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Alteration of the pupal diapause program and regulation of larval duration by photoperiod in the flesh fly *Sarcophaga similis* Meade (Diptera: Sarcophagidae)

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Abstract

Using a water treatment technique that extends the larval period of flesh flies, we investigated the effect of photoperiodic exposure on the alteration of the pupal diapause program and regulation of larval duration in the flesh fly *Sarcophaga similis* Meade (Diptera: Sarcophagidae). Exposure to only 4 cycles of long days successfully altered the developmental program from diapause to nondiapause, and short days altered the nondiapause developmental program to a diapause program. Larvae recognized all photoperiods of 14L10D, 15L9D, and 16L8D as long days, but their diapause-averting effects were different, i.e., 16L8D showed the strongest effect, 14L10D showed the weakest, and 15L9D showed an intermediate effect. These results indicate that larvae can discriminate long days quantitatively. *S. similis* also showed a clear photoperiodic response in the larval stage, i.e., larvae reared under short-day conditions pupariated later than those reared under long-day conditions. When diapause-destined larvae were exposed to 3 cycles of long days, some larvae altered their developmental program from diapause to nondiapause and pupariated significantly earlier than individuals destined to diapause.

Key words: Diapause; larval duration; photoperiodic response; quantitative time measurement

INTRODUCTION

The photoperiodic regulation of seasonally occurring events, such as the onset of diapause, is an important aspect of insect life cycle (Tauber et al., 1986; Danks, 1987). In the temperate zone, flesh flies enter pupal diapause in response to short days (Fraenkel and Hsiao, 1968; Denlinger, 1971; Vinogradova, 1976; Saunders, 1979; Kurahashi and Ohtaki, 1979; Tanaka et al., 2008). Stages sensitive to photoperiods have been reported by several authors and are primarily restricted from the late embryonic stage to the early larval stage (Denlinger, 1971; Vinogradova, 1976; Kurahashi and Ohtaki, 1979; Tanaka et al., 2008). In *Sarcophaga peregrina* (also known as *Boettcherisca peregrina*) and *Sarcophaga similis*, however, some sensitivity is retained throughout their development until pupariation (Kurahashi and Ohtaki, 1979; Moribayashi et al., 2002; Tanaka et al., 2008).

In flesh flies, pupariation is inhibited when mature larvae are subjected to wet conditions (water treatment), but their development resumes after the larvae were transferred to dry conditions (Ohtaki, 1966; Moribayashi et al., 1996, 2002; Yoder et al., 2006). Ohtaki (1986) and Moribayashi et al. (1992) showed that the terminal organ located at the anterior end of the first segment of the larval body recognizes water, and this organ then transmits a signal to prevent the release of ecdysteroids that promote pupariation. Moribayashi et al. (2002) extended the larval period of *S. peregrina*, which is sensitive to the photoperiod, using the water treatment technique, and altered their developmental program from diapause to nondiapause or *vice versa* by long-day or short-day exposure during water treatment. Using the same technique, we investigated the effect of photoperiodic exposure during water treatment on the alteration of the pupal diapause program and larval duration in *S.*

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similis to analyze the physiological mechanisms underlying photoperiodic responses.

MATERIALS AND METHODS

Insects. A colony of *Sarcophaga* (*Pandelleisca*) *similis* (Pape et al., 2007), also known as *Parasarcophaga similis* (Hirashima, 1989), originating from adults captured in Osaka city (34.60°N, 135.51°E), Japan, in 2002, was maintained as described by Denlinger (1972). Newly emerged adults under 16L8D (16-h light and 8-h darkness) at 25°C were transferred to 12L12D (12-h light and 12-h darkness) or 16L8D at 20°C on day 0 or day 1 and provisioned with water, sugar, and a piece of beef liver. Females larviposited 15 days after provision of the liver, and the larvae were continuously reared under the same conditions.

White fluorescent lights (FLR15W, FLR20SW/M, FLR30SW, or FL40SW; Panasonic Electric Works) were used as a white light source at an intensity of 0.8–1.3 $W \cdot m^{-2}$ on the surface of the container in the photophase; however, feeding larvae stayed within their food (beef liver), and mature larvae in wet wood chips at 10 mm depth during water treatment or concealed in dry wood chips to a depth of 30–50 mm after water treatment (see below); therefore, the light intensity actually perceived by the larvae depended on their positions.

