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

VIII. Experimental Studies of the Timing
Mechanism of Adult Eclosion¹

Yoshio HIRAI

*Entomological Laboratory, Tokyo University of Agriculture
and Technology, Fuchu, Tokyo, Japan*

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The adult eclosion of *Hyphantria cunea* of the summer generation under the field condition was concentrated for about 1 hr after dusk. Experiments analysing the timing mechanism of this event revealed that the eclosion was inhibited by the light and promoted by the change from light to dark. By experiments artificially shifting the dusk, the pupae kept under a field light condition were found to be ready to emerge by 14:00. Further experiments indicated that the time of eclosion was not determined by the absolute length of the light period prior to the dusk, but was dependent on the light and dark cycles repeated during the pupal stage. The latter entrains the endogenous circadian periodicity, which was clearly demonstrated under the continuous darkness, and when eclosion was fully prepared, the light-off stimulus acted as the direct inducer. Difference of the time of eclosion in the field between the overwintered and the summer generations was discussed in relation to the temperature drop in the afternoon.

INTRODUCTION

For the adult *Hyphantria cunea* DRURY, very conspicuous white moth, the timing of eclosion from the pupa is of a great significance from, at least, two points of view: firstly, if adult eclosion takes place in the day-time when many birds are active, as HASEGAWA and ITÔ (1967) already pointed out, almost all of the adults may be destroyed because of the predation by birds. Secondly the timing of eclosion seems to play an important role for ensuring the synchronization of mating behaviour. The present work was carried out to analyse experimentally the timing mechanism of adult emergence.

MATERIALS AND METHODS

The adult emergence in nature was observed in the campus of Tokyo University of Agriculture and Technology at Fuchu in Tokyo. All the moths just emerged were counted and captured immediately after their emergence. For the

¹ This is the 8th report from the study group on the biology and population dynamics of fall webworm, *Hyphantria cunea* in Japan.

observation, walls of several buildings, on which the adult eclosion occurred frequently, were selected. Counting of the moths was made every twenty min. The eggs and larvae used in the experiments were collected from cherry trees in the campus and reared in the laboratory. After pupation the pupae were kept for about ten days until emergence in the boxes which were set to various light conditions. The light source was 6-W fluorescent lamp (TOSHIBA) and regulated by the 24-hr time switch. The temperature was kept at about 25°C. Counting of the emerged moths under dark condition was made with the aid of a weak red lamp.

NATURAL DISTRIBUTION OF ADULT ECLOSION IN THE SUMMER GENERATION

Under the field conditions, the adult eclosion of the summer (second) generation of *H. cunea* began at around 18:00 and ended at around 21:00. After midnight only occasional adult eclosion was observed in the field. The maximum emergence occurred between 18:30 and 20:00 as shown in Fig. 1. In other words

Fig. 1. Distribution of the adult eclosion of *H. cunea* of the summer generation in the field and temperature change during the observation period (July 28, 1968, Fuchu, Tokyo).

the emergence was concentrated for about 1 hr after dusk. As shown in Fig. 1, there was no remarkable change in the temperature. This suggests that the adult eclosion may be induced by the light-off stimulus.

EFFECT OF LIGHT CONDITIONS

A group of pupae were continuously exposed to the light beyond the period in which the adult eclosion normally takes place. As shown in Fig. 2, the adult eclosion occurred randomly and lasted until the midnight. The clear peak of emergence like that observed under the field condition was not observed.

Fig. 2. Pattern of the adult eclosion under continuous light in *H. cunea* (summer generation).

Another group of pupae were kept under the light from just before dusk to 21:00, and then the light was switched off leaving the pupae in the continuous darkness (Fig. 3). Only a few moths emerged during the illuminated period. After the light was turned off, however, the adult eclosion occurred successively and the maximum peak of the eclosion was observed about 1 hr after the transition from light to dark.

These experiments indicated that the adult eclosion of the moth was inhibited by the light and promoted by the change from light to dark.

Fig. 3. Effect of light-off at 21:00 following prolonged illumination on the adult eclosion of *H. cunea* (summer generation).

The third group of pupae was kept under a photoperiod of 15 hr of light and 9 hr of dark (15L9D), which was similar to natural light condition for the entire pupal stage. On the expected day of emergence, the light was turned off at 12:00, 13:00, 14:00, 15:00, or 16:00. Thus the pupae were subjected to darkness earlier than usual. The results are shown in Fig. 4. Among those pupae which were artificially brought about to dusk at 12:00, i.e. 7 hr earlier than usual, the adult eclosion was not observed until 2 hr later. Afterwards the eclosion started and lasted randomly shownig no definite peak. Such a dispersed distribution of emergence was very similar to that observed under the continuous illumination.

Fig. 4. Pattern of adult eclosion in *H. cunea* (summer generation) when the time of dusk was shifted 3-7 hr earlier than expected from the photoperiod under which pupae had been kept. Expected dusk: 19:00.

The distribution changed, however, when the "artificial dusk" was shifted 1 hr later by turning off the light at 13:00. The eclosion was no longer random but showed considerable concentration around 14:00. This concentration of the adult eclosion around a peak appearing 1 hr after the "dusk" became more clear as the "artificial dusk" was shifted towards the natural dusk.

These results indicate that the pupae kept under a field light condition were

ready to emerge by 14:00. The light-off stimulus induced a concentrated eclosion only when it was applied after this time. It follows, therefore, that the light-off stimulus is not the sole factor for the timing of eclosion. There may be some prerequisites.

