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Change of Photoperiodic Sensitivity with Fat Body Development during Prediapause Period in the Twenty-Eight-Spotted Lady Beetle, *Henosepilachna vigintioctopunctata* FABRICIUS (Coleoptera : Coccinellidae)

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When adult beetles were reared normally on host plant foliage under a short day, they lost sensitivity to a long photoperiod after the 6th day of adult life, ceased feeding and entered diapause on about the 17th day. Fat bodies, including those surrounding the brain, developed markedly to fully occupy the body cavity during this period.

If the amount of feeding was limited to 35–40% of normal, the fat body development was delayed and photoperiodic sensitivity to a long day was maintained until the 12th day of adult life. Furthermore, limited feeding after photoperiodic sensitivity was lost, causing an elongation of the prediapause period.

In adults which responded to the photoperiodic change to a long day, paraldehyde fuchsin positive materials in the neurosecretory cells in the pars intercerebralis decreased and the diameter of the corpus allatum increased, showing a high activity of the brain-corpus allatum endocrine system. While in the adult showing no response, the neurosecretory cells and corpus allatum showed appearances of decreased activity.

It was concluded from these results that the neurosecretory cells in the pars intercerebralis lose their sensitivity to the long photoperiod in the course of the fat body development in adults exposed to a short day, and they enter diapause when the neurosecretory cells become more inactive.

INTRODUCTION

The adult beetle, *Henosepilachna vigintioctopunctata* FUBRICIUS, reared on host plant foliage, ceases to feed and enters diapause after 16 days of vigorous feeding in short-day photoperiods. The body weight of the adult increases markedly through the prediapause period of 16 days and the fat body develops simultaneously (KONO, 1980 b). These beetles begin to develop their ovaries if they are transferred to a long day within 5 days of adult life under a short day. But if the change of photoperiod occurs later, they enter diapause after a certain period of feeding even under a long day without ovarian development (KONO, 1979).

These phenomena, the loss of photoperiodic sensitivity and the entrance into diapause, are obviously delayed when the adults are fed on slices of potato. Females, for instance, continued to feed for more than 30 days under short-day conditions, and could respond to the long day by developing their ovaries even after a short-day exposure of 36 days (KONO, 1979). The fat body development under a short day is also delayed in the beetle fed on slices of potato owing to its nutritional deficiency. Analysis on the relationship between weight and photoperiodic sensitivity indicated that females gaining less weight were able to respond to a long day for a longer duration (KONO, 1980 b).

If a mechanism functions in the field population whereby beetles do not enter diapause until they fully develop the fat body in which various materials are reserved for energy resource and cold hardiness during diapause and post-diapause periods, the risk of being exhausted during diapause will be reduced.

In the present paper, therefore, in order to ascertain such a mechanism, changes of photoperiodic sensitivity were investigated in adults normally or limitedly fed on host plant foliage in relation to the weight gained, fat body development, and neurosecretory activity in the brain.

MATERIALS AND METHODS

A stock culture of *Henosepilachna vigintioctopunctata* was maintained on the foliage of Solanacean plants, *Solanum tuberosum* and *S. carolinense*, under a long-day condition (25°C, 16L–8D).

Adults used for experiments were taken from this stock culture just after emergence. Six adults (3♀, 3♂) or 8 adults (5♀, 3♂) were confined in a petri dish and were fed on fresh leaves of *S. carolinense*.

In order to reduce the weight gain of the beetles, limited amounts of the leaves were fed (limited feeding). Female adults were weighed individually or in groups of certain intervals, and the weight gain was represented as a percentage of that just after emergence.

Photoperiodic sensitivity was judged by the response to a change of photoperiod from a short day (10L–14D, 25°C) to a long day (16L–8D, 25°C). If the female responded to a long day, her ovaries began to develop. Ovarian development was checked under a dissecting microscope and graded according to the stage of the development as shown in Fig. 1, 10 days after the change of photoperiod.

