

# Biology of *Hyphantria cunea* DRURY (Lepidoptera : Arctiidae) in Japan

## X. Development of Larvae in Relation to the Induction of the Pupal Diapause

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### Introduction

In some species of insects, it has been known that the induction of diapause accompanies morphological, physiological or behavioural changes during the developmental stages preceding diapause. Remarkable examples are the selection of place for laying winter eggs by the red spider mite *Panonychus ulmi* (LEES, 1953), the formation of hibernacula in the fruit moth *Carposina sasakii* (TOSHIMA *et al.*, 1961) and particular additional ecdyses for hibernation in some tortricid larvae (OKU, 1966). There are also other examples of more frequent though less conspicuous, occurrence. Thus, a small but significant delay of pre-diapause development has been reported in the diapause-predestined individuals of *Telea polyphemus* (DAWSON, 1931), *Chilo suppressalis* (FUKAYA, 1950) or *Mamestra brassicae* (UCHIDA and MASAKI, 1953).

The purpose of this work is to examine whether there is any sign of this kind which indicates the future occurrence of diapause in *Hyphantria cunea*. If such a sign could easily be detected, it would facilitate the prediction of the seasonal life cycle of this species. This objective has not been achieved as yet, but the data obtained seem to provide information about the seasonal adaptation of the life cycle of *Hyphantria cunea*. Since the analysis of its adaptation in this country is one of the important subjects of the research project on *Hyphantria cunea*, an account of these data will be given below as the tenth report from the Research Group for the Study of Biology and Population Dynamics of *Hyphantria cunea* DRURY in Japan.

### Material and Method

Many egg batches deposited on leaves of the cherry *Prunus yedoensis* were collected in Hiratsuka city in May, 1968. They were incubated in the laboratory. Three of them, which hatched simultaneously on 28th May, were used for experiments. The newly hatched larvae were allotted at random to three different groups, each of which comprised of 300 individuals. In order to produce both diapause and non-diapause insects, one

group was exposed to a short photoperiod of 13 hours (per 24 hours), a second one to a long photoperiod of 16 hours, and the last one to an intermediate photoperiod of 14.5 hours. As the temperature was maintained at 25°C in the biotron where the larvae were grown, the intermediate photoperiod was very close to the critical value, at which about equal numbers of diapause and non-diapause pupae might be expected to occur (see MASAKI *et al.*, 1968). On the other hand, the short photoperiod would produce only diapause pupae, and the long one only non-diapause pupae. Each photoperiod was provided by a 10 W fluorescent tube which was controlled by a time switch.

The larvae were fed on cut shoots of *Prunus yedoensis*, which were inserted into water flasks, and kept in nylon-screen cages (40×40×50 cm). When they reached the end of the 6th instar, a sample of 52 individuals was randomly taken from each of the 13-hour and 16-hour photoperiodic groups, and a sample of 104 from the 14.5-hour group. The larvae were then individually reared in petri-dishes (10×2 cm) and provided with cut leaves of cherry. Each larva was transferred everyday to a clean container and given an adequate amount of food. When the larvae were fully fed, a glass tube (1×4.5 cm) covered with black paper was placed in the vials in order to provide pupation sites.

In the last instar, the body colour of the larvae was rated as described previously (UMEYA *et al.*, 1967). The number of feces excreted by each larva was counted, and the presence or absence of eating marks was noted everyday throughout the last instar. Individual records were also kept of the times of the initiation of cocoon spinning and pupation, both of which could easily be observed by removing the paper covering of the pupation tube. After pupation, each insect was checked for sex and transferred into a dark cabinet at 25°C. The occurrence of diapause was judged from observations continued for two months after pupation. If a pupa did not give rise to adult within a month, it was regarded as diapausing.

