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Photoperiodic Control of Adult Diapause in the Bean Bug, *Riptortus clavatus**

By

HIDEHARU NUMATA**

Department of Zoology, Faculty of Science, Kyoto University, Kyoto 606, Japan

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Abstract. 1) The role of photoperiod in the control of adult diapause was examined and the photoperiodic receptors were localized in the bean bug, *Riptortus clavatus* Thunberg. 2) Adults reared from eggs under a long-day photoperiod at 25°C showed prompt ovarian development and laid eggs. Under a short-day photoperiod, however, the ovarian development stopped, an adult diapause being induced. 3) The diapause was maintained for 30 days or more under a short-day photoperiod and promptly terminated when adults were transferred to a long-day photoperiod. 4) Diapause development proceeded gradually even under a diapause-maintaining short-day photoperiod. The critical daylength for prompt diapause termination was lowered and the preoviposition period after transfer to a diapause-terminating long-day photoperiod was shortened. 5) The compound eyes were demonstrated to be the principal photoperiodic receptors by excising them and by topically applying a phosphorescent paint on these organs.

Introduction

Many insects have two alternative programs of development (i.e., the diapause and non-diapause programs), and the environmental stimuli experienced at an earlier developmental stage influence the programming of subsequent development. These species may produce two or more generations per year, either actually or potentially (Beck 1980). Diapause in these species has been termed facultative diapause traditionally. With facultative diapause, moisture, diet, temperature and photoperiod have all been implicated as being involved in the diapause induction, although photoperiod has proved to be of the greatest importance (Lees 1955, Danilevskii 1961, Beck 1980).

Diapause was defined as being a state of arrested morphogenesis (Andrewartha 1952), arrested growth (Lees 1955) or supressed development (Beck 1980). However, it is now well known that the insect in diapause is not physiologically inactive. Andrewartha (1952) pointed out that the physiological processes involved in the completion of diapause can be looked upon as a gradual development which is influenced by temperature in much the same fashion as morphogenesis, and coined a term, diapause development for the processes. In addition to temperature,

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** Present address: Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi, Osaka 558, Japan

many factors (e.g., photoperiod, water, sensory stimuli, nutritive factors) have been shown to influence the rate of diapause development. Furthermore, response to photoperiod and/or temperature changes as diapause development proceeds in many species (Tauber and Tauber 1976, Beck 1980).

The bean bug, *Riptortus clavatus* Thunberg, is known as a soybean pest in Japan. This species produces two generations a year in Kyoto (Natuhara 1985) and overwinters as adults. Diapause in adult insect is manifested primarily as a supression of the reproductive function (Beck 1980). In *R. clavatus*, Kidokoro (1978) reported that the females reared under a short-day photoperiod entered an adult diapause and that mature eggs were observed only in those reared under a long-day photoperiod. The present paper aims to know the role of photoperiod in the control of adult diapause of *R. clavatus*, by examining the photoperiodic sensitivity systematically in various states of diapause, e.g., before entering the diapause, in the interim of the diapause development, and after the diapause termination. Other environmental conditions (temperature and food availability) were also examined.

Photoperiodism is regulated by a physiological mechanism containing three essential functional components: a receptor system, a clock system and an effector system (Beck 1980). The hormonal mechanism in the effector system for the control of adult diapause has been demonstrated in many insects. Adult diapause is due to the inactivity of the corpora allata to secrete juvenile hormone, which is controlled by the brain (see Raabe 1982). Diapause in *R. clavatus* may result from the same mechanism, because an application of a juvenile hormone analogue terminates diapause in this species also (Numata and Hidaka 1984c).

The receptor system for insect photoperiodism in seasonal development has been studied by many researchers. These studies suggest that the "organized" photoreceptors (compound eyes and ocelli) are not involved in the response to photoperiod (Saunders 1982).

However, Ferenz (1975) concluded from cauterization experiments that the compound eyes are the photoperiodic receptors in the regulation of the adult diapause in males of *Pterostichus nigrita*. Beck (1980) took this for the only example of a developmental photoperiodism in which the receptor system is apparently retinal. However, it is very difficult to destroy the retina totally without injuring the central nervous system. In fact, the lesion extended deeply into the optic lobes and was accompanied by a marked vacuolization of the neuropile when photoperiodic sensitivity was lost by the cauterization of compound eyes in *Megoura viciae*, in which the photoperiodic receptors were demonstrated to be in the brain (Lees 1964). Therefore cauterization experiments without histological observations are not sufficient to affirm or deny the involvement of a photoreceptor. Further examination of the retinal receptors in developmental photoperiodism appears to be necessary.

In the beginning of elucidating the receptor-clock mechanism for the control of adult diapause in *R. clavatus*, the location of photoperiodic receptors was examined with special reference to the role of retinal receptors.

Materials and Methods

Adults of *R. clavatus* were collected in legume fields in the northern part of the city of Kyoto from May to September in 1980–1983. The eggs of the first laboratory generation were used for

the experiments. Temperature was kept at 10±0.5°C, 15±0.5°C, or 25±1.5°C. Photoperiod was provided by a daylight-type fluorescent lamp (10 W) controlled by a time switch.

Nymphs were reared on soybean and water with a few immature pea pods (Kamano 1978 modified) in plastic pots, 15 cm in diameter and 9 cm in depth. The density of the nymphs was maintained at about 100 per pot in the first instar, and was lowered gradually to about 25 per pot in the last (fifth) instar. Two individuals of the adults emerged were reared in each 200 ml plastic cup with 10 grains of soybean and water, unless otherwise stated.

The ovarioles were examined by dissecting insects in 0.9% NaCl solution with fine forceps under a stereoscopic microscope, and the developmental stages were determined under a light microscope. Six developmental stages of ovarioles were distinguished, as shown in Fig. 1. Each ovary is composed of seven ovarioles, and their development is not synchronized. The most advanced ovariole in each ovary was taken to designate the developmental stage.

Testes were fixed in Bouin's solution, and embedded in paraffin using conventional methods. The paraffin blocks were sectioned 10 μm in thickness. The sections were stained with Mayer's acid-haemalaum and eosin, and observed under a light microscope.

Surgical operations were performed on adults according to Nishiitsutsuji-Uwo et al. (1967). Each adult was anaesthetized with CO_2 and secured dorsal side up on a plastic box with an inlet for CO_2. Each compound eye was removed with a micro-scalpel made from a razor blade fragment held in a blade holder (Inami). The wound area was then checked for remaining fragments of ommatidia. Excision of ocelli was performed in the same manner. "Wounding" was made by cutting out a triangle piece of integument posterior to each compound eye. The wound was sealed with melted wax.