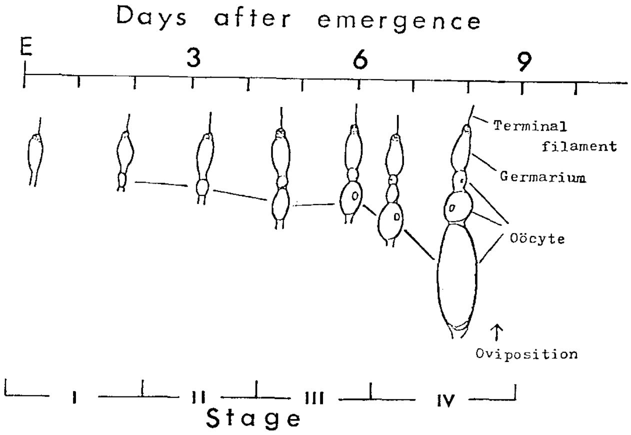


Fig. 1. Four stages of oöcyte development in the adult beetle reared on host plant foliage under a long-day condition (16L–8D, 25°C).

Fat body was observed under a dissecting microscope after taking off the dorsal cuticle of the adult body.

For observations of neurosecretory cells (NSC) in the pars intercerebralis (PIC) and corpus allatum (CA), opened heads of the female were fixed in formaldehyde solution or in Bouin's fixative and brain-corpus cardiacum-CA system was stained *in situ* with aldehyde-fuchsin (AF) (Dogra and Tandan, 1964). The diameter of CA was measured by an eyepiece micrometer along the long axis. Four or five preparations were observed in each experimental group.

Details are shown under Results.

RESULTS

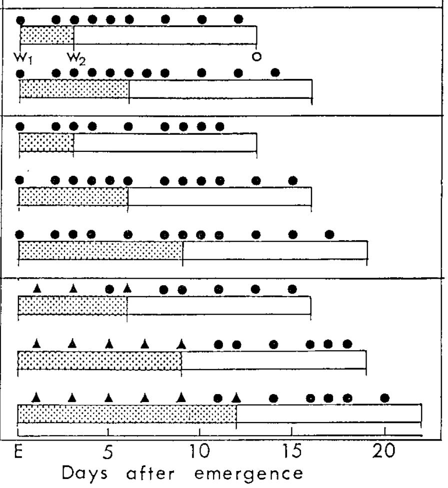
1. *Body weight–photoperiodic sensitivity*

Adults were divided into three groups each of which consisted of two or three subgroups as shown in Table 1. The first group (A) was confined in large petri dishes (12 cm in diameter) and supplied with a sufficient amount of foliage. One adult of this group consumed about 3 cm^2 of foliage per day on an average. The second group (B) was reared in small petri dishes (9 cm in diameter) with a sufficient supply of foliage. Adults of this group, however, consumed less of the food than those in A. The third group (C) was reared in small petri dishes with a reduced amount of foliage. Less than 40% of the amount consumed by A was taken by this group.

Subgroups consisting of four dishes (3♀, 3♂ in each dish) were exposed to different cycles of short-day followed by 10 days of long day-length. Female adults were weighed individually at the transference from the short day to the long day, and their ovarian development was checked after 10 days of long day-length.

Table 1. Conditions and schedules of feeding experiments shown in Fig. 2

Group[a]	Duration of short-day exposure	Diameter of petri dish	Amount of foliage supplied	Amount of foliage eaten[b]	Data of feeding
A	3	12 cm	sufficient	100	
	6				
B	3	9 cm	sufficient	65-85	
	6				
	9				
C	6	9 cm	half	35-40	
	9				
	12				



a Three females and three males were confied in each petri dish and raised with sufficient supply (●), or limited supply (▲) of foliage, first for different durations under short day-length (dotted bar), and then 10 days under long day-length (white bar).

b The amount eaten by animals of group A was taken as 100%.