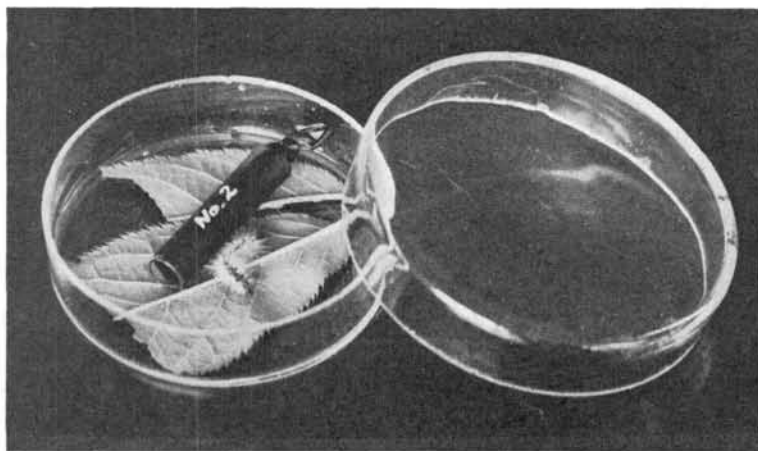


Fig. 1. A rearing container for last-instar larvae of *Hyphantria cunea*. Note a glass tube for pupation.

## Results

### *Incidence of Diapause*

The occurrence of diapause at the three different photoperiods is given in Table 1. The result was precisely as expected. All pupae diapaused at 13-hour photoperiod, all did not do so at 16-hour, and about equal numbers of diapause and non-diapause pupae occurred at 14.5-hour. This was in agreement with a previous finding (MASAKI *et al.*, 1968) and would enable to make adequate comparisons between diapausing and non-diapausing individuals.

Table 1. INCIDENCE OF DIAPAUSE IN *Hyphantria cunea* REARED ON *Prunus yedoensis* AT THREE DIFFERENT PHOTOPERIODS, 25°C AND 70% R.H.

Photoperiod (hr)		Diapause pupae	Non-diapause pupae
13.0	♂	39	0
	♀	11	0
	Total	50	0
14.5	♂	51	21
	♀	8	21
	Total	59	42
16.0	♂	0	21
	♀	0	25
	Total	0	46

### *Body Colour of Larvae*

As the larvae reached the last instar, they showed various degrees of melanization notably on the dorsal surface of their body. They were classified into five grades according to the degree of melanization. The numbers of larvae belonging to the five colour grades are illustrated in figure 2, which may suggest relations of body colour to photoperiodic treatments and the future occurrence of diapause. As reported previously, the larvae grown at the short photoperiod were darker in colour than those at the long photoperiod. Important information would be obtained from the rearing at the intermediate photoperiod. In this treatment, the distribution of colour grade was intermediate between the short and long photoperiods. However, the degree of melanization seemed to be different according to the future onset of diapause. Most of the diapause-predestined larvae at the 14.5-hour photoperiod were similar in colour to those at the 13-hour photoperiod. On the other hand, a considerable proportion of the non-diapause individuals occurring at the same photoperiod showed a lighter coloration.

### *Developmental Time*

The durations of development from hatching to the 6th ecdysis and also to pupation were set out in Table 2. The total duration of the larval stage was significantly longer at the short photoperiod than at the long. At the intermediate photoperiod, it was longer in the diapause-predestined individuals than in the others. The duration from hatching to the 6th ecdysis was only slightly longer at the short photoperiod than at the long. This duration showed no significant difference between diapause and

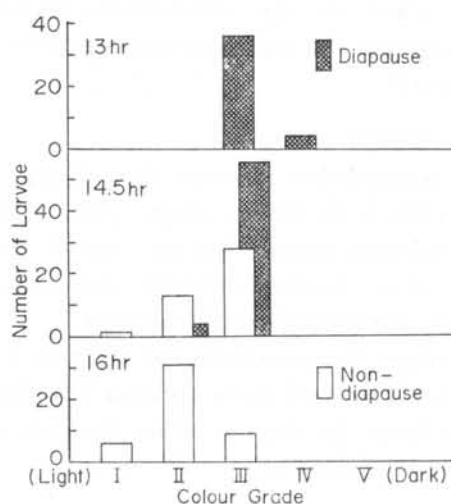


Fig. 2. Colour-grade frequency of last instar larvae of *Hyphantria cunea* reared at a short, long or intermediate photoperiod (25°C, 70% R.H.).

Table 2. THE DURATIONS IN DAYS FROM HATCHING TO THE 6TH ECDYSIS AND PUPATION IN *Hyphantria cunea*. REARING CONDITIONS AS GIVEN IN TABLE 1.

