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Effects of Temperature and Photoperiod on the Termination of Larval Diapause in *Lucilia sericata* (Diptera: Calliphoridae)

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ABSTRACT–Larvae of the blow fly, *Lucilia sericata* (Meigen), enter diapause in the third instar after cessation of feeding. The effects of temperature and photoperiod on the termination of diapause were examined. The diapause terminated spontaneously under the diapause-inducing condition of 20°C and LD 12:12, although pupariation was not synchronous. Diapause development proceeded under a low temperature of 7.5°C. Transfer to long-day conditions of LD 16:8 or to a high temperature of 25°C induced prompt and synchronous pupariation. Low temperatures in winter probably play a predominant role in the termination of diapause under natural conditions.

Key words: *Lucilia sericata*, photoperiod, temperature, diapause development

INTRODUCTION

The blow fly, *Lucilia sericata* (Meigen), enters diapause and overwinters as a post-feeding larva just before puparium formation (Davies, 1929). Cragg and Cole (1952) have shown that the diapause incidence of the progeny produced by wild females increases as the winter approaches when the progeny are reared under constant laboratory conditions. Moreover, we have recently shown in *L. sericata* that short-day and low-temperature conditions in the parental and larval generations synergistically act on the induction of diapause (Tachibana and Numata, 2004).

The effects of environmental factors on the termination of larval diapause have also been examined in *Lucilia* by several authors. Lees (1955) has suggested that low temperature is the normal stimulus required to terminate diapause under field conditions, based on the results of Roubaud (1922) in which a low temperature causes prompt pupariation after a return to high temperatures in *L. sericata*. In *L. caesar*, there are two contradictory results: Ring (1968) has shown a prominent effect of low temperature on diapause termination, although Fraser and Smith (1963) have observed little effect.

Diapause in many temperate insects can be terminated by photoperiod and high temperature as well as low temperature (Tauber *et al*., 1986; Danks, 1987; Hodek and Hodková, 1988; Hodek, 2002). In *Lucilia*, Ring (1968) has surmised that photoperiod is unlikely to play an important role in the termination of diapause, with no evidence supporting this assumption. Diapause termination by photoperiod and high-temperature has been reported only in *Calliphora vicina* among blow flies with larval diapause (Vinogradova, 1974). Therefore, the effects of environmental factors on termination of larval diapause in *L. sericata* are still open to argument.

The present study aims to clarify the effects of temperature and photoperiod on diapause termination in *L. sericata*, and the correlation between the duration of diapause and these environmental factors was also examined.

MATERIALS AND METHODS

A laboratory culture of *L. sericata* originating from adults captured on the campus of Osaka City University (34.7°N, 135.5°E), Japan in September 2001 has been maintained under LD 16:8 (16 hr light and 8 hr darkness) at 25°C, as previously reported (Tachibana and Numata, 2001), and their progeny was used for the experiments. In all experiments, temperature fluctuation did not exceed ±1°C.

Insects of the parental generation were reared under LD 12:12 at 20°C on an artificial diet (Tachibana and Numata, 2001). Newly hatched larvae produced by these parents were reared in a 500-ml beaker under LD 12:12 at 20°C. Mature larvae were transferred to a plastic container (150 mm in diameter, 50 mm in depth) full of damp wood chips kept under LD 12:12 at 20°C. Under these conditions, most larvae enter diapause (Tachibana and Numata, 2004). Fifteen days after cessation of feeding, the larvae that had not pupariated were regarded as being in diapause (Tachibana and

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Numata, 2004), and transferred to petri dishes (50 mm in diameter, 10 mm in depth) with moistened cotton wool.

These diapause larvae were kept under LD 12:12 at 7.5°C for 0-60 days or under LD 12:12 at 20°C for 10-30 days. They were then transferred to LD 12:12 at 20°C, LD 16:8 at 20°C, or LD 12:12 at 25°C. Some diapause larvae were transferred to five different photoperiods at 20°C, i.e., LD 12:12, LD 13:11, LD 14:10, LD 15:9, and LD 16:8. Newly formed puparia were counted daily.

