INDUCTION OF PUPAL DIAPAUSE AND PHOTOPERIODIC SENSITIVITY DURING EARLY DEVELOPMENT OF SARCOPHAGA PEREGRINA LARVAE*

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SUMMARY: Effects of long day and short day treatments during the embryonic and larval stages on induction of pupal diapause were studied on a diapausing race of Sarcophaga peregrina. Two long day (15L 9D) cycles during 2 days before or after the larviposition completely stopped the induction of pupal diapause on larvae which grow in short day condition before and after the long day treatment. The sensitivity appeared to decrease during the early stage of the third instar and to increase again to some extent in the prepupal stage.

INTRODUCTION

A certain photoperiod and temperature are indispensable factors for inducing pupal diapause of the larviparous fleshfly, Sarcophaga peregrina. Ohtaki and Takahashi (1972) found that only 4 long day cycles given in the larval stage of S. peregrina exclusively induced the pupal type to non-diapause, eliminating the effects of short day during the early developmental stage. These results imply that larvae of this species have a marked tendency to develop into nondiapause pupae. They also observed that larvae usually developed into the nondiapause type of pupae regardless of short day conditions in the larval stage if their parents were reared under long day condition (14 Light 10 Dark, 25 C). This suggests that the maternal influence or the sensitivity to day length and temperature in the embryonic stage may be involved in determination of the physiological state of pupa. Vinogradova and Zinovjeva (1972) interpreted it as the maternal influence on the physiological state of the progeny. Prior to these investigators, Denlinger (1971) concluded that the embryos of S. crassipalpis in the mother’s uterus have photoperiodic sensitivity, and that the photoperiodic experience by the embryos is directly effective on determination of pupa to diapause. The results of our crossing experiments between nondiapausing and diapausing races also suggested that the physiological state of the progeny is determined by combi-

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nation of genetic and environmental conditions (Kurahashi and Ohtaki, 1977). Vinogradova (1976) proved that the external photoperiodic information is directly perceived by the embryo in *S. similis* and *S. septentrionalis*. She also discovered that the photoperiodic regime is not important during the first 12 days after imaginal emergence, and that 4 short day cycles are enough to induce pupal diapause in *S. septentrionalis*. The present study was designed to confirm the analogous phenomenon in a diapause race of *S. peregrina* as that observed by Denlinger (1971) and Vinogradova (1977) and a special attention was paid to know whether the sensitivity of offspring to photoperiodic signals is constant or not during their embryonic and larval developments.

**Materials and Methods**

A diapausing race of *S. peregrina* (= *Boettcherisca peregrina*) maintained for thirteen years in our laboratory was used. The breeding procedure was detailed in the previous paper (Ohtaki, 1966). The original colonies were derived from a single wild female collected in Omiya near Tokyo in 1965. Almost all offspring of them undergo pupal diapause in response to a certain environmental condition of photoperiods (13L 11D–10L 14D) and temperature (20°C). Under a long day condition (15L 9D) at 20°C, they seldom enter diapause (Kurahashi and Ohtaki, 1977).

**Experiment 1:**

This experiment was concerned with the effects of photoperiod and temperature on the gravid female. Newly emerged males and females kept in each of six cages (A–F) were reared under a long day (16L 8D) room at 25°C for 8, 10, 12, 14, 16, or 18 days after emergence. After a long day and high temperature treatment, each cage was transferred to a short day (12L 12D) room at 20°C and left there until used in the experiments. In 14 days after emergence, the first instar larvae were dissected out from uterus and reared under a short day regime except for series E and F. Prepupae and pupae left under the 12L 12D regime until diapause and nondiapause pupae were counted in one week after pupation. On the other hand, females were dissected to observe the stage of ontogenesis and embryogenesis under the conditions of 12L 12D, 20°C or 16L 8D, 25°C.