Photoperiod (hr)		Till the 6th ecdysis		Till pupation	
		Mean	S. D.	Mean	S. D.
13.0	D	24.8	0.64	35.2	1.40
14.5	D	24.5	0.73	34.4	1.04
14.5	N	24.4	0.66	32.2	0.87
16.0	N	23.6	0.61	31.4	0.96

D=larvae giving rise to diapausing pupae.

N=larvae giving rise to non-diapausing pupae.

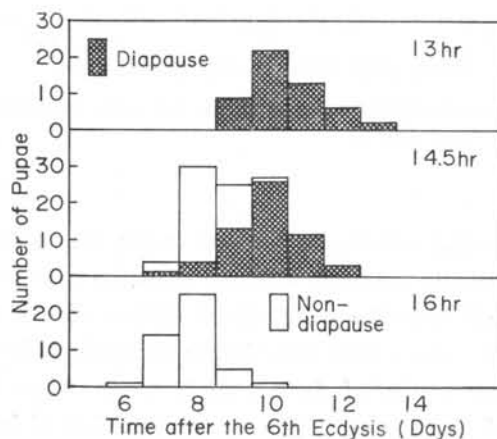


Fig. 3. Time required for pupation after the 6th ecdysis in *Hyphantria cunea*. Rearing conditions as given in Fig. 2.

non-diapause insects obtained at the intermediate photoperiod. Evidently, the significant delay of development in the diapause-predestined larvae mainly occurred in the last (7th) instar (figure 3).

#### *Time Sequence of Behaviour Change*

From the behavioural point of view, there are three distinct stages in the last instar of *Hyphantria cunea*. The first is the feeding stage. After fully fed, the larvae begin to wander around. This wandering characterizes the second stage. It ends when the larvae creep into pupation sites. In the third stage, they spin cocoons and prepare for pupation, which demarcates the completion of this stage.

The durations of these three stages are illustrated in figure 4. There was no apparent difference in the duration of the third stage between the diapause and non-diapause individuals. On the other hand, the diapause-predestined larvae required a longer time both in the feeding and wandering stages.

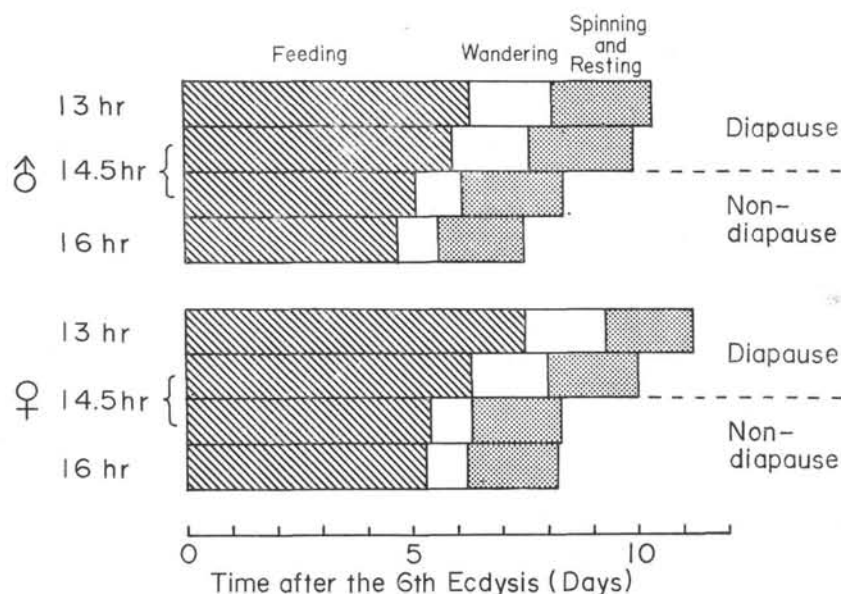


Fig. 4. The time sequence of behaviour change in the last instar of *Hyphantria cunea*. Rearing conditions as given in Fig. 2.

#### *Counts of Excreted Feces*

As a measure of feeding activity in the last instar, the numbers of excreted feces were counted, and the results obtained with the diapause and non-diapause individuals were compared. Figure 5 shows the accumulated daily counts of feces. It demonstrates that the excretory activity was a little lower in the diapause-predestined individuals than in the others. The total number of feces was, however, larger in the former than in the latter. This was of course due to the longer duration of feeding in the diapause-predestined larvae. The number of feces seemed to be correlated, to a certain extent, with the duration of feeding.