In addition, newly hatched larvae produced by parents kept under LD 12:12 at 20°C were reared under the above five photoperiods at 20°C, and the diapause incidence was examined.

RESULTS

Fig. 1 shows the effect of low temperature on the termination of larval diapause. When diapause larvae were continuously kept under LD 12:12 at 20°C, pupariation was not synchronous and the median of larval duration was 61.5 days. In larvae returned to LD 12:12 at 20°C after exposure to a low temperature of 7.5°C under LD 12:12 for 10 days, larval duration after the return to 20°C was significantly shorter than that in insects without exposure to the low temperature, although their pupariation was still asynchronous. In larvae exposed to the low temperature for 20 days, larval duration was significantly shorter and pupariation was more synchronous. As the period of low temperature was longer, larval duration after the return to 20°C was shorter and pupariation was more synchronous. All the larvae pupariated within 8 days of the return to 20°C from exposure to the low temperature for 30 days or more.

When diapause larvae were transferred to LD 16:8 at 20°C without exposure to low temperature, larval duration was significantly shorter than that in larvae kept continuously under LD 12:12 at 20°C (Mann-Whitney *U* test, $P<0.01$). The median of larval duration in the former condition was 15 days. When larvae were transferred to LD 16:8 at 20°C after exposure to a low temperature of 7.5°C, the larval duration at 20°C was shorter as the period of low temperature lengthened (Fig. 2).

The effects of high temperature on the termination of larval diapause were examined by the transfer of diapause larvae to a high temperature of 25°C under LD 12:12 (Fig.