One of the prerequisites might be the length of light period that the pupae have passed prior to the light-off. Indeed, in the pupae that were subjected to the light-off at 12:00, the light period was only 8 hr instead of about 15 hr in the field. Light period of 8 hr might be too short for the preparatory period.

The pupae were kept, therefore, under 8L16D photoperiod during the entire pupal stage. The result shown in Fig. 5 clearly demonstrated that 8-hr light period was sufficient for preparing eclosion or sensitizing the pupae to the light-off stimulus; the adult emergence occurred concentrically within about 1 hr after the "dusk" just as under the natural photoperiodic condition. It is, therefore, likely that the potential time of eclosion is not determined by the absolute length of the light period prior to the dusk, but is dependent on the light and dark cycles repeated during the pupal stage.

Fig. 5. Pattern of the adult eclosion in *H. cunea* (summer generation) subjected to an 8L16D photoperiod throughout the pupal period.

A group of pupae kept under a field photoperiodic condition for the most part of pupal development were transferred to a different photoperiodic condition, where the light phase began at 21:00 and ended at 12:00. On the day of the transfer, the adult eclosion occurred randomly as shown in the foregoing experiments. On the 2nd day, however, the concentration of the eclosion around a clear peak was observed.

On the 3rd and 4th days, the peaks were clear (Fig. 6). This was confirmed also by YUSHIMA and NOGUCHI (unpublished).

After several days under the field light condition, the light and dark cycle was shifted to 12 hr. Namely, the diurnal cycle was nearly reversed by this shifting. It took at least 3 or 4 days before full entrainment to the newly provided cycle was established.

Fig. 6. Random distribution of the adult eclosion in *H. cunea* (summer generation) following transfer of pupae to a new light-and-dark cycle and its consecutive concentration.

These facts indicated that the adult eclosion depends not only on the external light condition but also on an endogenous rhythm which can be reset and entrained by the shift of the time-cue photoperiodic stimulus.

FREE-RUNNING RHYTHM IN THE CONTINUOUS DARKNESS

The suggestion mentioned above was confirmed by the fact obtained from the experiments as described below. The pupae were entrained to a field photoperiod of 15L9D during the pupal stage until the day in which the earliest eclosion in the group was observed. Then the pupae were transferred to the continuous darkness. As shown in Fig. 7, the first peak of adult eclosion occurred about 1 hr

after the onset of the darkness. The consecutive peaks of eclosion came at approximately 24 hr interval. It is now clear that the adult eclosion of *H. cunea* reflects an endogenous circadian periodicity under continuous darkness and the endogenous periodicity might be established by the photoperiod during the pupal stage and carried over the time of the adult eclosion.

Fig. 7. Pattern of the adult eclosion in *H. cunea* (summer generation) under 2 days continuous darkness after 7 days of 15L9D photoperiod with the dusk at 19:00.

NATURAL DISTRIBUTION OF ADULT ECLOSION IN THE OVERWINTERED GENERATION

As stated above, the maximum adult eclosion of the summer generation of this moth in the field occurs shortly after dusk. That of the overwintered generation, however, was reported to occur much earlier in the afternoon (HASEGAWA and ITÔ, 1967).

Actually the eclosion was observed in the field from 15:00 in the overwintered generation (Fig. 8).

During observation period extending to two weeks, the peak of eclosion fluctuated between 17:00 and 18:00 by the day. This was not encountered in the case of the summer generation. This difference in the pattern of eclosion between two generations offer further questions on the timing mechanism of the adult eclosion.

The adult eclosion of the overwintered and of the summer generation occurs just one month before and after the summer solstice respectively. The difference can not, therefore, be attributed to the difference in the photoperiodic conditions under which adult eclosion takes place.

ITÔ (personal communication) suggested that the difference in the pattern of eclosion might be due to different temperature condition of the two seasons. Under the field condition, indeed, a clear difference of the temperature drop in the afternoon was observed between two seasons: 2°C or less in July-August (summer generation), about 5°C in early June (overwintered generation) (Figs. 1 and 8). These field data strongly suggest that the drop of temperature induces earlier eclosion. Some preliminary experiments seem to support this suggestion. Detailed experiment is now in progress.

Fig. 8. Distribution of the adult eclosion of *H. cunea* of the overwintered generation in the field and temperature change during the observation period (May 21, 1968, Fuchu, Tokyo).

DISCUSSION

About the timing mechanism of the adult eclosion in moths, several works have been reported (*e.g.*, MORIARTY, 1959; BANARJEE and DECKER, 1966; BECK, 1968). In those reports the discussion seems to be focused to the problem of the circadian periodicity and its entrainment.

From the present experiments, it is obvious that the timing mechanism of the adult eclosion in *H. cunea* of the summer generation involves a circadian rhythm entrained by the photoperiodic cycles during pupal development as the basic process, and a light-off stimulus as the direct inducer. Although the circadian cycle of this moth shown under the continuous darkness was approximately 24 hr or a little shorter, the preparation for the eclosion seems to be established already 20 hr after the preceding dusk. This seems to prove a strong modifying effect of light-off stimulus. Temperature condition will also exert a modifying effect on the timing of eclosion, as it is suspected particularly in the case of the overwintered generation.

In both generations the adult eclosion occurs within limited hours. This appears to be an interesting fact, for the following mating behaviour of the moths takes place within only about half an hour at the next dawn (HIDAKA *et al.*, 1967). The concentrated distribution of the adult eclosion will permit synchronization of sexual activity. This includes an adaptive significance in the regulation of the

dynamics of the field population.

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