Water treatment. Five days after larviposition, larvae that were regarded as "mature larvae" were placed in wet wood chips at a depth of 10 mm for various periods under various photoperiodic conditions at 20°C. After water treatment, the larvae were transferred to dry wood chips 30–50 mm in depth under 12L12D at 20°C to allow them to pupariate and pupate, unless otherwise stated. Ten or more days later, the developmental status (diapause/nondiapause) of the pupae was determined according to the standard described by Fraenkel and Hsiao (1968).

Alteration of pupal diapause program. Mature larvae reared under 12L12D or 16L8D were subjected to water treatment for various periods and exposed to 12L12D, 16L8D, constant darkness (DD) or constant light (LL). Or, mature larvae reared under 12L12D were subjected to water treatment and exposed to 12L12D, 13L11D, 14L10D, 15L9D, or 16L8D for 4, 6, 8, or 10 days.

Duration of the larval period. We examined the time of pupariation to check whether larval duration was affected by photoperiodic conditions during water treatment. Mature larvae reared under 12L12D or 16L8D were subjected to water treatment for 0–6 days and exposed to either 12L12D or 16L8D. In this experiment, larvae were kept under 12L12D or 16L8D after water treatment. Puparia were counted every day.

Statistical analyses. Diapause incidences were compared by Tukey-type multiple comparisons for proportion (Zar, 1999, pp. 563–564) and larval durations by Mann-Whitney's *U* test (Zar, 1999, pp. 146–149).

RESULTS

Photoperiodic induction of diapause

S. similis showed a clear photoperiodic response, i.e., all individuals reared under 12L12D throughout their development entered pupal diapause, while those reared under 16L8D throughout did not (Fig. 1 *A*, *B*). When mature larvae reared under 12L12D were maintained under the same photoperiodic conditions during water treatment, they entered diapause, irrespective of the duration of water treatment (Fig. 1 *C1–C7*). When mature larvae reared under 12L12D were subjected to water treatment under 16L8D cycles for 1 or 2 days, they did not respond to the long days and most larvae entered diapause (Fig. 1 *D1*, *D2*); however, larvae averted diapause when they were exposed to 16L8D cycles for 3 or more days (Fig. 1 *D3–D7*).

When mature larvae reared under short-day conditions were exposed to 4 cycles of long days and then to additional short days during water treatment (Fig. 1 *E1–E3*), the incidence of diapause increased as did the number of short days. When diapause-destined larvae were exposed to an additional 2–6 cycles of short days and then to 4 cycles of long days during water treatment, the incidence of diapause was always low (Fig. 1 *F1–F3*).

Figure 1 *G1–G6* show that both DD and LL promoted the nondiapause program, but the former was less effective than the latter and 16L8D. Long-day exposure from the embryonic stage to day 5 larval stage confirmed the nondiapause program, which was not altered by an additional 5 or 10 cycles of short days during water treatment (Fig. 1 *H1*, *H2*).