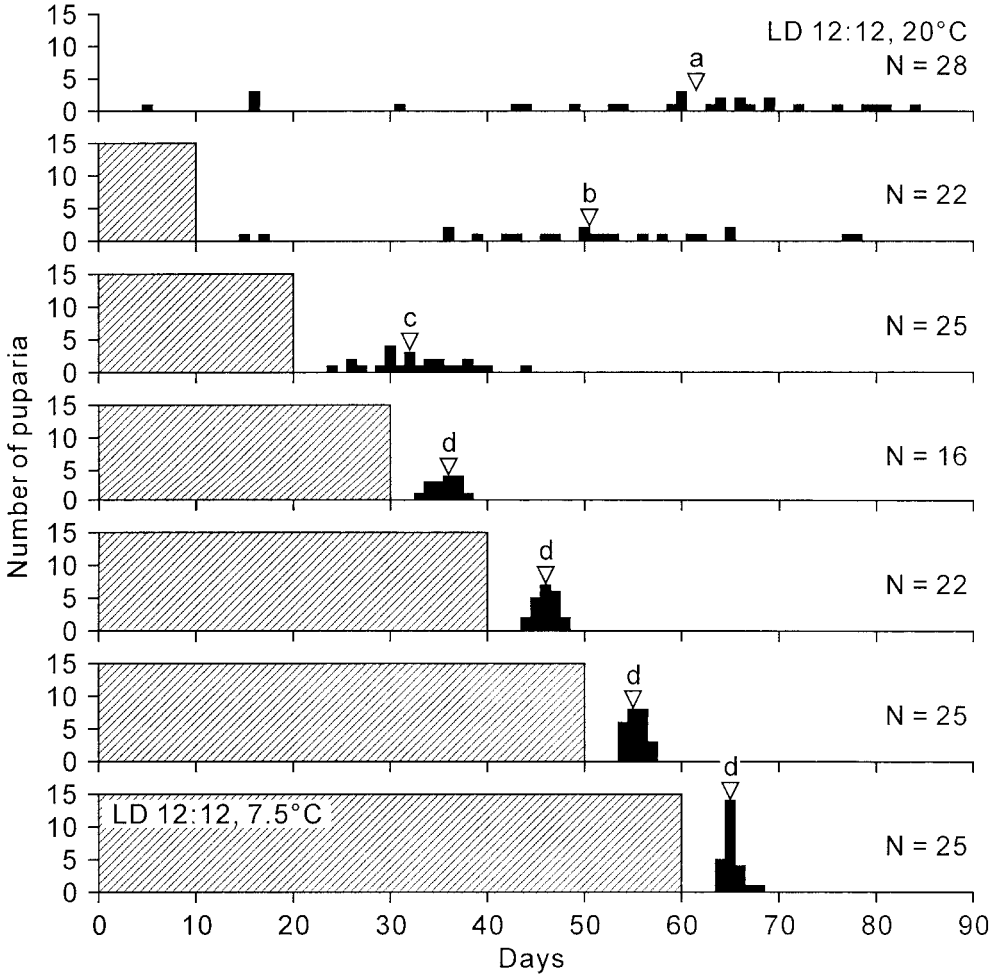

Fig. 1. Frequency distribution of pupariation in diapause larvae of *Lucilia sericata* under LD 12:12 at 20°C after exposure to LD 12:12 at 7.5°C for 0–60 days. Both parental flies and progeny larvae were reared under LD 12:12 at 20°C, and diapause larvae 15 days after cessation of feeding were used. Triangles indicate the medians. Larval duration after return to LD 12:12 at 20°C was not significantly different between series with the same letter ($P>0.05$, Steel-Dwass test). N=16–28.

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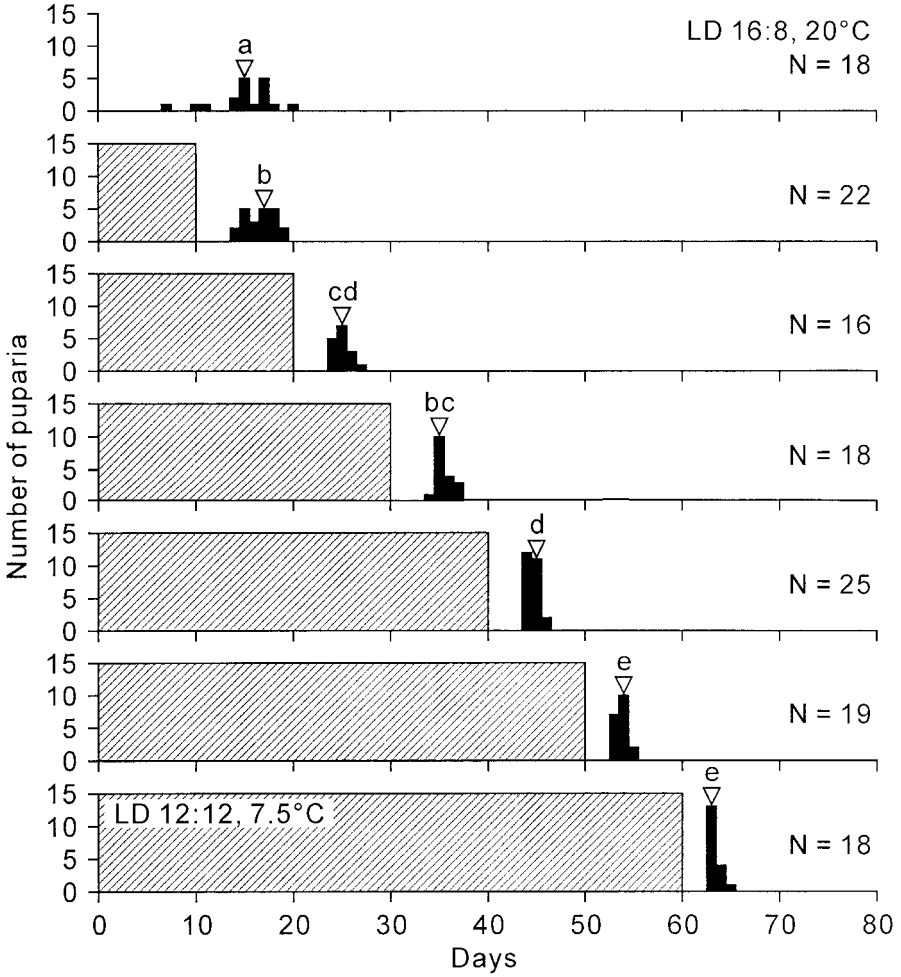

Fig. 2. Frequency distribution of pupariation in diapause larvae of *Lucilia sericata* under LD 16:8 at 20°C after exposure to LD 12:12 at 7.5°C for 0–60 days. Both parental flies and progeny larvae were reared under LD 12:12 at 20°C, and diapause larvae 15 days after cessation of feeding were used. Triangles indicate the medians. Larval duration after transfer to LD 16:8 at 20°C was not significantly different between series with the same letter (P>0.05, Steel-Dwass test). N=16–25.

