Sensitive stages for photoperiodic induction of pupal diapause in the flesh fly Sarcophaga similis (Meade) (Diptera: Sarcophagidae)

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Abstract

Photoperiod is a major factor in the induction of pupal diapause in the ovoviviparous flesh fly, Sarcophaga similis. This species entered diapause when kept under short-day conditions throughout the embryonic and larval stages. Exposure to long-day conditions for the whole embryonic or whole larval stage, however, prevented diapause. Moreover, most insects did not enter diapause when they were exposed to long-day conditions for only 2 days in the embryonic stage just before larviposition. When the uterus containing embryos was removed from the mother’s body and kept in vitro under long-day conditions, most of these embryos became nondiapause pupae even under subsequent short-day conditions. When postfeeding larvae were exposed to long-day conditions for 3 days, 50% entered diapause. From these results, it is concluded that sensitivity to photoperiod is highest in the later embryonic stage and embryonic sensitivity to photoperiod is independent of the mother.

Key words: Embryo; flesh fly; photoperiodic sensitivity; pupal diapause; sensitive stage

INTRODUCTION

Many temperate insects enter diapause before the arrival of unfavorable seasons, and the regulation of diapause is mostly governed by photoperiod. Photoperiodic sensitivity for the induction of diapause in these insects is generally restricted to a particular developmental stage in their life cycles, most often a dormant stage itself or a stage preceding it (Danks, 1987).

The flesh fly Sarcophaga spp. is ovoviviparous and photoperiodically enters diapause as coarctate pupae (e.g. Denlinger, 1971; Kurahashi and Ohtaki, 1979). Most of these species use feces of carnivores and carcasses as resources for growth, development and reproduction under natural conditions (Kurahashi, 1997). Larvae produced by females of these species promptly burrow into these resources and remain in them until the cessation of feeding. It is likely that such spatially closed environments during the larval period would be unfit for receiving external photoperiodic signals and temperature for diapause induction. It should therefore be considered that the progeny of flesh flies mainly perceive information in the female’s uterus as embryos (Denlinger, 1971; Kurahashi and Ohtaki, 1979).

Although the sensitive stages for photoperiodic induction of diapause in flesh flies were reported by several authors (Denlinger, 1971; Vinogradova, 1976; Kurahashi and Ohtaki, 1979), each result differed slightly among species in the sensitive phase and its duration, and some examinations did not consider the effect of temperature on development. Moreover, two flesh flies, Sarcophaga peregrina and S. similis, are common and sympatrically distributed throughout Japan (Kano and Shinonaga, 1997; Moribayashi, 2001), but the photoperiodic response to diapause induction in the latter species is unknown. Such information would be useful for comparing adaptive strategies between species sharing a common niche. In the present study, the crucial phase in the sensitive stages for photoperiodic induction of diapause and the intensity of sensitivity in these stages were therefore examined under constant temperature in S. similis.
MATERIALS AND METHODS

Insects. Adult females of *S. similis* were captured with a polyethylene container trap (Kurahashi et al., 1984; Tachibana and Numata, 2006) on the campus of Osaka City University (34.59°N, 135.50°E), Japan, in April 2001. A laboratory culture originating from these adult females was maintained under long-day conditions of 16 h light and 8 h darkness (16L : 8D) at 25°C, and the second to eighth generations in the laboratory were used for experiments. In all experiments, temperature fluctuation did not exceed ±1°C.

Experimental culture. Adult flies were maintained in groups of about 50 in a plastic container (15 cm in diameter, 9 cm in depth) covered with nylon netting. Water, granulated sucrose and small pieces of beef liver were provided throughout their adult lives. Larvae deposited by adult females on liver samples were placed in a 500 ml beaker with a 150 g lump of artificial diet (Tachibana and Numata, 2001) within 24 h. After the cessation of feeding, larvae were transferred to plastic containers filled with dry wood chips as the pupariation medium and covered with nylon netting. Puparia were maintained in containers until adult emergence.

Photoperiodic-sensitive stage. After adult emergence, the insects and larvae deposited by these insects were reared at 20°C under different combinations of long-day and short-day (12L : 12D) conditions in specific periods of their life cycles.