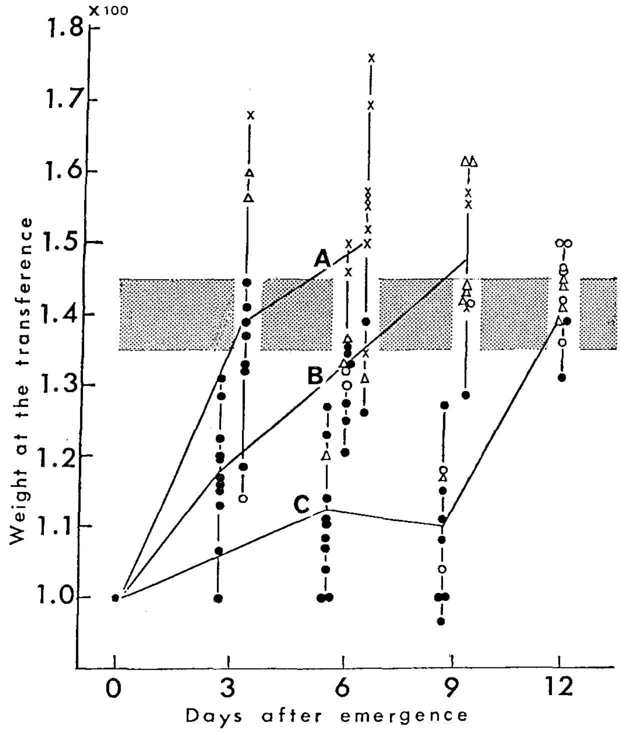


Fig. 2. Effects of feeding on increase of body weight and ovarian development in several groups of adult beetles differently reared as shown in Table 1. Ordinate : adult stage of being exposed to a short-day condition before being transferred to a long day-length. Abscissa : percentage weight gain under short-day exposure (see Table 1). Transverse lines bound the mean values of body weight of subgroups belonging to same group. Vertical lines bound the result belonging to same subgroup. X, △, ○, and ● represent ovarian development stages I, II, III, and IV in Fig. 1, respectively, at the end of long-day exposure.

The weight gained at the transference and the degree of ovarian development in respective females are plotted in Fig. 2. Body weight increased normally in A to be above 150% on an average 6 days after emergence. Most of the females responded to a long-day photoperiod by developing their ovaries when the change of photoperiod took place at the end of the 3rd day (3 days after emergence, A-3), but only 2 out of 11 females responded when they were transferred to a long day at the end of the 6th day (A-6). Weight in B increased more slowly than in A. Eight out of 12 females in the small petri dishes averaged a weight gain lower than 135% at the 6th day, showing the response to a long day after 6 days of short-photoperiod (B-6). By 9 days of short-photoperiod in the small dishes when averaged weight exceeded 145%, photoperiodic sensitivity was lost (B-9)

The weight hardly increased when the feeding was seriously limited (C-6, 9). All females except 2 exposed to short-photoperiod within 9 days maintained photoperiodic sensitivity. Weight prominently increased to an average 140% level by sufficient feeding for only one day during 12 days of short-day exposure (C-12, see Table 1). Most of the females in this group could respond to a long day with slight delay in ovarian development.

It can be concluded from Fig. 2 that the females whose weight gains did not exceed 135–145% of the initial level during short-day exposure, responded to a long

Table 2. Relationship between weight gain under short day-length and ovarian development after 10 days exposure to a long day thereafter[a].

Developmental stage of ovary[b]	No. of females	Weight gain (%) mean ± S.D.
I	14	155.0 ± 11.1
II	16	142.2 ± 13.3
III	13	132.2 ± 16.3
IV	45	120.7 ± 13.2
I+II	30	148.1 ± 12.1
III+IV	58	123.3 ± 13.8

[a] Results given in Fig. 2 were rearranged with weight gain and ovarian development.
[b] Results of t-test, I-II:s**, II-III:n.s., III-IV:s*, (I+II)-(III+IV):s***.

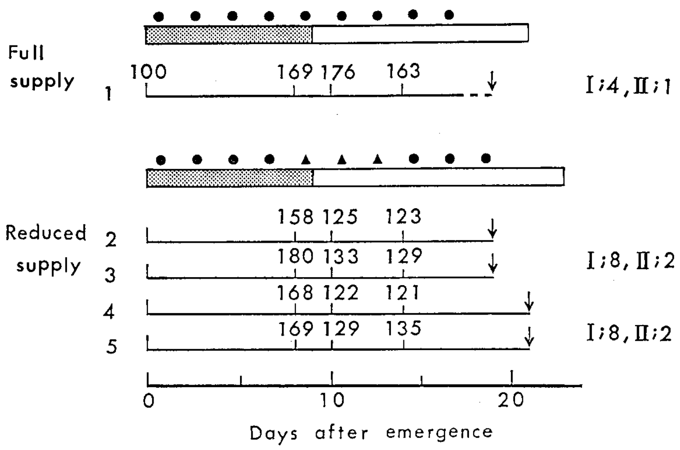


Fig. 3. Effects of limited feeding on photoperiodic sensitivity in animals whose weight once exceeded 150% of level at emergence. Five females and 3 males were confined in a petri dish. Dotted bar : short-day; white bar : long-day exposure; ▲ : reduced feeding; ● : normal feeding. Values on lines show weight gains over the initial level. Numbers of females in different stages of ovarian development at age indicated by arrows are shown at right. Dotted line represents cessation of feeding.