3). When transferred to the high temperature, the larval duration was significantly shorter than that in larvae kept continuously under LD 12:12 at 20°C (Mann-Whitney U test, P<0.01). The median of larval duration in the former condition was 8.5 days. When larvae were transferred to the high temperature after exposure to a low temperature of 7.5°C, larval duration at 25°C was shorter as the period of the low temperature lengthened to 40 days. All the larvae pupariated within 3 days at 25°C after exposure to the low temperature for 30 days or more. However, larval duration after 60 days' exposure to the low temperature was a little longer than that after 40 days' exposure.

Furthermore, the effects of the long-day photoperiod and high temperature without exposure to low temperature were examined. Diapause larvae were kept under the conditions in which they were raised (LD 12:12 at 20°C) for 10, 20, or 30 days, and then transferred to LD 16:8 at 20°C or LD 12:12 at 25°C. There was no significant difference in larval duration after transfer to the long-day conditions between insects with and without exposure to a low temperature (Figs. 2 and 4A; Mann-Whitney U test, P>0.05). After the transfer to the high temperature, a significant difference was found in larval duration only between insects kept at 20°C and those kept at 7.5°C for 30 days (Figs. 3 and 4B; Mann-Whitney U test, P<0.01). Thus, the effects of the long-day photoperiod and high temperature drastically shortened larval duration, and the effects of low temperature were not detectable in most cases.

Larval duration without exposure to low temperature was quite different between diapause larvae kept continuously under LD 12:12 at 20°C (Fig. 1) and those transferred to LD 16:8 at 20°C (Fig. 2). Therefore, larval duration was examined in diapause larvae transferred to various photoperiods at 20°C (Fig. 5A). Larval duration was significantly longer in insects kept continuously under LD 12:12 than that in any other photoperiod. There was no significant difference in larval duration among LD 14:10, LD 15:9, and LD 16:8. Larval duration in larvae transferred to LD 13:11 was intermediate between those kept under LD 12:12 and those transferred to LD 14:10, LD 15:9, or LD 16:8.