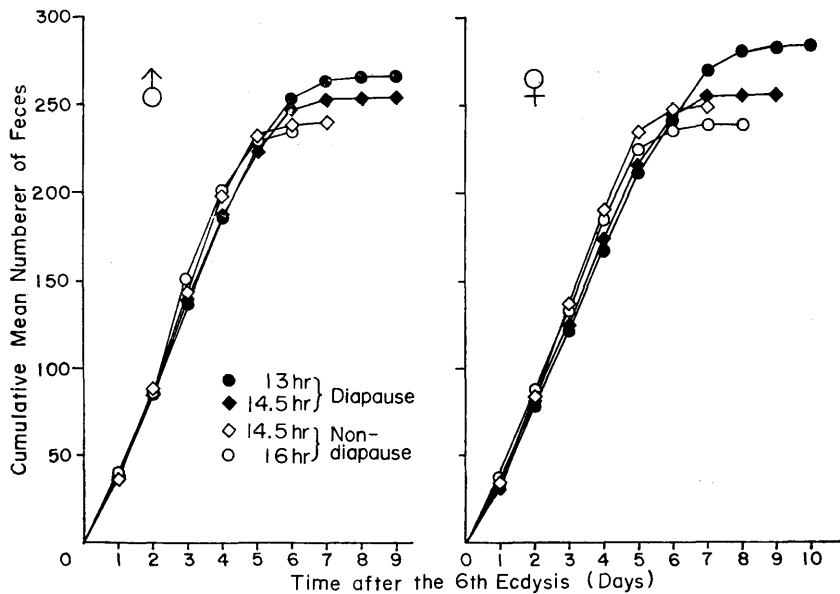


Fig. 5. The cumulative mean numbers of excreted feces in the last instar of *Hyphantria cunea*. Rearing conditions as given in Fig. 2.

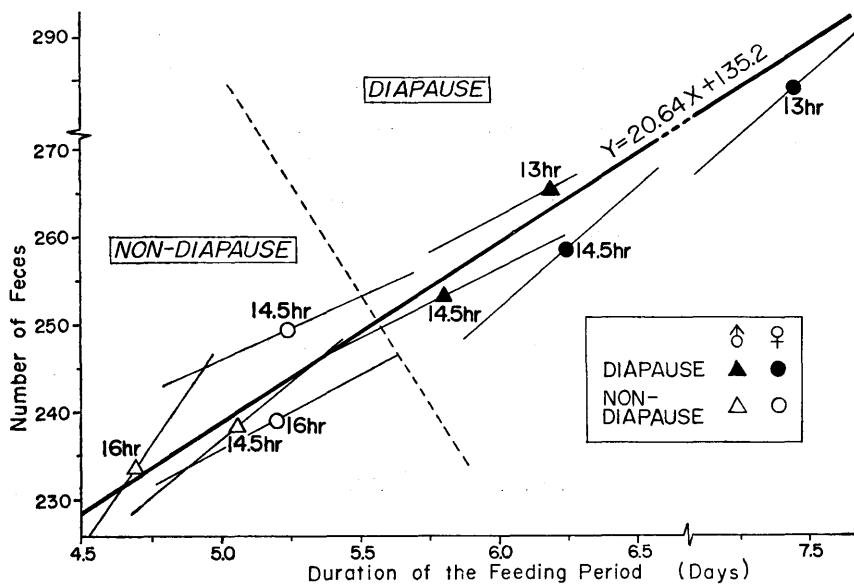


Fig. 6. Correlation between the duration of feeding and the number of excreted feces in the last instar of *Hyphantria cunea*. Each symbol indicates the mean for each group of larvae; the thin lines represent the regression within groups, and the thick line the common regression.

The relation between the duration of feeding and the total counts of feces was therefore examined. The test animals were classified into eight groups according to the photoperiodic treatment, the incidence of diapause and sex. Analysis of covariance shows that there was no significant deviation of the intra-group regression from the inter-group regression ( $n_1=7$ ,  $n_2=188$ ,  $F=1.24$ ,  $P<0.05$ ). The regression lines are given in figure 6. The thin lines represent the regression within groups and the thick one the common regression. The correlation coefficient for the pooled data is 0.62 ( $n=197$ ). These results presumably signify that most of the diapause pupae had taken a larger amount of food in their larval stage than had the non-diapause ones.

