INDUCTION AND TERMINATION OF PUPAL DIAPAUSE IN RELATION TO THE CHANGE OF ECDYSONE TITER IN THE FLESHFLY, SARCOPHAGA PEREGRINA*

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SUMMARY: Larvae of Sarcophaga peregrina entered pupal diapause under a certain short day condition through embryonic stage to mature larvae. The critical day length for 50% induction of pupal diapause was 13 L: 11 D cycles at 22°C, but the effect of the short day was enfeebled by higher temperature. In order to break the diapause, pupae had to be exposed to low temperature for 8 weeks or more, though spontaneous termination of diapause occurred by placing the pupae for a longer term at 25°C.

Ecdysone contents of the whole body varied with their developmental stage from the mature larvae to pupae. In both diapause and nondiapause pupa, the ecdysone titer began to increase after transferred to dry condition and reached a maximum 12 hr after pupariation. About 40 hr after pupariation, the ecdysone titer decreased to minimum values. Afterwards, only in non-diapause pupae, the titer began to increase again at the time of larval-pupal ecdysis. The second peak of ecdysone titer is thought to be responsible for adult development, since the peak was not seen in diapause pupae.

INTRODUCTION

In higher Diptera, ecdysone, a moulting hormone, is known to provoke pupariation and subsequent pupation when secreted in fully grown larvae. After the mature larvae of Sarcophaga peregrina attained a favorable site for pupation, their ring glands begin to release ecdysone. From this moment, the ecdysone content of whole body of larvae gradually increases toward pupariation which takes place 17 hr after transferred to dry condition at 25°C. However, the effect of ecdysone necessary for pupariation reaches a critical level already at the half way to it, i.e., in 8.5 hr. In this stage, ecdysone titer in the larval body is still relatively low, and a drastic increase in the titer occurs several hours before pupariation (Ohtaki, Milkman and Williams, 1968).

We interpreted these phenomena as that the ecdysone titer in the larval body represents the balance between secreted and inactivated ecdysone and that pupariation requires the accumulated effects of ecdysone but not ecdysone itself.

In the study on the ecdysone titer from immature larvae to adult of the blowfly, Shaaya and Karlson (1965) described two maxima of ecdysone titer; one arises in

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several hours after pupariation and the other in 2 days after pupation. They thought that the first maximum of ecdysone titer was for larval-pupal ecdysis and the second one for adult development.

Recently, Fraenkel and Hsiao (1968 a,b) and Saunders (1971) reported that some species of sarcophagid flies undergo facultative pupal diapause which is induced by certain photoperiods. It should be possible to clarify the role of the high ecdysone titer around pupariation in larval-pupal ecdysis or adult development by using diapausing pupae in which the development stops just after larval-pupal ecdysis.

The present paper describes the conditions necessary for induction and termination of pupal diapause by a sarcophagid species and determination of ecdysone titers in both diapause and nondiapause pupae. Some relationships between the ecdysone titer and the morphogenetical events through pupation to adult are also discussed.

**MATERIALS AND METHODS**

Larvae of the fleshfly, *Sarcophaga peregrina*, were reared and maintained as detailed previously (Ohtaki, 1966). Adults originating from nondiapause pupae were used in the present experiment. If parents were placed under a long day condition, the offspring developed into only nondiapause pupa regardless of light regime in their larval stage. Therefore, adults were reared in a short day room except in a combination experiment (Experiment 2, Fig. 2 A).

In the experiment of diapause induction, 20 of newly deposited larvae were transferred into a glass vessel (480 ml in volume and 8 cm in diameter). Then the vessels were put into 5-gallon cans equipped with a 5-watt fluorescent lamp. Illumination of this lamp was controlled by an outside time switch. The inside temperature of each can was controlled by an aerator. Illumination time was previously programmed as following seven different light regimes; continuous lighting, 14-hr light (14 L): 10-hr dark (10 D), 13 L:11 D, 12 L:12 D, 11 L:13 D, 10 L:14 D, and continuous darkness. These cans were placed in rooms at constant temperatures of 19 C, 22 C and 25 C. For larval food, a piece of pig liver was supplied daily. In the case of continuous darkness, the larvae were exposed to light for a few minutes every day to exchange the food. The larvae reached their maximal sizes in 3 days at 25 C, 4 days at 22 C and 5 days at 19 C. These fully grown larvae were left for additional 4 days in the same vessels where distilled water was supplied daily to keep the inside wet. Then, the animals were transferred to dry vessels for pupation and kept for further 2 weeks under the same photoperiod and temperature.