day after that, while those with gained weights over 140–145% could not respond. This conclusion was confirmed by statistical analysis of the relationship between weight gain and ovarian development in this series of experiments (Table 2). The lighter was the female at the time of changing day-length, the more developed the ovary under a long day. Difference in the weights between those females which developed ovaries (III+IV) and those which did not (I+II) was quite significant.

Body weight was lost by limited feeding in females whose weight had once exceeded 150% so that photoperiodic sensitivity was supposed to have been lost at the same time. The response to a long day was tested in these insects (Fig. 3). The females no longer responded even if their weight fell below 130%. Ovarian development was not observed in the ill-fed insects nor was it in the control animals. The former continued to feed until they were sacrificed for the observation of the ovary, while the latter ceased feeding at the 18th day.

2. *Body weight–entrance into diapause*

The time of entrance into diapause was judged by cessation of feeding and a comparison between normal insects and those which had gained weight over 150% but then were fed limitedly was made (Fig. 4). The normal adults fed on sufficient food ceased feeding between the 16th and 18th day (Fig. 4, 1–3) under the short-day condition. It took about ten days longer to enter into diapause in the partly starved insects than in the normal insects (Fig. 4, 4–6).

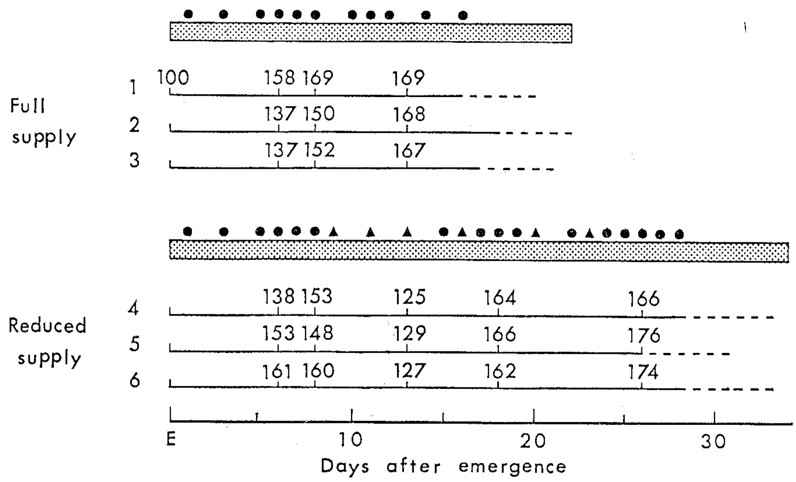


Fig. 4. Effects of limited feeding on entrance into diapause in animals whose weight once exceeded 150% of level at emergence. Five females and 3 males were confined in a petri dish. Symbols same as in Fig. 3.

These results suggest that sufficient intake of food is necessary not only for the loss of photoperiodic sensitivity but also for entrance into diapause.

3. *Fat body development–weight gain*

During the experiment shown in Table 1, observation of the fat body was also carried out simultaneously. Figure 5 shows the dorsal view of the abdominal body cavity after stripping off the cuticle.

In normally fed females reared under short-photoperiod, fat body occupied most of the abdomen by the 7th day and the lobe enlarged its volume between the 7th and 13th day (Fig. 5–2, 3, 4).

In the limitedly fed females (C-6, 9, 12 in Table 1), the fat body showed only weak development and did not cover other organs in the body cavity even after 12 days under short-day condition (Fig. 5–7). It seemed that the lobes of fat body stopped hypertrophy 3 days after emergence (Fig. 5–5).