Embryonic sensitivity. Our preliminary observation showed that fertilized eggs in females usually moved into the uterus 10 days after adult eclosion at 20°C. The uterus including eggs was therefore removed from the female 10 days after adult emergence. Eggs were dissected from the uterus and maintained in an artificial uterus, consisting of a moistened cotton sheet with 0.9% NaCl solution (2–3 mm in thickness) covered with filter paper in a small Petri dish (3 cm in diameter, 1 cm in depth) (Denlinger, 1971). The Petri dish was sealed with Parafilm® (American National Can Co.) to maintain a moist uterine-like environment. In this experiment, only larvae that hatched 5 days after separation from the mother, i.e. 15 days after adult emergence of the mother, were used.

Determination of diapause status. The antennae of pupae were used for the evaluation of diapause status as reported by Fraenkel and Hsiao (1968). Ten days after pupariation, the anterior caps of puparia were removed to expose the head region of the pupae. Pupae with pigmented antennae were considered as nondiapause, whereas those whose antennae had no sign of pigmentation were regarded as entering diapause.

RESULTS

Figure 1 shows the sensitive stages for photoperiodic induction of diapause in all developmental stages. Most insects entered diapause when they were continuously reared under short-day conditions (Fig. 1A), whereas diapause was prevented under long-day conditions (Fig. 1G). A high incidence of diapause was also induced when insects were transferred from long-day to short-day conditions when fertilized eggs were shifted to the uterus in the bodies of females (Fig. 1B); however, diapause was prevented when insects were transferred to short-day conditions after larviposition (Fig. 1C), and also when insects were transferred from short-day to long-day conditions during the shifting of fertilized eggs to the uterus (Fig. 1D). Diapause was also prevented when insects experi-
enced 5 long-day cycles only at the embryonic stage (Fig. 1E), or were transferred from short-day to long-day conditions at larviposition (Fig. 1F). These results indicate that sensitivity to photoperiod for the induction of diapause exists in both the embryonic and larval stages.

Photoperiodic sensitivity during the larval stage was then examined in detail (Fig. 2). Long-day exposure for two or three days was applied between periods of short days. Insects were sensitive to photoperiod throughout the larval period, but diapause was not completely prevented by long-day treatments. Diapause incidence was significantly higher in insects that experienced long-day cycles immediately before the cessation of feeding (Fig. 2C) than in insects that experienced them in the early, middle or post-feeding stage (Fig. 2A, B and D, respectively). Thus, sensitivity to photoperiod was lower in the larval stage than in the embryonic stage.

Next, sensitivity to photoperiod for the induction of diapause during embryonic development was examined in detail (Fig. 3). Embryonic development was usually completed 5 days after transfer to the uterus at 20°C. Most insects entered diapause when they were reared under short-day conditions throughout the developmental stages whether they were kept in the female’s body or in an artificial uterus (Fig. 3A and B, respectively). On the other hand, most insects averted diapause when they experienced three or more long-day cycles during embryonic development (Fig. 3C, D and E). The proportion of insects entering diapause, however, was different among the experimental series when the insects experienced 2 long-day cycles during embryonic development: diapause was prevented when eggs were exposed to long-day conditions for the last 2 days (Fig. 3F), whereas most insects entered diapause when eggs were kept under long-day conditions for the first 2 days (Fig. 3G), and about a half of the insects entered diapause when they experienced 2 long-day cycles in the middle of embryonic development (Fig. 3H). Moreover, only a single long-day cycle had a significant effect on averting diapause when given on the fourth day in the artificial uterus (Fig. 3I), but had only a small statistically insignificant effect when given on the last (fifth) day in the artificial uterus (Fig. 3J). In the later period of embryonic development, insects were thus highly sensitive to long-day con-
To examine whether there is a maternal effect on inducing diapause in offspring via the uterus, uteri containing fertilized eggs that had just shifted to the uterus were removed from the mother, and the eggs were incubated in an artificial uterus either still within or after being removed from the uterus (Table 1). All larvae were transferred to short-day conditions after hatching. When eggs were maintained under short-day conditions during embryonic development, most insects entered diapause irrespective of the presence of the uterus (Table 1B and C), as was the case for insects that developed normally in a female body during the egg stage (Table 1A). When eggs without the uterus were kept under long-day conditions, however, no insects entered diapause (Table 1D).