A phosphorescent paint (RMLC-G1A, Dainippon Toryo-Sinloihi) was used for the experiment. The phosphorescent pigment (LC-G1A, Sinloihi; the main conponent is ZnS) is a substance that absorbs light energy, and discharges its own green phosphorescence. Brightness

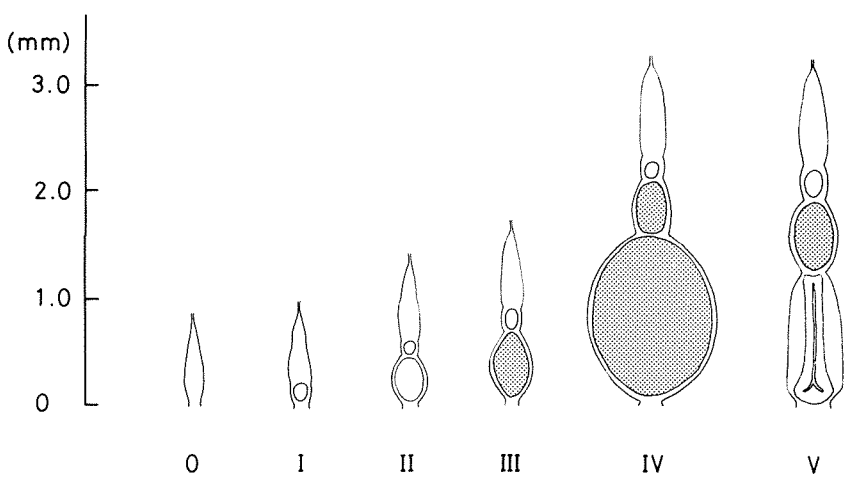

Fig. 1. Developmental stage of oocytes in *Riptortus clavatus*. Stage 0: no oocytes in vitellarium. Stage I: one oocyte in vitellarium. Stage II: two transparent oocytes. Stage III: two oocytes in vitellarium; the basal one filled with opaque, light-blue yolk. Stage IV: three oocytes; the lower two light blue. Stage V: the mature eggs ovulated.

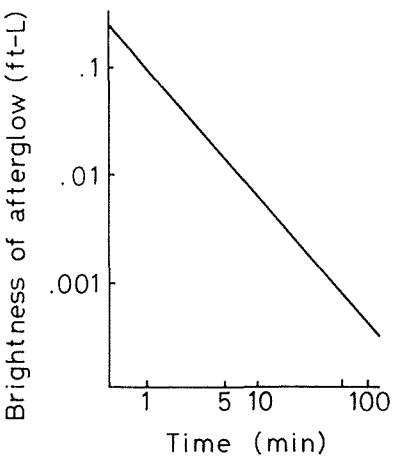

Fig. 2. Brightness of afterglow of the phosphorescent pigment, LC-G1A after arrest of illumination, determined by Lambert method (communicated by Sinloihi Co., Ltd).

of afterglow of this pigment decreases after arrest of illumination, promptly in the beginning but slowly afterwards (Fig. 2).

Statistical analyses were carried out for the preoviposition period by Mann-Whitney *U* test or by Kruskall-Wallis test, or for the proportion of diapause-terminated individuals by Fisher's exact probability test.

Experiments and Results

Experiment 1

Development of gonad was compared between adults under a long-day and those under a short-day photoperiod. Nymphs were reared under a long-day photoperiod (16L-8D) or under a short-day photoperiod (10L-14D) at 25°C. Females and males were separated from each other on the day of adult emergence. Ovaries and testes were examined at various ages.

In adults reared from eggs under a long-day photoperiod, the ovaries developed promptly,

Table 1. Ovarian development in *Riptortus clavatus* reared under 16L-8D and 10L-14D at 25°C

Photoperiod	Adult age (day)	No.	Developmental stage of ovaries					
			0	I	II	III	IV	V
16L-8D	0	12	9	3	0	0	0	0
	1	12	3	9	0	0	0	0
	2	12	0	6	5	1	0	0
	3	12	0	0	3	8	0	1
	4	12	0	1	0	5	4	2
	5	12	0	0	1	3	2	6
	6	12	0	0	1	1	1	9
	7	12	0	0	0	2	1	9
10L-14D	0	12	11	1	0	0	0	0
	7	12	0	3	9	0	0	0
	28	20	0	20	0	0	0	0

reaching the III–V stage within a week. After ovulation, the empty follicle underwent changes to form green corpus luteum at the posterior end of the vitellarium. Eggs were stored in the lateral oviducts temporarily and pigmented brown before being laid. They began to lay eggs even without copulation. However, ovarian development in adults reared under a short-day photoperiod stopped at stage I, although ovaries of some individuals reached to stage II (Table 1). The light-blue yolk deposition did not occur in these individuals, and the fat body developed within a week in them instead. Therefore, it is concluded that the adults reared under a short-day photoperiod entered reproductive diapause.

On the contrary, no difference was observed in testes between adults reared under a long-day photoperiod and those reared under a short-day photoperiod, either on the day of emergence or seven days after that. Sperms were found even in the testes of newly emerged adults reared under either photoperiod.

Experiment 2

The diapausing males were distinguished from non-diapausing ones only by the lack of mating activity, which was not easy to monitor. Diapause assessment in the following experiments was therefore limited to the females.

The experiment was carried out to obtain photoperiodic response curve of diapause induction and to determine the critical daylength for it. Nymphs were reared under various photoperiods at 25°C. The adults were kept in male/female pairs from the day of emergence. Oviposition was recorded daily. Ten days after the adult emergence, the developmental stage of their ovaries was examined.

Ovarian stages were classified as follows: (−) no yolk was deposited in oocytes (stage I–II in Fig. 1); (+) light blue yolk was deposited in oocytes (stage III–IV in Fig. 1); (++) mature eggs were ovulated into the oviduct (stage V in Fig. 1). Individuals which had ovaries in stage (+) or (++) were considered to be nondiapausing because ovaries remained in stage I–II and light-blue yolk deposition never occurred in diapausing females (Table 1).