The same features were observed in the fat body surrounding the brain in the head capsule (Fig. 6). Sheath width of the fat body thickened and became opaque to cover the brain by the 7th day in normal adults (Fig. 6–1). At the 13th day, the fat body was packed in the head capsule (Fig. 6–2). Those in females fed on a limited amount of foliage remained lucent until the 13th day (Fig. 6–3, 4).

4. *Endocrine activity–photoperiodic sensitivity*

In the normally and limitedly fed animals, NSC in PIC and CA were also com-

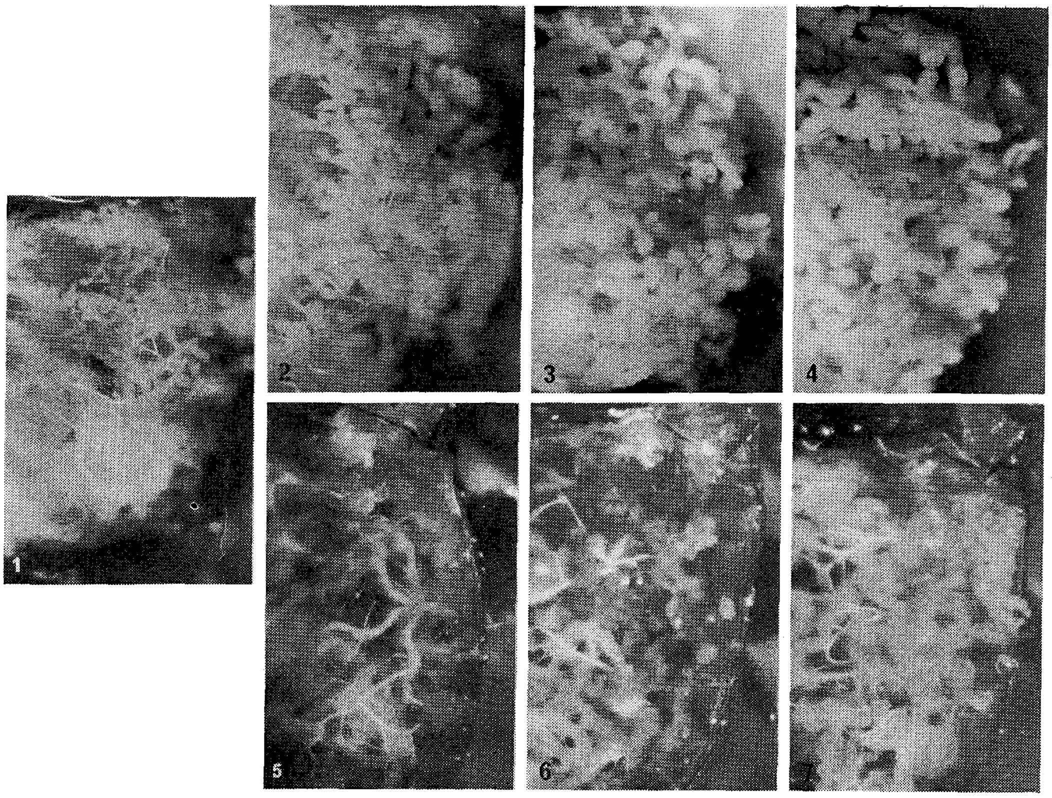


Fig. 5. Comparison of fat body development between fully fed and limitedly fed beetles. 1, 2, 3, and 4 show fat body in normally fed females reared under short day-length for 0, 6, 9, and 12 days after emergence, respectively. 5, 6, and 7: fat bodies in limitedly fed females reared for 6, 9, and 12 days under short day-length, respectively (ref. C-6, 9, 12, in Table 1). (×8)