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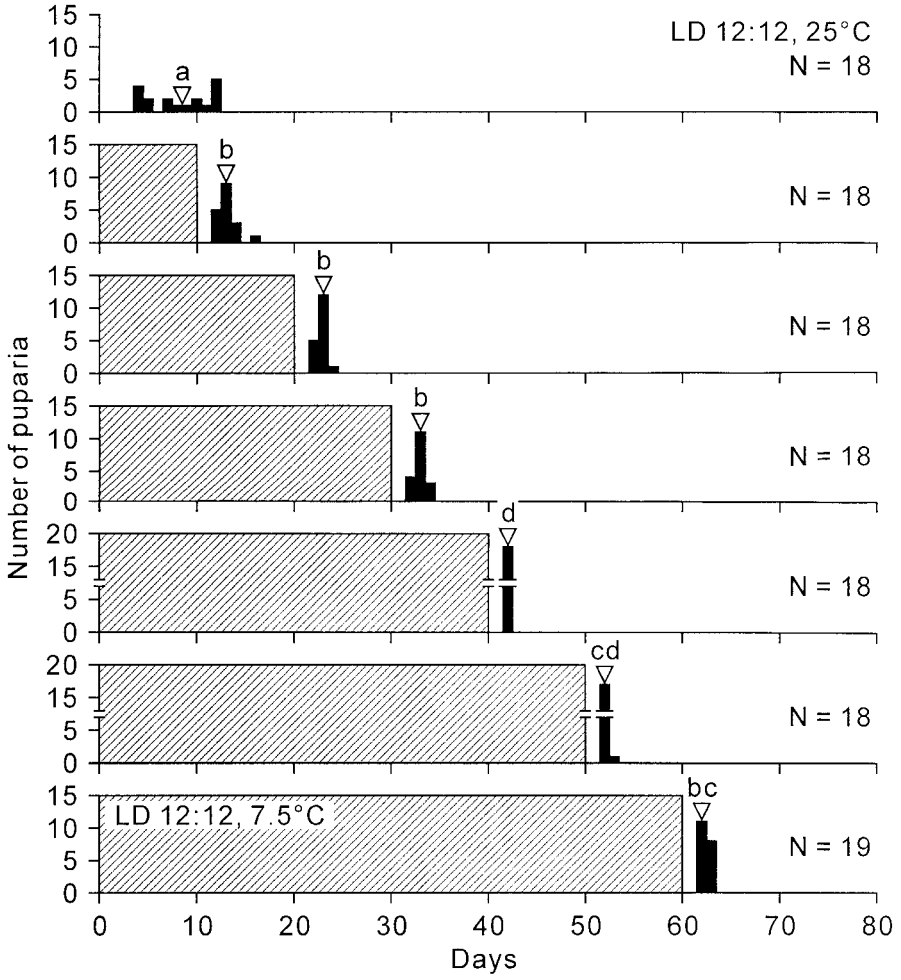

Fig. 3. Frequency distribution of pupariation in diapause larvae of *Lucilia sericata* under LD 12:12 at 25°C after exposure to LD 12:12 at 7.5°C for 0–60 days. Both parental flies and progeny larvae were reared under LD 12:12 at 20°C, and diapause larvae 15 days after cessation of feeding were used. Triangles indicate the medians. Larval duration after transfer to LD 12:12 at 25°C was not significantly different between series with the same letter (P>0.05, Steel-Dwass test). N=18–19.

The effects of various photoperiods on the induction of larval diapause were also examined (Fig. 5B). The diapause incidence in larvae reared under LD 14:10, LD 15:9, and LD 16:8 was very low (0–1.2%). More than 60% of insects entered diapause under LD 12:12. The diapause incidence under LD 13:11 was intermediate between that of LD 12:12 and that of longer photoperiods.

DISCUSSION

In many insects in temperate regions, diapause development is promoted and completed by low temperature, and morphogenesis then resumes when temperatures rise, so that hatch or emergence are synchronized in spring (Danks, 1987). However, the requirement of low temperature for the completion of diapause development has been overgeneralized (Hodek and Hodková, 1988). In many species, diapause terminates spontaneously under the conditions above a lower thermal threshold (Tauber *et al*., 1986; Danks, 1987; Hodek and Hodková, 1988; Hodek, 2002). In *L. sericata* also, larval diapause terminated spontaneously when kept continuously under the diapause-inducing condition of 20°C and LD 12:12, although pupariation was not synchronized.

Although diapause in *L. sericata* terminated without exposure to a low temperature, a low temperature induced prompt and synchronous pupariation. The necessary period of low temperature exposure for diapause termination is usually approximately 10 weeks or more (Tauber *et al*., 1986; Danks, 1987). In *L. caesar*, the optimal period of exposure to a low temperature for diapause termination is approximately 12 weeks and exposure of 4 or 8 weeks has no or little effect (Ring, 1968). In *L. sericata*, however, low temperature exposure leading to diapause termination was much shorter than that in *L. caesar*, and even 10-day exposure to a low temperature had a significant effect for shortening the duration of diapause. Because Ring (1968) used 5°C as a low temperature, which is different from the low temperature of 7.5°C in the present study, it is not appropriate to compare the results of these two studies directly. However, one possible reason for the different responses is