Not only long-day photoperiods, 14L-10D to 24L-0D, but also very short-day photoperiods, 0L-24D to 4L-20D, prevented diapause. The median preoviposition period was 6–7 days and

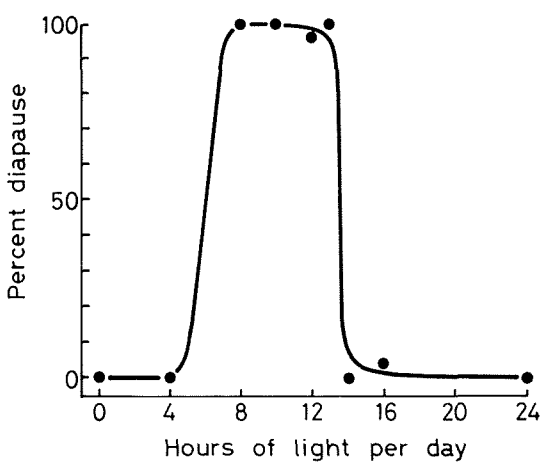

Fig. 3. Effect of photoperiod on the induction of adult diapause in *Riptortus clavatus* at 25°C. Each plot represents 21–25 pairs.

the minimum was 5–6 days under each nondiapause-inducing photoperiod. Diapause was induced by photoperiods from 8L-16D to 13L-11D (Fig. 3).

Experiment 3

Preliminary experiments showed that diapause was maintained under a short-day photoperiod and terminated when transferred to a long-day photoperiod. Experiment 3 was carried out to determine the critical daylength for the termination of diapause, and to know whether the diapausing adults respond to the absolute duration of photophase or the direction of change in photoperiod.

Nymphs were reared under various diapause-inducing photoperiods at 25°C. The diapausing adults were kept in isolated male/female pairs from the day of emergence, and exposed to various photoperiods at 25°C. Oviposition was recorded daily. Thirty days later, the developmental stage of their ovaries was examined. The diapause status (i.e., whether maintained or terminated) was judged by the same standards as in Experiment 2.

Under a long-day photoperiod, 14L-10D or 16L-8D, diapause was terminated in most individuals for all the nymphal photoperiods. Short-day photoperiod, 10L-14D or 12L-12D, did not terminate diapause, regardless of the nymphal photoperiod. Under 13L-11D, diapause was not terminated in the adults reared as nymphs under 12L-12D or 13L-11D, although it was terminated in some of those reared as nymphs under 8L-16D or 10L-14D. The difference in the percentage of diapause-terminated individuals between these two groups was significant ($P<0.01$) (Table 2). Thus, diapause was terminated under a photophase longer than the critical value (between 13 hr and 14 hr at 25°C in newly emerged adults), although an increase in photophase from a very short one to another which was a little below the critical value also had an effect on diapause termination.

The preoviposition period in the group transferred from 13L-11D ot 14L-10D was signifi-

Table 2. Effect of photoperiod on the termination of adult diapause induced under different photoperiods at 25°C in *Riptortus clavatus*

Photoperiod		No.	Diapause-terminated (%)	Preoviposition period (day)	
Nymph	Adult			min.	med.
8L-16D	12L-12D	22	0	—	—
	13L-11D	22	23	24	—
	14L-10D	22	86	13	18
10L-14D	10L-14D	20	0	—	—
	12L-12D	20	0	—	—
	13L-11D	20	25	24	—
	14L-10D	20	85	12	19
	16L-8D	20	85	15	22.5
12L-12D	12L-12D	21	0	—	—
	13L-11D	21	5	—	—
	14L-10D	22	100	13	20
13L-11D	12L-12D	21	0	—	—
	13L-11D	21	0	—	—
	14L-10D	23	96	8	12

cantly shorter than in each of the groups transferred from 8L-16D, 10L-14D or 12L-12D to 14L-10D ($P<0.01$) (Table 2). Thus, diapause was less intense when induced under a photophase near the critical value than under more typical short-day photoperiods.

Experiment 4

Experiment 3 showed that diapause was terminated when adults were transferred to a long-day photoperiod on the day of emergence, except for some individuals remaining in diapause for 30 days or more (Table 2). Experiment 4 was carried out to examine the photoperiodic sensitivity of diapausing adults which experienced various temperatures for various period under a short-day photoperiod.

Nymphs were reared under a diapause-inducing photoperiod of 10L-14D at 25°C. Ten males and 10 females of adults were reared in each plastic pot, 15 cm in diameter and 9 cm in depth, with soybean grains and water under the same conditions. Seven days after adult emergence, they were exposed to various temperatures under 10L-14D, and then their photoperiodic sensitivity was examined. The following regimes were employed as the first exposures before the photoperiodic sensitivity tests: *Group 25°–0*, without first exposure; *Group 25°–30*, 30 days at 25°C; *Group 15°–30*, 30 days at 15°C; *Group 10°–30*, 30 days at 10°C; *Group 25°–90*, 90 days at 25°C; *Group 15°–90*, 90 days at 15°C. Soybean grains were replaced every 30 days, and examined whether they were fed on or not.

The photoperiodic sensitivity of these adults after the first exposure was examined by keeping them in isolated male/female pairs and subjecting them to 10L-14D, 12L-12D, 13L-11D or 14L-10D at 25°C. Oviposition was recorded daily. Thirty days later, the developmental stage of their ovaries was examined. The diapause status was judged by the same standards as in Experiment 2.

Diapausing adults continued feeding at 25°C, and ceased it immediately after transfer to 10°C. They fed a little only during the first 30 days at 15°C. In *Group 25°–90*, 11 out of 94 females started oviposition before transfer to various photoperiods. No females in the other groups oviposited before the photoperiodic sensitivity test.

The results are summarized in Fig. 4. In seven-day-old diapausing adults (*Group 25°–0*), the critical daylength for diapause termination at 25°C was a little shorter than 13 hr, although some individuals maintained diapause even under 14L-10D.

After 30 days' exposure to 10L-14D at 25, 15 or 10°C, diapause was terminated in all the individuals transferred to 14L-10D and 25°C, and the percentage of diapause-terminated individuals under 13L-11D at 25°C was also increased. The preoviposition period after transfer to 14L-10D decreased ($P<0.01$ between *Group 25°–0* and *25°–30*, $P=0.07$ between *Group 25°–0* and *15°–30*, $P<0.05$ between *Group 25°–0* and *10°–30*). There was no significant difference in preoviposition period under 14L-10D among *Groups 25°–30*, *15°–30* and *10°–30* ($P=0.53$). Under 13L-11D, the preoviposition period was significantly shorter in *Group 25°–30* than in *Groups 25°–0* ($P<0.01$) and *15°–30* ($P<0.01$).