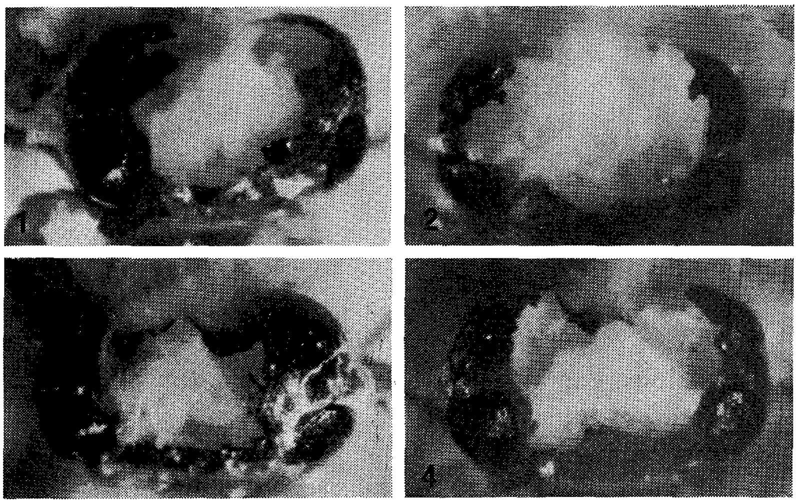


Fig. 6. Comparison of fat body development in the head capsule between fully fed and limitedly fed beetles. 1, 2: 6 and 12 days after emergence in fully fed females. 3, 4: 6 and 12 days after emergence in limitedly fed females reared under same conditions as shown in Fig. 5. (×24)

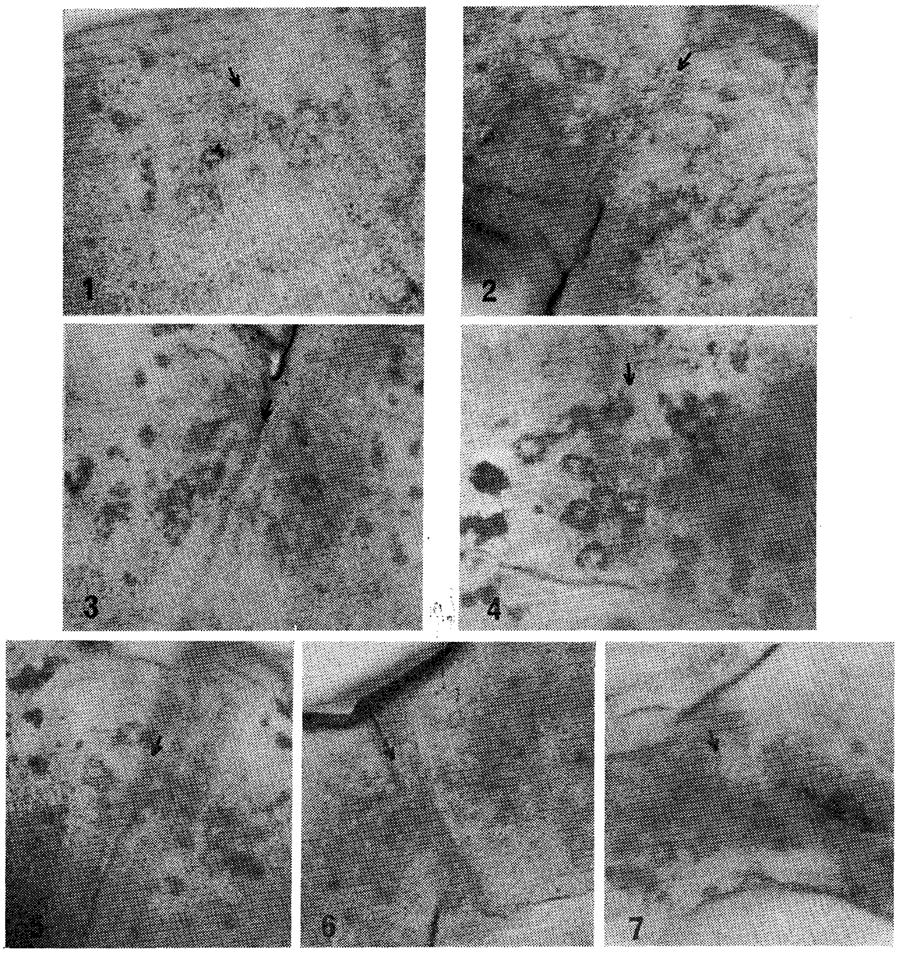


Fig. 7. Structure of NSC in PIC before and after long-day exposure. (×300) 1 : after 4 days of short-day photoperiod, fully fed (4SD); 2 : after 4 days of short-day photoperiod and 6 days of long-day photoperiod, fully fed (4SD–6LD); 3 : after 8SD, fully fed; 4 : after 8SD–6LD, fully fed; 5 : after 8SD, limitedly fed (LF8SD); 6 : after LF8SD–6LD (stage of ovarian development was III–IV); 7 : after LF8SD–6LD (stage of ovarian development was I–II). Arrow indicates midline of brain.