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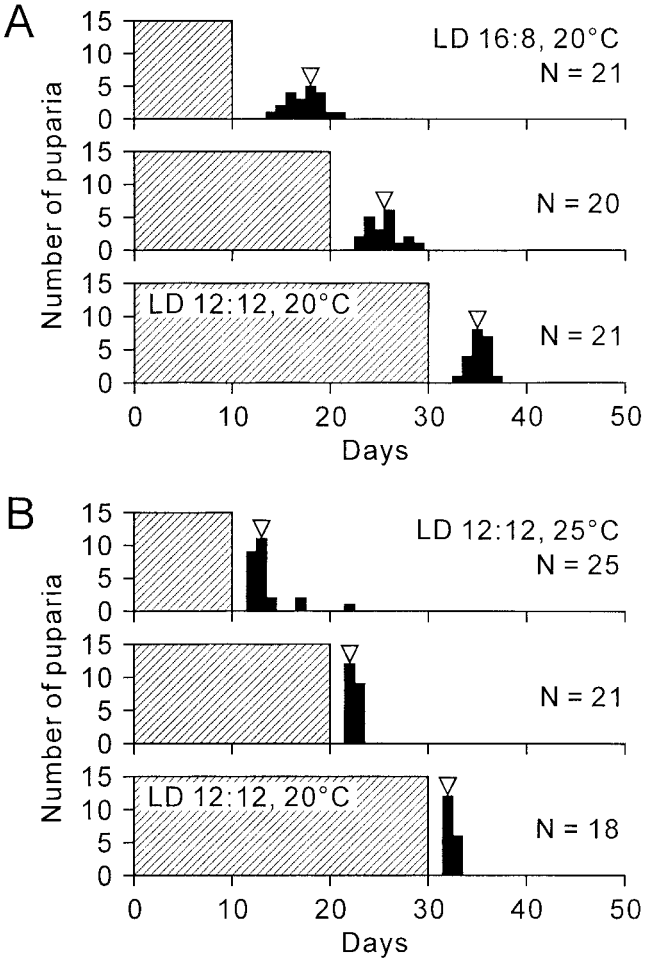

Fig. 4. Frequency distribution of pupariation in diapause larvae of *Lucilia sericata* under LD 16:8 at 20°C (A) and LD 12:12 at 25°C (B) after being kept under LD 12:12 at 20°C for 10–30 days. Both parental flies and progeny larvae were reared under LD 12:12 at 20°C, and diapause larvae 15 days after cessation of feeding were used. Triangles indicate the medians. N=18–25.