After 90 days' exposure to 10L-14D at 25°C or 15°C, diapause was terminated in all individuals transferred to 13L-11D or 14L-10D at 25°C, and even in many of those transferred to 10L-14D or 12L-12D at 25°C. The preoviposition period in *Group 25°–90* or *15°–90* under 13L-11D or 14L-10D at 25°C was significantly shorter than that in *Group 25°–30* or *15°–30*, respectively ($P<0.01$). Under 10L-14D or 12L-12D, the percentage of diapause-terminated

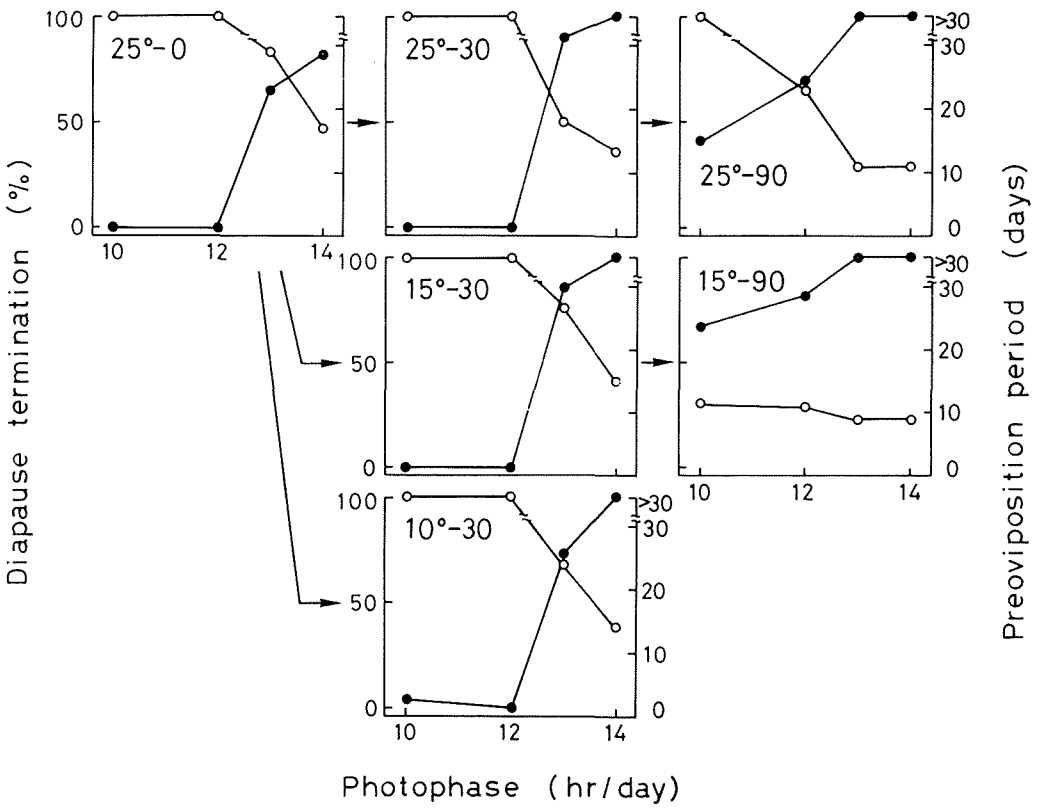

Fig. 4. Photoperiodic sensitivity of diapausing adults of *Riptortus clavatus*. Diapausing adults (7-days old) were first exposed to various temperature conditions under 10L-14D (0 days; 25°C 30 days; 15°C 30 days; 10°C 30 days; 25°C 90 days; 15°C 90 days), and then transferred to 10L-14D, 12L-12D, 13L-11D or 14L-10D at 25°C. Closed circle: the percentage of individuals in which diapause was terminated within 30 days. Open circle: median preovposition period after transfer. Each plot represnts 21–25 pairs.

individuals was higher and the preoviposition period under each photoperiod was shorter in *Group 15°–90*, than in *Group 25°–90*, although the difference was not statistically significant in either photoperiod (P=0.11–0.37).

Thus, diapause development in *R. clavatus* proceeded gradually even under a diapause-maintaining short-day photoperiod. The critical daylength for diapause termination was lowered and the preoviposition period after transfer to diapause-terminating long-day photoperiods was shortened. The rate of diapause development was a little higher at 25°C than at 15°C or 10°C during the first 30 days, and a little lower at 25°C than at 15°C during the next 60 days.

Experiment 5

Gradual diapause development under a short-day photoperiod proceeded even after the cessation of feeding in Experiment 4. In Experiment 5, effect of food on prompt diapause termination under a long-day photoperiod and on post-diapause ovarian development was examined.

Table 3. Developmental stage of ovaries in *Riptortus clavatus* kept under various combinations of food and photoperiod (25°C)

Conditions*	Period (day)	No.	Stage of ovaries			
			−	±	+	++
10L-14D, fed	14	20	20	0	0	0
16L-8D, fed	14	20	3	0	6	11
16L-8D, starved	14	20	17	2	1	0
16L-8D, starved	21	20	9	7	3	1

* Diapausing adults (35-days old) were transferred to these conditions.

Nymphs were reared under a diapause-inducing photoperiod of 10L-14D at 25°C. Both male/female pairs and females separated from males of adults were transferred to various regimes of food supply (soybean) and photoperiod (10L-14D or 16L-8D) at 25°C, 35 days after emergence. The development of ovaries was examined in females separated from males. In Experiment 2,