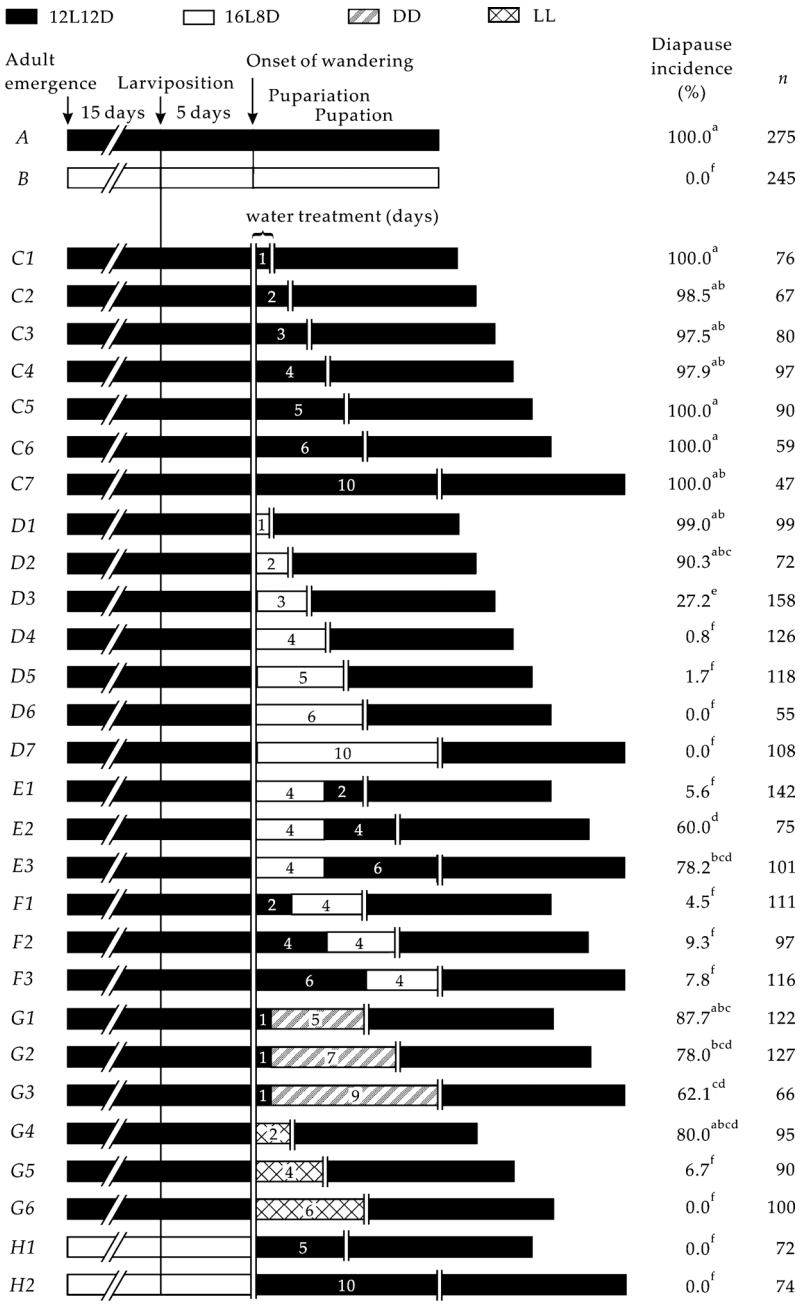

Fig. 1. Photoperiodic induction of diapause in *Sarcophaga similis* with or without water treatment. The experimental designs are also shown. Mature larvae reared under either 12L12D or 16L8D were subjected to water treatment and exposed to various photoperiodic conditions for various periods. After water treatment, they were transferred to dry wood chips to induce pupariation and pupation under 12L12D or 16L8D. The same lower-case letters denote that diapause incidences did not significantly differ (Tukey-type multiple comparisons for proportion, $p>0.05$).

Photoperiodic response curves

Mature larvae reared under 12L12D were subjected to water treatment under various photoperiods for various periods. Water treatment for 6, 8 and 10 days produced identical photoperiodic response curves, i.e., differences among diapause incidences under a given photoperiod were less than 4%. Thus, we presented response curves derived from 4 and 6 days of water treatment only (Fig. 2). Diapause was averted when larvae were exposed to 6 days of 14L10D, 15L9D or 16L8D, whereas some entered diapause when they were exposed to

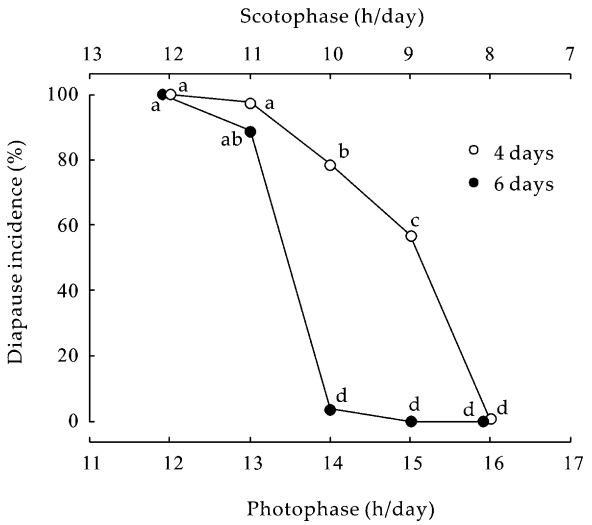

Fig. 2. Photoperiodic response curves in *Sarcophaga similis* subjected to water treatment. Mature larvae reared under 12L12D were subjected to water treatment and exposed to various photoperiodic conditions (12L12D, 13L11D, 14L10D, 15L9D, or 16L8D) for 4 or 6 days. After water treatment, they were transferred to dry wood chips under 12L12D (n=55–126). The same letters denote that diapause incidences did not significantly differ (Tukey-type multiple comparisons for proportion, $p>0.05$).