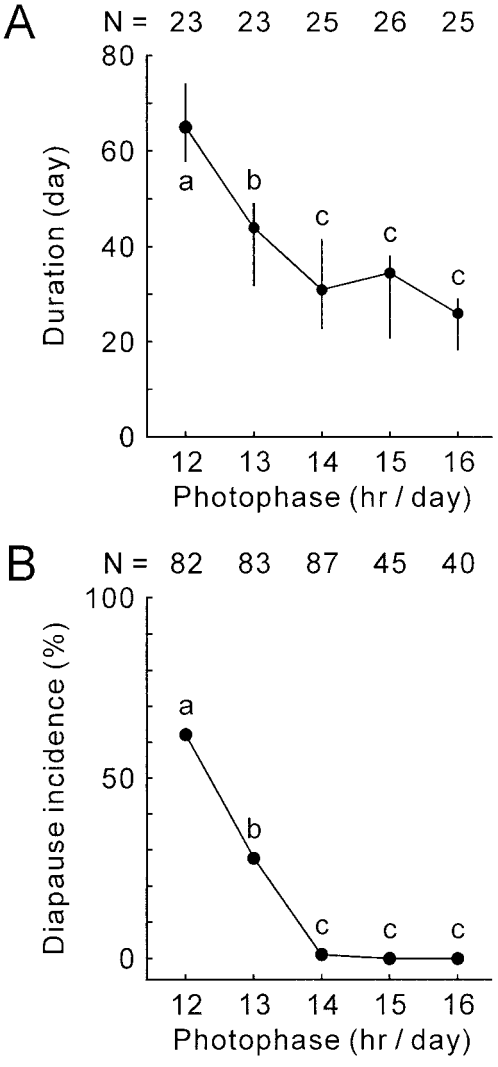

Fig. 5. Effects of photoperiod on the termination (A) and induction (B) of larval diapause in *Lucilia sericata*. (A) Both parental flies and progeny larvae were reared under LD 12:12 at 20°C, and diapause larvae 15 days after cessation of feeding were transferred to various photoperiods at 20°C. Larval duration is shown as median/interquartiles. Larval duration was not significantly different between conditions with the same letter (P>0.05, Steel-Dwass test). N=23–26. (B) Parental flies were reared under LD 12:12 at 20°C, and the progeny larvae were reared under various photoperiods at 20°C. The incidence of diapause was not significantly different between conditions with the same letter (P>0.05, Tukey-type multiple comparison test for proportions). N=40–87.

that the climate in the origin of these laboratory cultures is different: *L. caesar* used by Ring (1968) originated from Glasgow (55.8°N), and *L. sericata* in the present study was from Osaka (34.7°N) with moderate winter. The mean monthly temperature is lower than 10°C for 7 months in Glasgow and 4 months in Osaka.

In many insects, diapause can be terminated not only by exposure to low temperatures but also by transfer to higher temperatures (Tauber *et al*., 1986; Danks, 1987; Hodek and Hodková, 1988). However, an increase in temperature is inevitable for the resumption of morphogenesis after exposure to a low temperature. It is therefore difficult to distinguish the effects of high temperature in the later period from the effects of the increase in temperature (Danks, 1987). For example, a transfer of diapause larvae in *C. vicina* from 5°C to 20 or 25°C without changing the photoperiod induces prompt and synchronous pupariation, although a transfer from 5°C to 12°C is not as effective (Vinogradova, 1991; see Hodek, 2002 also). It is not clear whether higher temperatures or larger increases in temperature are effective for prompt diapause termination. In *L. sericata*, however, not only a transfer from 7.5°C to 25°C but also a transfer from 20°C to 25°C induced prompt and synchronous pupariation. Moreover, a transfer from 20°C to 25°C was much more effective than a transfer from 7.5°C to 20°C. Therefore, a high temperature of 25°C itself has a stimulating effect on diapause termination in this species. In *C. vicina*, high temperatures prevent the induction of diapause programmed by the short-day conditions in the parental generation (Vinogradova and Zinovjeva, 1972;

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Saunders *et al*., 1986). Chernysh *et al*. (1995) have shown that the sensitivity to high temperatures is restricted to the post-feeding stage, and regard this response as an adaptation for producing an additional generation in a relatively warm autumn. In *L. sericata*, however, it is unlikely that larvae enter diapause once under short-day conditions and moderate or low temperatures in autumn, and then terminate it in response to unusually high temperatures in late autumn to produce an additional generation within the year.

In *Lucilia*, it has been thought that photoperiod does not play an important role in the termination of diapause (Ring, 1968) because diapause larvae of *Lucilia* usually burrow and overwinter in the soil (Davies, 1934). The present results indicate that long-day conditions are effective in the termination of diapause in *L. sericata*. Moreover, this species responded quantitatively to photoperiod in diapause termination, with an intermediate duration of diapause under LD 13:11, in which the diapause incidence was also intermediate between LD 12:12 and longer photoperiods. This coincidence suggests that the same mechanism underlies the photoperiodic responses for the termination and induction of diapause. Such a coincidence of the two parameters is found in many other species (Tauber *et al*., 1986).

It has been shown in many temperate species with winter diapause that long-day conditions terminate diapause in the laboratory. In most of these species, however, insects usually show a progressive loss in their sensitivity to photoperiod as diapause development proceeds, and by midwinter photoperiodic sensitivity has ceased under natural conditions (Tauber *et al*., 1986; Danks, 1987). In *L. sericata* also, larvae enter diapause under short-day conditions and low or moderate temperatures in autumn, and diapause development proceeds at low temperatures in winter. Therefore, long-day conditions in spring probably play no role in the termination of diapause under natural condition, even if diapause larvae can receive photoperiodic information in the soil.

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