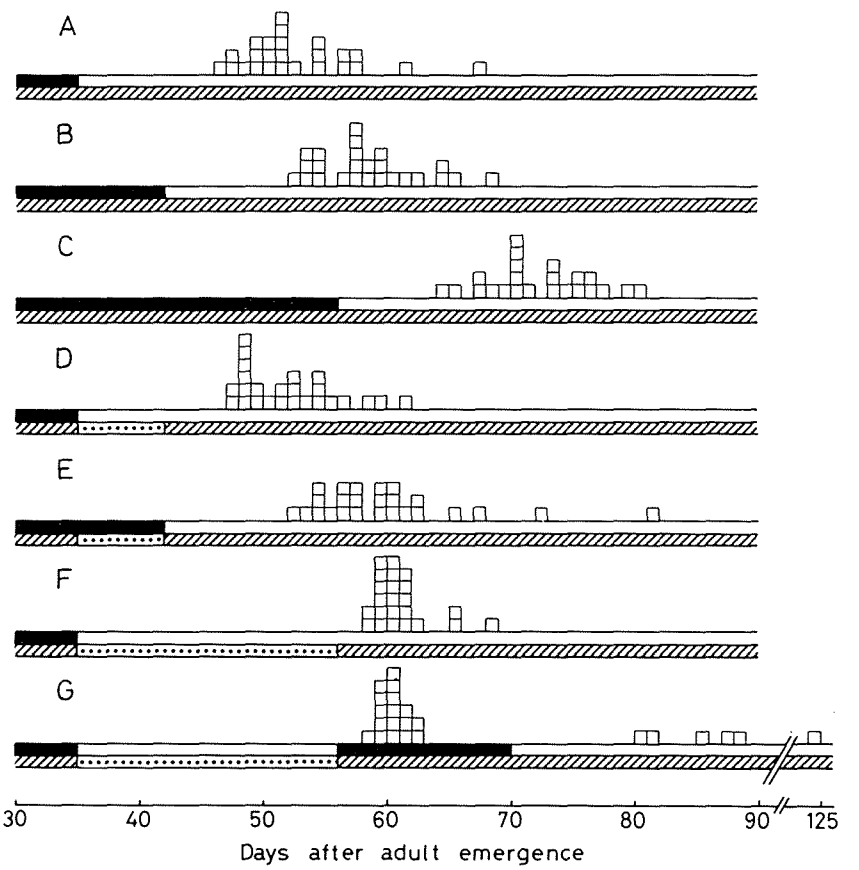

Fig. 5. Oviposition onest after adult diapause in *Riptortus clavatus* under various regimes of food and photoperiod (25°C). Each block represents oviposition onset of an individual. Solid bar: 10L-14D, open bar: 16L-8D, hatched bar: food available, dotted bar: no food.

ovaries were classified into three developmental stages: (−), (+) and (++). In Experiment 5, the basal oocyte was slightly tinged with light blue in some individuals. Such ovaries were classified as (±). In male/female pairs, oviposition was recorded daily.

In continuously fed females, ovaries remained undeveloped for 14 days under a short-day photoperiod, and developed promptly under a long-day photoperiod. Ovarian development proceeded also in starved females under a long-day photoperiod, although it was much delayed as compared to that in fed ones (Table 3).

Diapause was terminated and oviposition started promptly in continuously fed females when they were transferred from a short-day photoperiod to a long-day one (Fig. 5-A, B, C). There was no significant difference in the period from the transfer to the first oviposition among these three groups ($P=0.60$).

Females starved for seven days after transfer to a long-day photoperiod started oviposition as prompt as continuously fed insects did (Fig. 5-A, D) ($P=0.70$). There was also no significant difference in preoviposition period between females starved during the last seven days under a short-day photoperiod and those continuously fed (Fig. 5-B, E) ($P=0.55$). Thus, the photoperiodic sensitivity persisted without food, and the starvation did not affect the diapause development. In groups A–E (Fig. 5), about 80% of females started oviposition within 21 days from the transter to the long-day photoperiod.

When females were starved for 21 days under a long-day photoperiod, they did not start oviposition (Fig. 5-F, G), although their ovaries developed gradually (Table 3). When food was

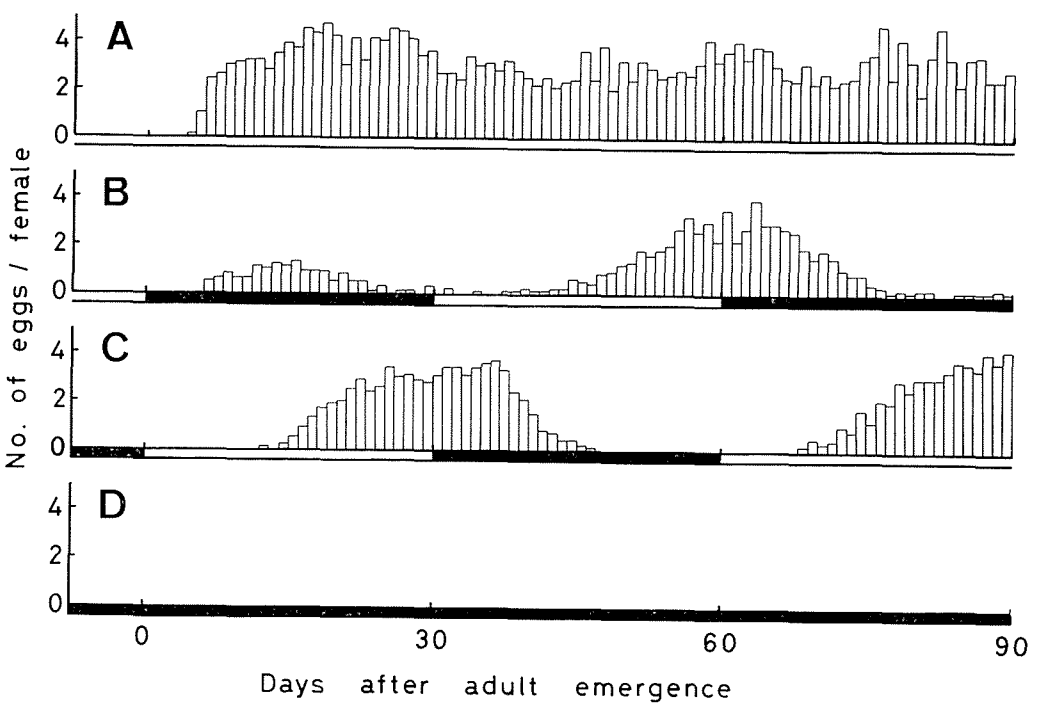

Fig. 6. Effect of repeated reversal of photoperiod on the oviposition of *Riptortus clavatus* at 25°C. Solid bar: 10L-14D, open bar: 16L-8D. The initial number of pairs were 34 (group A), 34 (B), 34 (C) and 31 (D). The number of females surviving at the end of the experiment were 9 (A), 21 (B), 21 (C) and 21 (D).

supplied 21 days after the long-day transfer, all females soon started oviposition (Fig. 5-F) and most females did so even after re-transfer to the short-day photoperiod (Fig. 5-G). In the latter case, diapause development had been completed under the long-day and starved conditions although their post-diapause ovarian development was very slow.

Experiment 6

Experiment 3 and 4 showed photoperiodic response in diapausing adults (Table 2). In Experiment 6, photoperiodic transfers were made repeatedly to examine the photoperiodic sensitivity of nondiapausing and post-diapausing adults. Nymphs were reared under a long-day photoperiod (16L-8D) or under a short-day photoperiod (10L-14D) at 25°C. The adults were kept in isolated male/female pairs from the day of emergence. Photoperiodic transfers were made 0, 30 and 60 days after adult emergence. The number of eggs was recorded and the adult viability was checked daily for 90 days. When the male in a pair died, another one reared under the same condition was introduced.