pared (Fig. 7 and Table 3). In the normally fed animals 4 days after emergence under short day when they were still sensitive to photoperiod, only fine granules stained with PF could be seen in NSC (Fig. 7–1). The diameter of CA was 47.2 μm on an average. After exposure to 6 cycles of long-day following 4 cycles of short-day, the ovaries developed to stages III–IV. NS materials were very few in NSC suggesting an active release of NS materials from them (Fig. 7–2) and CA diameter increased, also implying the active functioning of CA.

In normally fed females after 8 days of short-photoperiod when they had already lost photoperiodic sensitivity, many small stainable materials remained scattered in NSC of PIC (Fig. 7–3) and CA remained small. Six days of long-day exposure in these animals no longer induced ovarian development (stage I–II). NS materials increased during this period to pack in the cytoplasm (Fig. 7–4). CA diameter was 47 μm.

After 8 days exposure to short day, stainable materials were a little fewer in the females fed on limited food than in those fed normal food (Fig. 7–5), and CA diameter

Table 3. Change of CA diameter before and after the long-day exposure.

Condition[a]	CA diameter, mean±S.D. (μm)		
	Before the exposure	After the exposure	
		Ovarian development stage	
		I–II	III–IV
4SD–6LD	47.2±3.0	—	65.1±6.1
8SD–6LD	42.5±3.2	46.9±12.1	—
LF·8SD–6LD	46.2±1.9	46.2± 1.5	60.3±3.8

[a] The animals were reared under short day-length for 4 days (4SD), 8 days (8SD) with sufficient supply of food, or 8 days with limited supply (LF·8SD), before being exposed to long photoperiod for 6 days with full supply of food (6LD).

was the same with both groups. When the adults of limited feeding were subsequently exposed to a long day for 6 days, the ovaries showed different development ranging from I to IV stages. Stainable materials decreased to a scarcely detectable level in the females whose ovaring were in stages III–IV (Fig. 7–6), while the materials increased to pack in cytoplasm in those whose ovaries had failed to develop (Fig. 7–7). Large CA was always accompanied by NSC, having a small amount of secretory material.

DISCUSSION

It had already been shown that photoperiodic sensitivity was maintained for a longer period by the feeding of slices of potato to those insects which seemed to have nutritional deficiency (Kono, 1980 b). Present data with limited feeding on host plant foliage showed that insufficient intake of food evoked elongation of the photosensitive stage in the prediapause period. Furthermore, it was indicated that photosensitivity was closely correlated with weight gain and fat body development. If weight gain once exceeded 140–145% of the initial level it usually caused hypertrophy of the fat body under short-day conditions, photoperiodic sensitivity was lost and diapause entrance became inevitable.

From these findings, the mechanism of photoperiodic induction of diapause in this species can be summarized as follows. Photoperiods influence firstly NSC in PIC to regulate their activity. This can be supported by the previous study that PF stainable materials are less in NSC of 5 day-old adults under a long day than in those under a short day (Kono, 1980 a). Few secretory materials in NSC seem to indicate their active phase. A decrease of NS activity in PIC entrained by a short day suppresses JH release from CA. Reducing activity in both NSC of the brain and CA, in consequence, triggers physiological events characteristic of prediapause period such as atrophy of the ovary and hypertrophy of the fat body.

As the fat body develops, the beetle loses its sensitivity to a long day, but is not in a diapause state and still feeds. For the entrance into diapause, accumulation of more reserves in the fat body lobes are needed. NSC in PIC are inactivated after the completion of the fat body development, and the beetles enter diapause.

This mechanism seems well adapted for long diapause life in winter, reducing the risk of death by a shortage of reserves.

NSC reduced their secretory materials but showed active release whenever the beetle responded to a long day to develop the ovary. Photoperiodic sensitivity can be considered, therefore, to be the ability to respond to a long day stimulus in NSC.

The present study was not able to elucidate what causes the disappearance of responsiveness of NSC to the photoperiod.

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