Judgement for diapause and nondiapause pupae was made according to the method described by Fraenkel and Hsiao (1968 b).

To study the effects of chilling or photoperiodism on the break of diapause, groups of diapausing pupae were placed in cold rooms at 5 C, 8 C and 10 C for certain periods, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 weeks. After cold treatment, the pupae were transferred to a long day (16 L:8D) room at 25 C. The other groups of diapausing pupae were placed under a short day (12 L:12 D) at 20 C or a long day (14 L:10 D) at 25 C for a long period. Number of emerged flies and date of the emergence were recorded. In this experiment, individuals emerging within 2 weeks were regarded as perfectly reactivated animals, because the adult usually hatches out from nondiapause pupae on the 11th or 12th day of pupariation at 25 C.
To decide the sensitive stage of the larvae for induction of diapause, a series of experiments was carried out in combination of two light regimes, short day and long day. The larvae were reared under either short day (12L:12D) at 20°C or long day (14L:10D) at 25°C. After the larvae ceased feeding, half of them was kept under the same conditions and the other half transferred to the opposite light regime, namely, from the short day to long day or vice versa. After being left in wet vessels for certain days, the larvae were transferred to dry vessels for pupation. Two weeks later, the number of diapause and nondiapause pupae were counted.

For determination of the ecdysone titer in larvae and pupae, a batch of newly laid larvae were divided into two groups; one group was reared under a short day condition (8L:16D) at 20°C, and the other under a long day (16L:8D) at 25°C. In the former group, 97-100% of the larvae entered diapause; whereas, in the latter, larvae developed absolutely into nondiapause pupae. Ecdysone was extracted from the larvae or pupae in the following developmental stages; 0 hr dry, the half way between 0 hr dry and pupariation, and 0, 12, 24, 36, 48, 72, 96 and 168 hr after pupariation. In each case 100 larvae were pooled and homogenized. The procedure for extraction and estimation of ecdysone contents in the extract were described in detail in the earlier paper (Ohtaki et al., 1968). The experiments were repeated 3 times, and average values were presented in results.

RESULTS

Experiment 1. Effects of Photoperiodism and Temperature on Induction of Diapause

When larvae were reared at temperatures below 22°C under short day conditions (lighting for 12 hr or less and in darkness for 12 hr or more), almost all larvae entered pupal diapause, as shown in Fig. 1. At 25°C, incidence of diaposing pupae was lower than those at lower temperatures, the maximum being about 70% under the short day condition of 10L:14D (Fig. 1).
Experiment 2. The Photosensitive Stage

In next series of experiments, the larvae were placed in combinations of two different light regimes and temperatures, to know the sensitive period during their growth for induction of diapause.

The pupae developed into the nondiapause type regardless of photoperiod in larval stage, if their parents were reared under a long day condition (14 L : 10 D) (Fig. 2 A).

On the other hand, when the adult flies were placed in a short day (12 L : 12 D) room, pupal type of the offsprings varied depending upon the photoperiods in their larval stage. Nine cycles of short day induced nearly 100 % of diapause pupae (Fig. 2 B). However, thus preordained type to diapause was easily modified into nondiapause by a further long day treatment during the postmaturation stage, and incidence of diapause decreased toward 0 %, depending on the duration of the long day treatment (Fig. 2 C). When the larvae were transferred to a long day room just after being laid and reared in it during their feeding stage, the pupal type was conclusively decided to nondiapause (Fig. 2 E) and the destiny was not modified by the subsequent short day condition during the postmaturation stage even if it was relatively long (Fig. 2 D).

The results of these experiments show that only 4 cycles of the long day condition in the larval stage absolutely decide the pupal type to nondiapause and that the effects of short day in feeding stage are altered easily by a further long day treat-
ment, implying that larvae of this species tend strongly to develop into nondiapause pupae.

Experiment 3. Termination of Diapause

When the diapause pupa was kept at 25°C either in a short day (8 L:16 D) or in a long day (16 L:8 D), the adult development never occurred during the first 3 months. Afterward, adult flies emerged occasionally from the diapause pupae under both long and short day conditions, and within half a year more than 30% of the pupae initiated adult development, the rate of emergence fluctuating from batch to batch, but not by photoperiods. Spontaneous break of diapause was also described in other species of the fleshfly by Frankel and Hsiao (1968a), and Saunders (1971).