**DISCUSSION**

The pupal diapause of *Sarcophaga similis* was induced under short-day conditions at a moderate temperature in the present study, as has been shown in other *Sarcophaga* spp. (Denlinger, 1971; Saunders, 1971; Vinogradova, 1976; Kurahashi and Ohtaki, 1979); however, sensitivity to photoperiod in *S. similis* differed from other *Sarcophaga* spp. Induction of diapause in *S. similis* primarily depended on the photoperiod that they experienced in the later embryonic stage, but some sensitivity to photoperiod was retained throughout their development until pupariation. In *S. peregrina*, sensitivity to photoperiod is high in the later stages of the embryo and post-feeding larva, but disappears in young third instar (Kurahashi and Ohtaki, 1979). In *S. crassipalpis*, moreover, sensitivity to photoperiod exists in the later embryonic stage to the stage just after larviposition, but is subsequently lost (Denlinger, 1971). The difference in the duration of the embryonic period may be related to the difference in photoperiodic sensitivity, because the duration of embryonic development in *S. peregrina* is 9 days at 20°C (Moribayashi et al., 2002), which is much longer than that in *S. similis* (5 days) in the present study; however, it is still unclear why the photoperiodic-sensitive stage for the induction of diapause is different among species.

Female flesh flies lay first-instar larvae on materials of animal origin, such as carcasses and faeces. The larvae immediately burrow in, and stay there until the cessation of feeding. The larvae then leave the animal-derived materials and immediately burrow into the soil to pupate (Moribayashi, 2001). The period during which insects receive accurate photoperiodic information is therefore limited to the embryonic stage in the mother’s body and the post-feeding larval stage. In addition, diapause incidence is influenced by temperature throughout larval development in *S. crassipalpis* (Denlinger, 1971). As Denlinger (1971) pointed out, the temperature of food materials into which larvae crawl is usually higher than the external temperature, and larvae in these closed environments cannot accurately obtain information on ambient temperature. The higher sensitivity before larviposition in *S. similis* therefore seems to be ideal for receiving precise photoperiodic and temperature information to determine subsequent development.

Moreover, higher sensitivity at the end of embryonic development may also include developmental constraints. It is generally accepted that measurement of night length, accumulation of inductive cycles, and storage of this information are required in a photoperiodic response (Saunders, 2002), and

<table>
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<tr>
<th>Conditionsa</th>
<th>Number of pupae</th>
<th>Incidence of diapause (%)b</th>
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<tr>
<td></td>
<td>Diapause</td>
<td>Nondiapause</td>
</tr>
<tr>
<td>A in situ</td>
<td>12L : 12D</td>
<td>69</td>
</tr>
<tr>
<td>B in vitro</td>
<td>+ uterus</td>
<td>12L : 12D</td>
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<tr>
<td>C in vitro</td>
<td>- uterus</td>
<td>12L : 12D</td>
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<tr>
<td>D in vitro</td>
<td>- uterus</td>
<td>16L : 8D</td>
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a Eggs developed in the mother’s body (*in situ*) or in an artificial uterus after removal from the mother (*in vitro*). + uterus, eggs were in the uterus; – uterus, eggs were removed from the uterus. After hatching, all larvae were reared under 12L : 12D.

b Values followed by the same letter did not differ significantly at the 5% level (G test with Bonferroni correction).
therefore the response seems to be impossible without a highly organized nervous system. Denlinger (1971) suggested that the lack of response to photoperiod in the very early embryonic stages is attributable to the immature nervous system in the early phase of embryonic development in *S. crassipalpis*. In *Drosophila melanogaster*, the central nervous system is formed in the middle of embryonic development (Campos-Ortega and Hartenstein, 1985). It is conceivable that in *S. similis* photoperiodic sensitivity is also lacking until the middle of embryonic development because the central nervous system is not yet formed.

In many insect species, the offspring’s phenotype can be affected by the environments experienced by the mother (Mousseau and Dingle, 1991). The transmitting factors and mechanisms of the mother’s experience of the environment to her offspring are mostly unknown, but it is thought that the phenotype of the offspring is changed via cytoplasmic factors (e.g. yolk amount, hormones and mRNAs) originating in the mother (Mousseau and Fox, 1998). Diapause is prevented even under diapause-inducing conditions in offspring produced by mothers that have experienced short days in *S. bullata* and *S. similis* (Henrich and Denlinger, 1982; M. Tanaka, S.-I. Tachibana and H. Numata, unpublished), so maternal factors for preventing diapause can be transmitted to offspring of *Sarcophaga* spp. In offspring derived from non-diapause females, however, the incidence of diapause is determined by the photoperiodic conditions experienced by offspring during embryonic development in both *S. similis* and in *S. crassipalpis* (Denlinger, 1971; present results). We therefore suggest that the photoperiod is directly received by the embryo through the cuticle and arthrodial membrane of the mother’s abdomen, and no maternal factors for inducing diapause are transmitted to the offspring.

**REFERENCES**


