insects during the summer.

In the Hokkaido population, adult diapause was averted under 16L:8D or longer photoperiods at 25°C, showing that insects can produce more than one generation a year. Hori (1984) reported that *D. baccarum* was observed from May to July and in October in central Hokkaido, suggesting that the insect has a bivoltine lifecycle. This observation is not in conflict with the present findings that adults started reproduction under long-day photoperiods at high temperatures. However, we cannot exclude the possibility that a partial bivoltine lifecycle might be common in eastern Hokkaido because adults of the first generation collected in August in 1996, 1998 and 2000 were in diapause (Nakamura, unpublished). Even if some adults of the first gen-

eration enter diapause in eastern Hokkaido, a certain proportion of insects can reproduce in the summer under long daylengths.

The critical photoperiod in the Hokkaido population was between 15 and 16 h, which was longer by 2 h than that in the Osaka population (Fig. 2). In many insect species, populations at higher latitudes have longer critical daylengths for the induction of diapause (Danilevskii, 1961; Tauber et al., 1986; Danks, 1987). The present study exhibits that *D. baccarum* shows a similar geographical cline for the induction of adult diapause. The critical photoperiod increases about 1 h/5° of latitude in many insects (Danilevskii, 1961; Danks, 1987). In *Cletus punctiger* (Heteroptera: Coreidae), the critical photoperiod is 12 h in Ishigaki (24.3°N), between 13 and 14 h in Fukuyama (34.5°N) and Tsukuba (36.0°N), and 14.5 h in Shiraishi (38.0°N) (Ito, 1989). The increase in critical photoperiod is 1 h/5.48° in *C. punctiger*. From the present results, the difference in the critical photoperiod in *D. baccarum* is 1 h/4.75° of latitude, which is slightly less than that in *C. punctiger*. Because the cline is similar to many other insects, the timing of diapause induction in *D. baccarum* has a close relation to the arrival of winter in each locality. The cline can also be applied to the insects of Europe. In the population from southern Norway (61°30'N) where the longest photophase is more than 22 h, all adults reproduce under constant 18L:6D (Hodková et al., 1989), but diapause is induced under 16L:8D (Conradi-Larsen and Sømme, 1978). These findings show that *D. baccarum* has adapted to local climates by changing its photoperiodic response for the induction of adult diapause.

Temperature can modify the photoperiodic responses in *D. baccarum* of European populations (Conradi-Larsen and Sømme, 1978; Hodek and Hodková, 1993). Most insects of a population from southern Norway entered diapause under 16L:8D or 8L:16D at 21°C, whereas more than half of the adults started oogenesis within 18 d when the temperature was increased from 21 to 30°C after adult emergence, in either long-day or short-day conditions (Conradi-Larsen and Sømme, 1978). In a population from Prague (52°N), Czech Republic, more than half of the females oviposited even under short-day conditions when reared at 26°C or after an increase in temperature from 20°C to 26°C (Hodek and Hodková, 1993). In the present study,

lower temperatures tended to induce diapause under 16L : 8D, especially in the Hokkaido population (Fig. 3). In contrast, high temperatures such as 27.5 or 30°C did not avert diapause under 12L : 12D, contrary to the results in European populations. However, the duration of diapause under the short-day conditions was significantly shorter at 30°C in the Osaka population (Fig. 4). Therefore, even if high temperature does not avert diapause, it may accelerate diapause development.

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