Females kept continuously under a long-day photoperiod began to lay eggs within 20 days after emergence, except for one which took 59 days to lay her first eggs. They oviposited almost everyday until death or the end of the experiment (Fig. 6-A).

Twenty-three females transferred from a long-day to a short-day photoperiod on the day of emergence also began to lay eggs within 20 days, but most of them stopped oviposition within 30 days after emergence. Only one individual oviposited intermittently until the end of the experiment. Seven females did not lay eggs during the first 30 days of their adult life. When the insects of this group were returned to a long-day photoperiod after 30 days' exposure to a short-day photoperiod, 24 females oviposited within 30 days. They stopped oviposition again after re-transfer to a short-day photoperiod, except for the one mentioned above. Four females did not lay eggs at all in the course of the experiment (Fig. 6-B).

Adults reared as nymphs under a short-day photoperiod entered diapause, even when transferred to a long-day photoperiod on the day of emergence. Twenty-three females terminated diapause and started to lay eggs within 30 days. When returned to a short-day photoperiod, they stopped oviposition. Five females did not lay eggs for 60 days. Re-transfer to a long-day photoperiod after 30 days' exposure to a short-day photoperiod induced 20 females to oviposit within 30 days. Three females remained in diapause throughout the experiment (Fig. 6-C). Females kept continuously under a short-day photoperiod did not oviposit for 90 days (Fig. 6-D).

Thus, photoperiodic sensitivity persisted and diapause can be repeatedly induced by a short-day photoperiod and terminated by a long-day photoperiod.

Experiment 7

Experiment 1–6 revealed that photoperiod is a major environmental factor in controlling adult diapause of *R. clavatus*. In Experiment 7, the location of photoreceptors for the photoperiodism was examined by excising photoreceptors.

Nymphs were reared under a diapause-inducing photoperiod of 10L-14D at 25°C. Females were separated from males on the day of adult emergence and kept under the same condition. Surgical operations were performed on the diapausing adults, seven days after emergence.

Table 4. Effect of removal of photoreceptors on the termination of adult diapause in *Riptortus clavatus* (25°C)

Treatment	Photoperiod	No. used	No. died	Stage of ovaries			Diapause-terminated (%)
				−	+	++	
Intact	10L-14D	24	0	24	0	0	0
	16L-8D	25	1	7	6	11	71
	0L-24D	30	2	15	7	6	46
Both compound eyes removed	10L-14D	24	4	0	1	19	100
One compound eye removed	10L-14D	23	3	20	0	0	0
	16L-8D	20	0	0	0	20	100
Both ocelli removed	10L-14D	23	3	20	0	0	0
Wounded	10L-14D	21	4	17	0	0	0
	16L-8D	19	0	1	0	18	95

After 21-day exposure to a long-day photoperiod (16L-8D), a short-day photoperiod (10L-14D) or continuous darkness (0L-24D), the developmental stage of their ovaries was examined. The diapause status was judged by the same standards as in Experiment 2.

In intact animals, diapause was maintained under a short-day photoperiod, while it was terminated in most individuals under a long-day photoperiod. Under continuous darkness, diapause was terminated, although the percentage of diapause-terminated individuals was lower than under a long-day photoperiod (Table 4).

Under a short-day photoperiod, diapause was maintained even when both ocelli were removed, although it was terminated when both compound eyes were removed. However, the percentage of diapause-terminated individuals in this group was significantly higher than in intact individuals under a long-day photoperiod ($P<0.05$) or under continuous darkness ($P<0.01$). Removal of one compound eye or "wounding" did not induce diapause termination under a short-day photoperiod, but raised the percentage of diapause-terminated individuals under a long-day photoperiod ($P<0.05$ and $P=0.10$ respectively) (Table 4).

It is concluded that the ocelli are not involved in the response to photoperiod, and wounding accelerates diapause termination and/or post-diapause ovarian development under diapause-terminating photoperiodic conditions. Furthermore, these results suggest that the compound eyes play a role in the reception of photoperiod, although the effect of wounding obscures the exact role.

Table 5. Effect on the termination of adult diapause of exposing the selected region to a longer photophase than the rest of the body surface by applying a phosphorescent paint in *Riptortus clavatus* (25°C)

Portion painted	Photophase (hr)	No.	Stage of ovaries			Diapause-terminted (%)
			−	+	++	
None	12.5	20	20	0	0	0
	12.75	20	17	2	1	15
	13.0	20	11	3	6	45
Compound eyes	12.5	20	12	1	7	40
	12.75	20	6	3	11	70
	13.0	20	1	1	18	95
Ocelli and vertex	12.75	20	19	0	1	5

Experiment 8

The experiment was conducted to clarify the role of the compound eyes in the reception of photoperiod, free from the effect of wounding, using a phosphorescent paint.

Nymphs were reared under a diapause-inducing photoperiod of 10L-14D at 25°C. Females were separated from males on the day of adult emergence and kept under the same condition. Seven-day-old diapausing adults were used for the experiment. To test for photoperiodic sensitivity, the selected region was exposed to a longer photophase than the rest of the body surface by applying a phosphorescent paint. They were exposed to three photoperiodic conditions at 25°C: 12.5L-11.5D, 12.75L-11.25D and 13.0L-11.0D, because the critical day-length for the termination of diapause in seven-day-old diapausing adults which were reared as nymphs under 10L-14D at 25°C was about 13 hr (Fig. 4). The light intensity of photophase was kept between 5 and 10 lux by covering the lamp with black semitransparent polyethylene sheets, in order to ensure the best use of the effect of phosphorescent paint. After 21-day exposure to those photoperiods, the developmental stage of their ovaries was examined. The diapause status was judged by the same standards as in Experiment 2.

The results are shown in Table 5. The percentage of diapause-terminated individuals was significantly higher in the group, in which the compound eyes were painted, than in the intact group under each condition ($P<0.01$). Phosphorescent paint on compound eyes shortened the critical daylength by about 0.5 hr. On the contrary, phosphorescent paint on the ocelli and vertex, beneath which lies the pars intercerebralis, had no effect on diapause termination ($P=0.60$). Thus, the compound eyes are the principal, if not the only, photoperiodic receptors in the control of adult diapause in *R. clavatus*.