### Discussion

In *Hyphantria cunea*, such larval traits as body colour and developmental time have been known to vary under the influence of photoperiod (UMEYA *et al.*, 1967 MASAKI *et al.*, 1968). Two different processes might be suggested for the manifestation of these effects of photoperiod. First, the photoperiod may exert its effect through the determination of pupal diapause. In other words, the darker colour and slower maturation rate of larvae may be consequences of the induction of diapause. Second, the photoperiod may act upon the larvae quite independently of this induction.

The occurrences of both diapause and non-diapause insects at the critical photoperiod may throw light on this question. The data show that the two types of insects occurring at this photoperiod were definitely different in their developmental time and body colour. The darker colour and the slower maturation may therefore be regarded as results of the induction of diapause. The expression of these traits is, however, a little more conspicuous at the short photoperiod than at the critical photoperiod, even if the comparison was made only among the diapause individuals. There might, therefore, be direct influences of photoperiod on the body colour and developmental time, more or less independent of the determination of diapause. A diapause-inducing photoperiod may give a darker coloration and a slower maturation rate through its direct action upon the larvae as well as through its effect upon the induction of diapause.

Although the observed delay in pupation time under diapause-inducing conditions was only a few days, this may be of some importance for the survival of the species. The prolonged wandering of the diapause-predestined larvae would ensure more time than otherwise to seek suitable shelters for hibernation. The larger amount of food intake, as supposed from the prolonged feeding and larger number of fecal excretion, of the diapause-predestined individuals may also favour the winter survival. It would result in an increased food reserve of the hibernating pupae.

### Summary

Larvae of *Hyphantria cunea* DRURY were reared at a short (13 hr), long (16 hr) or critical (14.5 hr) photoperiod in a biotron maintained at 25°C and 75% R.H. Their

development and behaviour before the manifestation of diapause were recorded individually. All the individuals at the short photoperiod entered diapause, all did not do so at the long, and both diapausing and non-diapausing pupae occurred at the critical photoperiod. The body colour in the last larval instar was darker at the short photoperiod than at the long photoperiod. At the critical photoperiod, there was a variation in larval colour which seemed to be related to the future occurrence of diapause. The larvae which would enter diapause later as pupae were darker in colour than those which would not. As a consequence of predetermination of diapause, the periods of feeding and wandering before spinning the cocoon were prolonged for a few days. There was, however, no significant difference in the duration of the cocoon-spinning stage between the diapause and non-diapause insects. A positive correlation was found between the duration of feeding period and the number of excreted feces in the last instar. The diapause-predestined larvae thus excreted a larger number of feces. These characteristics of the larvae that had been determined to enter diapause after pupation may favour the winter survival.

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## 摘 要

## アメリカシロヒトリの生物学的研究

## X. 休眠誘起と幼虫の特性

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アメリカシロヒトリの蛹休眠の有無は幼虫前半期の日長によって決定されるが、この休眠の前決定は終令幼虫の特性にどのような影響を与えるかを調べるため、幼虫を温度 25°C、湿度 70% に保った当所バイオトロン内で、短日 (13 時間)、長日 (16 時間)、臨界日長 (14.5 時間) のそれぞれ異なる日長条件で飼育した。その結果、短日では全個体が休眠したが、長日ではその逆となり、臨界日長では休眠と非休眠の個体がともに出現した。幼虫の体色は短日で飼育したもののほうが長日のそれよりもより黒化したが、臨界日長で飼育したもので

も、休眠の決定された個体の方が非休眠のそれよりも黒化する傾向が見られた。また休眠の前決定は終令幼虫の摂食日数と摂食終了後からまゆつむぎまでの期間の延長をもたらしたが、蛹化前のまゆ期の長さは休眠と非休眠で違いが見られなかった。幼虫の終令期間の摂食日数と排糞数との関係では飼育された全個体を通じて正の相関が見られ、休眠の前決定された幼虫のほうが排糞数が多く、より多量の摂食を行っていることが推量された。このような休眠の決定された幼虫の特性は越冬適応の 1 断面を示しているものと思われる。