TABLE I
Effects of cold temperature on termination of diapause.
Percentage emergence from diapause pupae after chilling

<table>
<thead>
<tr>
<th>Temperature of chilling</th>
<th>Period of chilling (weeks)</th>
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<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>5°C</td>
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<tr>
<td>8°C</td>
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<td>10°C</td>
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Each group consisted of 30 diapause pupae, except those with asterisks, which consisted of 20 pupae.

Fig. 3. Ecdysone contents of larval body in diapause and nondiapause pupae (equivalent to ecdysterone).
The chilling experiment shown in Table I reveals that critical period of chilling for 50% break of diapause is about 8 weeks and suggests that the temperature of 5 to 8°C gives slightly higher rate of perfect reactivation of the diapause than at 10°C.

Experiment 4. Ecdysone Titer in Diapausing and Nondiapausing Pupae

As shown in Fig. 3, ecdysone titer changed with development from wet larvae to adult. There is no virtual difference in the titers between diapausing and nondiapausing pupae until larval-pupal ecdysis. Thereafter, ecdysone titer in diapausing pupae remained rather unchanged, but that in nondiapausing pupae increased dramatically after the time of larval-pupal ecdysis.

Larval-pupal ecdysis in both diapause and nondiapause took place about 40 hr after pupariation at 25°C, and the ecdysone titers fell down drastically several hours before larval-pupal ecdysis.

DISCUSSION

The pupal diapause of Sarcophaga peregrina is induced under a short day condition compensated with low temperature, as observed by Saunders (1971) in Sarcophaga argyrota. In S. peregrina, however, critical daylength for induction of diapause was shorter and incidence of diapause was higher under the same conditions.

The results of Experiment 2 indicate that light cycles continue to affect the determination of pupal type to either diapause or nondiapause through whole developmental stage beginning from the embryo to mature larvae (Fig. 2). A short day condition during embryonic development is exceedingly important to induce diapause in S. peregrina as well as in S. crassipalpis (Denlinger, 1971).

![Fig. 4. Seasonal change of daylength and temperature in Tokyo. The lower curve shows average temperature. The arrows show the times of adult emergence from overwintering pupae (A) and onset of diapause (B).](image-url)
The critical day length for induction of diapause under experimental conditions well corresponds to the length of photophase in late September to early October in central Japan. In autumn days all larvae enter pupal diapause in the field (Fig. 4). The chilling experiment suggests that overwintering pupae are expected to initiate adult development in early January. In spite of that, emergence of adult fly usually occurs in late April in the field. For adult development, high temperature must be indispensable (Fig. 4).

Fleshfly larvae usually pupate in the soil around the breeding place. Insensitivity of the diapausing pupae to the light cycles for the break of diapause is explained by considering the dark pupation site. Reactivation of diapausing pupae by chilling, but not by short day, in the fleshfly is similar to that of *Hyalophora cecropia*.

Fraenkel (1935, 1938) showed in his classic experiments that, when *Calliphora* larvae were ligatured after a critical period, the hind part could pupate inside the pupal shell. In our experiments, critical time of the true pupation proved to be 12 hr after drying, namely 5 hr before pupariation, while ecdysone titer of this stage is still relatively low (Ohtaki, Ohmori and Sakurai, in preparation). Furthermore, the titer in ligatured hind part remained extremely low despite that the hind part resulted in perfect pupariation (Ohtaki, 1972). Comparing the morphogenetic status in diapausing pupae with those in ligatured hind part, Fraenkel and Hsiao (1968b) postulated that the arrest of the adult development in both cases was ascribed to the lack of ecdysone, and they demonstrated that adult development could be initiated by injection of a certain amount of ecdysone. Their interpretation was substantially supported by the results of the Experiment 4.

Under these circumstances, the first peak of ecdysone titer, which is generally recognized as responding to pupation seems to be required for neither pupariation nor true pupation. Significance or role of the first peak of ecdysone titer is still unknown, though the second one has proved to be responsible to adult development. These facts remind us of that the adult male of *Hyalophora cecropia* contains considerable amount of juvenile hormone, though it is thought to be entirely inert on the male moth.

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REFERENCES