Discussion

Photoperiodic response curve of the diapause induction

Riptortus clavatus exhibits a facultative adult diapause. Adults of this insect reproduced under long-day photoperiods (14L-10D to 24L-0D), while they entered diapause under short-day photoperiods (8L-16D to 13L-11D) (Fig. 3). This type of response, the long-day response, is most frequent in insects and particularly common in multivoltine species with facultative hibernal diapause (Danilevskii 1961, Saunders 1982).

In *R. clavatus*, very short-day photoperiods (0L-24D to 4L-20D) prevented diapause as well as long-day photoperiods (Fig. 3). Danilevskii (1961) has pointed out that the portion towards the left-hand side of the curve, 0L-24D to 10L-14D, is of no ecological significance, because such photoperiods are never met with in natural conditions, or they occur in the depth of winter when insect morphogenesis is at a stand still. Nevertheless, the response of insects to experimental photoperiod with extremely short photophases have a physiological significance, e.g., in attempts to determine the mechanism of time measurement (Beck 1980, Saunders 1982).

On the contrary, the portion towards the right-hand side of the curve, particularly on either side of the critical point, represents the daylengths which occur naturally during that part of the year when the temperature and other climatic factors are suitable for insect development. Therefore, this part of the curve has an adaptive significance and is a product of natural selection

(Danilevskii 1961). It is considered that adult diapause in *R. clavatus* is induced in September and October in Kyoto, because the critical daylength for diapause induction was between 13 hr and 14 hr (Fig. 3), which corresponds to the daylength from late August to September in Kyoto (35°N).

Mode of diapause development

Despite the commonly accepted generalization that low temperature accelerates diapause development in insects, there are many species in which hibernal diapause can be terminated by long-day photoperiods without intervention of low temperatures (Tauber and Tauber 1976, Beck 1980). In most of these species, diapause development proceeds even under short-day photoperiods at a low rate: e.g., *Ostrinia nubilalis* (McLeod and Beck 1963), *Chrysopa carnea* (Tauber et al. 1970), *Pyrrhocoris apterus* (Hodek 1971), *Antheraea pernyi*, *Antheraea polyphemus* (Mansingh and Smallman 1971), *Aelia acuminata* (Hodek 1975) and *Toxorhynchites rutilus* (Bradshaw and Holzapfel 1977).

Recently, Hodek (1983) proposed the new terms, "horotelic process" for diapause development under short-day photoperiods and "tachytelic process" for prompt diapause termination under long-day photoperiods. Adult diapause in *R. clavatus* was promptly terminated in most individuals under a long-day photoperiod with no exposure to low temperatures (Table 2), and the diapause development proceeded even under a short-day photoperiod (Fig. 4). *R. clavatus* also belongs to this type of insects which have horotelic as well as tachytelic processes in their diapause development.

In these species, the period required for tachytelic process was shortened as horotelic process advanced (McLeod and Beck 1963, Tauber and Tauber 1973a, Hodek 1971, 1975, Mansingh and Smallman 1971, Bradshaw and Holzapfel 1977). In *T. rutilus*, Bradshaw and Holzapfel (1977) demonstrated that one of the effects of horotelic process was to lower the critical daylength for the onset of tachytelic process. In *R. clavatus* also, horotelic process brought both lowering of the critical daylength for the onset of tachytelic process, and shortening of the period required for tachytelic process (Fig. 4).

Thermal requirement for diapause development

Insects show diversity in their thermal requirements for diapause development, closely connected with climate and geographical distribution (Lees 1955, Danilevskii 1961). In hibernal diapause of insects distributed in temperate zones, the effective temperature range for diapause development has been reported to be much lower than that effective for nondiapause or post-diapause development (Andrewartha 1952, Lees 1955, Danilevskii 1961, Beck 1980). It has been reported that a low-temperature treatment enhanced the horotelic process even in some species in which diapause can be terminated by tachytelic process (McLeod and Beck 1963, Mansingh and Smallman 1971, Hodek 1975, 1978, Bradshaw and Holzapfel 1977).

In *R. clavatus*, however, the horotelic process at a temperature (25°C) favoring nondiapause and post-diapause development was not so delayed as compared to that at lower temperatures (10°C and 15°C) (Fig. 4). Danilevskii (1961) proposed that insects can be classified into three types based on the thermal requirement of morphogenesis and diapause development and discussed the relation between these types and their geographical distributions. *R. clavatus* is categorized to the type of *Philosamia* (=*Samia*) *cynthia* in which the temperature ranges for

morphogenesis and diapause development overlap broadly. He concluded that the distribution of this type of insects contains tropical and subtropical zones, and their diapause originates from the adaptation for dry, hot seasons. *R. clavatus* distributes from subtropical Taiwan to cool temperate Hokkaido (Miyamoto 1965). Further investigations (e.g., experiments on cold hardiness, observations of life cycle in subtropical zones) are needed to discuss the origin of diapause in this species.

Effect of food on post-diapause development

Oviposition under a long-day photoperiod was significantly delayed when artificial food, instead of aphids, was given to diapausing adults of *Semiadalia undecimnotata* (Hodek 1970) or *Chrysopa mohave* (Tauber and Tauber 1973b). These authors concluded that availability of prey was an important factor in diapause termination. However, it is still unknown in these species whether the quality of food affects the diapause development or the post-diapause development.

In *R. clavatus*, the post-diapause ovarian development was delayed (Table 3), and diapausing adults did not start oviposition for 21 days (Fig. 5-F, G) under long-day and starved conditions, as reported for *Pyrrhocoris apterus* (Hodková 1982). Thus, food affects the onset of post-diapause oviposition, not only in carnivorous species but also in phytophagous species like *P. apterus* and *R. clavatus*.

In diapausing larvae of *Chaoborus americanus*, a combination of a long-day photoperiod and food supply induced pupation (Bradshaw 1969). In this species, food and photoperiod interact synergistically to trigger the prompt diapause termination: The input of food and a long-day photoperiod must be simultaneous rather than sequential for inducing post-diapause development, although short-day and starved conditions do not retard development once initiated (Bradshaw 1970).

In *R. clavatus*, however, horotelic process went on even after the cessation of feeding in Experiment 4, and the rate of tachytelic process was not affected by starvation (Fig. 5-A, D). Furthermore, food and long-day photoperiod are not simultaneously required for post-diapause development, because feeding under a short-day photoperiod induced oviposition in most individuals after 21 days of starvation under a long-day photoperiod (Fig. 5-G). Starvation suppresses the post-diapause development but not the diapause development in this species.