4 days of 14L10D or 15L9D.

Photoperiodic response for larval duration

Figure 3 shows the time of pupariation after the transfer of larvae to dry conditions and their developmental program. When larvae were reared under 16L8D throughout development, even during water treatment, they pupariated within a few days after transfer to dry conditions and averted diapause. When larvae were reared under 12L12D throughout their developmental period, even during water treatment, they pupariated later than those reared under 16L8D (Mann-Whitney's U test, $p<0.05$) and entered diapause. Next, mature larvae reared under 12L12D were subjected to water treatment and exposed to 16L8D for 1–6 days. When larvae were exposed to 3 cycles of long days during water treatment, some individuals altered the diapause developmental program to a nondiapause program and pupariated significantly earlier than individuals destined to enter diapause (Mann-Whitney's U test, $p<0.05$). When larvae were exposed to 4 or 6 cycles of long days, all averted diapause and pupariated earlier.

DISCUSSION

S. similis larvae discriminated long from short days during water treatment and altered their developmental program from diapause to nondiapause. They also discriminated short from long days during water treatment and altered their developmental program from nondiapause to diapause (see also Goto and Numata, 2009).

Four cycles of long days were highly effective in preventing diapause (Fig. 1 *D4*, *F1–F3*), whereas exposure to 4 cycles of short days was less effective in promoting diapause (Fig. 1 *E2*). This suggests that photoperiodic information for long and short days is accumulated in a different manner as reported in many insects (reviewed by Saunders, 2002). It should be noted that 4 cycles of long days followed by 4 cycles of short days during water treatment produced a high incidence of diapause, whereas 4 cycles of short days followed by 4 cycles of long days during water treatment produced a low incidence of diapause (Fig. 1 *E2*, *F2*). Similar results were obtained when 4 cycles of long days and 6 cycles of short days were applied during water treatment (Fig. 1 *E3*, *F3*). These results indicate that 4 cycles of long days do not simply promote the nondiapause phenotype, but erase all the short-day information that has been accumulated.

Conventionally, the photoperiodic time measurement system has been described as a qualitative or all-or-none mechanism, i.e., assuming that the photoperiodic clock distinguishes only long from short days or short from long days (Denlinger et al., 2005); however, recent models for photoperiodic time measurement incorporate a quantitative concept (reviewed by Saunders, 2002). Such quantitative time measurement was indeed observed in some insect species (Hardie, 1990; Kimura, 1990; Spieth and Sauer, 1991; Numata and Kobayashi, 1994). *S. similis* larvae recognized all photoperiods of 14L10D, 15L9D, and 16L8D as long days, but their diapause-averting effects were different, i.e., 16L8D showed the strongest effect, 14L10D showed the weakest, and 15L9D showed an intermediate effect. These results indicate that larvae can discriminate long days quantitatively and accumulate photoperiodic information daily. Goto (2009) found that this species can also discriminate short days quantitatively, i.e., the wild-type strain enters diapause both under 10L14D and 12L12D,