**Experiment 2:**

To study the change of sensitivity to photoperiod during the early developmental stage of progeny, newly emerged flies were reared at 20°C under short day length (12L 12D) for 14 days after emergence. When mature eggs were transferred from the ovary to the uterus, the uterus was dissected out and the eggs cultured in vitro for 6 days by the method described by Denlinger (1971). The 6 days' old embryos, prenatal first instar larvae incubated in the uterus, were transferred to
a glass jar and reared with hog liver for 6 days. The matured larvae were kept in dry sawdust for pupation. Percentage of diapause pupae was counted a week later. The dissected uterus was placed on cotton matt wetted in a small petridish (6 cm in diameter by 2 cm in height), and covered with a moist filter paper. Ten series (A–J) of cultures were prepared for this experiment. Each series comprised five vessels. All vessels kept in a short day condition (12L 12D, 20 C) up to the end of experiment except for two long day (15L 9D) impulses. Series A, however, were kept in a short day regime throughout the experimental period for control. In series B, the embryos were exposed to a long day regime 14–15 days after the mothers emerged. Afterwards, we expressed the age of progeny simply as 14–15 days' old. The long day treatments were given at the ages indicated in parentheses in the following; series C (16–17 days' old), series D (18–19 days' old), series E (20–21 days' old), series F (22–23 days' old), series G (24–25 days' old), series H (26–27 days' old), series I (28–29 days' old), and series J (30–31 days' old). The culture and rearing the animals for all experiments were carried out at 20 C.

Results

Experiment 1:

Twenty-day mature larvae (60–90 individuals) were transferred to glass vessels containing dry sawdust for pupation. Diapause and nondiapause pupae were counted in each series of rearings. The experiment was repeated three times. The average incidences of diapausing pupae are illustrated in Fig. 1. The egg maturation stage, i.e. first 8 days after imaginal emergence, was independent of the effect of the long day treatment (series A) and all offspring underwent pupal diapause in response to the subsequent short day treatment. However, when the long day regime was extended to 10 days as in series B, the diapause incidence decreased to 10%. Extension of the long day regime showed an increased number of nondiapause pupae. No offspring underwent pupal diapause in series E and F where the long day regime was extended to 14 days or longer after imaginal emergence. Anatomical evidences indicated that the first 7 days corresponded to the stage of oogenesis at 25 C. After oogenesis, the eggs were moved from the ovary, fertilized, and then transferred into the uterus. The embryos were incubated within the uterus until hatching. At 20 C the period from the 8th to 14 days after imaginal emergence was related to embryogenesis and the following prenatal first instar. The first instar larvae were then deposited. Then it took 6 days for progeny to complete its larval development at 20 C (postnatal first instar for 1 day, 2nd instar for 1 day, and 3rd instar 4 days).

Experiment 2:

In this series, the temperature was kept constant at 20 C throughout the experiments. The adults were reared under a short day regime (12L 12D). The
Fig. 1. Incidence of diapause observed with rearing series A–F in experiment 1. The percentage of pupae entering diapause is indicated by the filled portion of the circle. Three experiments were performed and the results averaged.

The results presented are closely similar to those obtained with other species of Sarcophagine flies (Denlinger, 1971; Vinogradova, 1976). Our results
show that the progeny developing within the uterus can directly receive the external photoperiodic signal. The most sensitive period was observed in the stages before and after larviposition, which was related to the first instar. The photoperiod to which the progeny of S. peregrina was exposed during this stage appears to be the main factor determining whether it enters pupal diapause or not. The photoperiodic information given during the prepupal stage may help the determination. The stage of oogenesis may not be concerned with the photoperiodism determining pupal diapause. Thus, determination of pupal diapause in this fly may depend upon perception of the external photoperiodic signals through the dark abdominal integment of the mother fly. However, since the embryo and first instar larvae in the uterus may sometimes be visible through the connective membrane between the sternites and the tergites when it is expanded by the gravid uterus, it may be sufficient for the progeny in the uterus to receive the photoperiodic stimulus. First instar larvae in the uterus seem to easily adapt to the circumstance concerning the photoperiod and temperature which their mother fly did select. Adult flies can more easily find a suitable condition of life than their progeny. The physiological state of the progeny is apparently determined by interaction of the environmental factors acting both on the parental and on the daughter generation. The young third instar, on the other hand, has less sensitivity. This is suggested by the fact that their behavior is based upon a negative phototaxis during the stage. They exert themselves to
avoid light under or in the shade of medium. The temperature of food materials also easily changes by heat of decomposing matter.

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REFERENCES