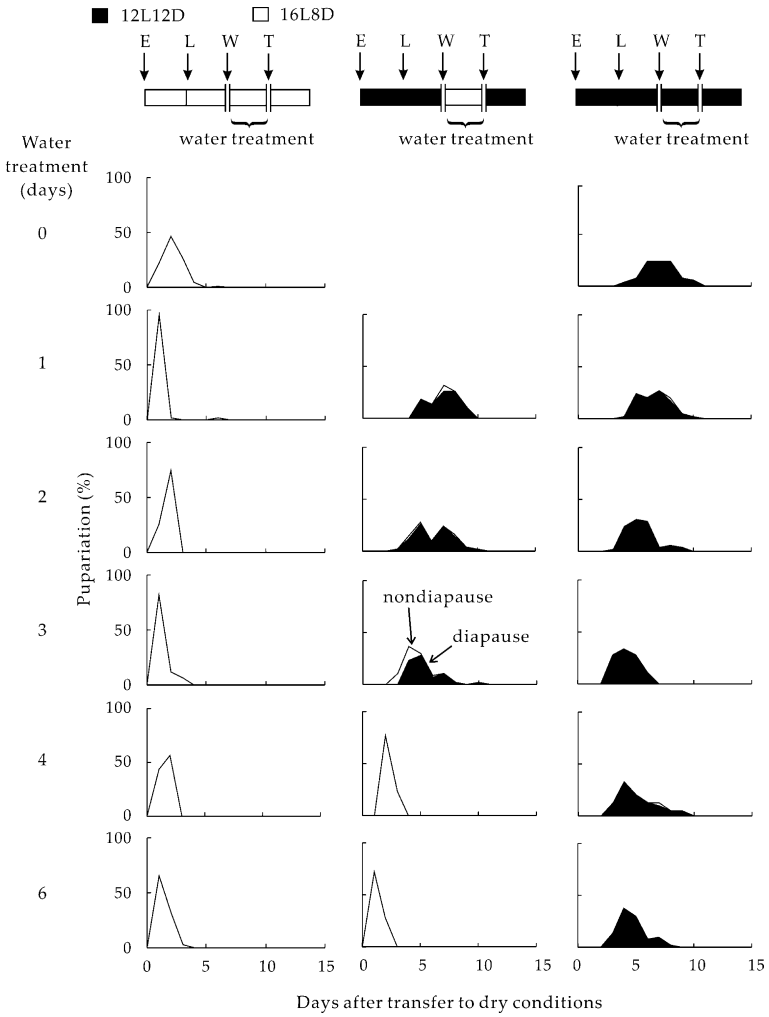

Fig. 3. Time of pupariation and developmental program (diapause or nondiapause) in *Sarcophaga similis*, shown in stacked area graphs. Experimental designs are shown in upper panels. E: eclosion of parental adults, L: larviposition, W: onset of water treatment, T: transfer of larvae to dry conditions. Mature larvae reared under either 12L12D or 16L8D were subjected to water treatment for 0–6 days and exposed to either 12L12D or 16L8D. Thereafter, they were transferred to dry wood chips under 12L12D or 16L8D. Closed and open polygons in graphs indicate diapause and nondiapause, respectively (n=33–100).

whereas a variant strain, which was established by artificial selection to show a low incidence of diapause even under short-day conditions, discriminates between 10L14D and 12L12D and shorter days are recognized as stronger diapause-inducing stimuli.

S. similis shows a clear photoperiodic response not only in the pupal stage (diapause) but also in the larval stage, i.e., larvae reared under short-day conditions pupariated later than those reared under long-day conditions (see also Goto, 2009). Such photoperiodic responses in the larval period were also reported in several other flesh fly species (Henrich and Denlinger, 1982; Bradley and Saunders, 1986; Moribayashi et al., 1988, 2008). Interestingly, when diapause-destined larvae of *S. similis* were exposed to 3 cycles of long days, some larvae altered their developmental program from diapause to nondiapause and pupariated significantly earlier than individuals destined for diapause. Thus, the present study revealed the close physiological association between the time of pupariation (larval duration) and diapause developmental program, as reported in *S. argyrostoma* (Saunders, 1971, 1973). Giebultowicz and Denlinger (1986) found that both the pupariation time and developmental program (diapause or nondiapause) are determined by the brain-ring gland com-

plex in *Sarcophaga crassipalpis*, and that transplantation of the complex from one larva to another can transfer the physiological characteristics of the donor to the host. Goto (2009) found that the larval photoperiodic response is also genetically associated with the pupal photoperiodic response in *S. similis*, i.e., loss of the larval photoperiodic response was co-selected by artificial selection for nondiapause. Thus, larval duration and the diapause/nondiapause program are physiologically and genetically inseparable, and are possibly controlled by the same mechanism, at least in part.

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