Factors controlling post-diapause oviposition in the field

In *Wyeomia smithii* and *Meleoma signoretti*, the critical daylength for terminating diapause does not change throughout autumn and winter, and ultimately spring daylength which exceeds the critical value are responsible for diapause termination in the field (Smith and Brust 1971, Tauber and Tauber 1975). However, most long-day insects complete diapause development by early winter and the long-day photoperiods in spring do not play a role in the field (Tauber and Tauber 1976, Beck 1980). This seems to be the case even in those species which terminate diapause under long-day photoperiods in the laboratory: e.g., *Pyrrhocoris apterus* (Hodek 1971), *Chrysopa carnea* (Tauber and Tauber 1973a), *Chryaopa harrisii* (Tauber and Tauber 1974), *Psylla pyricola* (McMullen and Jong 1976) and *Aelia acuminata* (Hodek 1979). When field samples were transferred to laboratory conditions in winter, there was no difference in the preoviposition period between samples under a long-day photoperiod and those under a short-day one in these species.

Adult diapause in *R. clavatus* is considered to be induced in September and October in

Kyoto as discussed above. In this species, after 97 days' exposure to a short-day photoperiod in the laboratory, the critical daylength for prompt diapause termination was shorter than 12 hr, being close to the daylength in late February in Kyoto, although photoperiodic sensitivity was not lost completely (Fig. 5). It is suggested that the critical daylength for prompt diapause termination decreases below the winter daylength, and the long-day photoperiods in spring do not appear, therefore, to play a role in diapause termination in the field.

Adults of *R. clavatus* start to lay eggs in legume fields from late May in Kyoto (Natuhara 1985). This timing of oviposition after hibernation cannot be explained only by temperature conditions. It is noted that mature legume pods become available for the first time as food at this period. In this species, post-diapause ovarian development is suppressed by starvation as discussed above. Therefore, appearance of food plays the most important role in timing the onset of oviposition after hibernation in *R. clavatus*.

Effect of change in photoperiod

In the study of insect photoperiodism, one subject of discussion has been whether insects respond to the direction of change in photoperiod or to the absolute duration of photophase (Beck 1980). Danilevskii (1961) concluded that insect photoperiodism depends on the absolute duration of the photophase, either above or below a critical value, rather than the direction of change in photoperiod, although the latter may have an additional effect.

However, some insects respond to the sequence of two different photoperiods (i.e., a long-day at the early stage of development and then a short-day at the later stage, or vice versa): e.g., *Nomadacris septemfasciata* (Norris 1965), *Heliothis zea* (Wellso and Adkisson 1966) and *Pterostichus nigrita* (Ferenz 1977). Furthermore, Tauber and Tauber (1970) demonstrated that *Chrysopa carnea* responds to photoperiodic change itself. This insect can perceive both decrease and increase in photophase without crossing the critical value defined by a stationary photoperiod. *R. clavatus* is considered to respond primarily to the absolute duration of photophase, although an increase from a very short photophase to one a little below the critical value is also effective for diapause termination (Table 2). The present results show another example of the response to the change in photoperiod.

Diapause induced under a photophase near the critical value was terminated significantly earlier under a long-day photoperiod than those induced under more typical short-day photoperiods (Table 2). This low intensity of diapause induced under a photophase near the critical value was reported in *Ostrinia nubilalis*, also. In *O. nubilalis*, diapause is induced in small portion of larvae under 15L-9D, and is terminated significantly earlier than those induced under 13L-11D or 9L-15D (McLeod and Beck 1963). It is interesting that diapause was induced in all individuals under 13L-11D (Fig. 3), although the intensity was low (Table 2) in *R. clavatus*.

Thus, the termination of adult diapause by tachytelic process in *R. clavatus* is related to the photoperiodic history of the individuals in two different ways, i.e., an effect of an increase in photophase and an effect of photoperiod which induced diapause on the intensity of diapause. However, ecological significance of these effects is unclear, because diapausing adults in the field do not encounter the increase in photophase until the winter solstice, when horotelic process may have brought the decrease of the critical daylength (see Experiment 4).

Reversibility of diapause

Although photoperiodic effects on prediapause and non-diapause development were reported for a number of insects, photoperiod generally does not affect post-diapause morphogenesis. Furthermore, post-diapause oviposition under long-day photoperiods is similar to that under short-day photoperiods in some species (Tauber and Tauber 1976). Beck (1980) asserted that diapause is not a rhythmic event and occurs once in the life of an individual insect, although few insects have been found to be capable of diapausing at more than one point in their life cycles.

However, specific photoperiodic requirement for continuing oviposition have been reported in post-diapausing females of some species since 1970s (see Hodek 1983). In *R. clavatus*, photoperiodic sensitivity persisted after diapause termination by tachytelic process, and diapause was re-induced by a short-day photoperiod and re-terminated by a long-day photoperiod (Fig. 6). Re-induction and re-termination of adult diapause in the laboratory was recorded in a few species, e.g., *Pyrrhocoris apterus* (Hodek 1974), *Aelia acuminata* (Hodek 1977) and *Oedipoda miniata* (Orshan and Pener 1979). Hodek (1979) presumed that photoperiod affects the post-diapause reproductive activity of many insects when they are kept for long periods under a diapause-promoting photoperiod after the termination of diapause. In fact, *Leptinotarsa decemlineata* adults hibernating for a second time are assumed to renew photoperiodic sensitivity later in the season, although post-diapause oviposition is not affected by photoperiod for 50 days in the laboratory (de Wilde et al. 1959).

It is still unclear whether *R. clavatus* can hibernate more than once: After hibernation, the critical daylength may have decreased in adults, because horotelic process has advanced sufficiently in them. In *P. apterus*, diapause was re-induced by photoperiod only in adults in which diapause was terminated by tachytelic process (Hodek 1971, 1974). Resumption of photoperiodic response is required to re-induce diapause in *R. clavatus* after horotelic completion of diapause, as reported in *A. acuminata* (Hodek 1979). Such an effect in *R. clavatus* will be studied in near future by examining the photoperiodic sensitivity of adults in which diapause was terminated under natural conditions, for a long period.

Effect of wounding

Wounding has been reported as a diapause-terminating stimulus by several authors (see Hodek 1983). In *R. clavatus*, however, wounding did not terminate diapause without the photoperiodic transfer, although it raised the percentage of diapause-terminated individuals after transfer to a long-day photoperiod (Table 4). It is concluded that wounding does not affect the change from horotelic process to tachytelic one, but affects the rate of tachytelic process and/or post-diapause development.

In diapausing pupae of *Hyalophora cecropia*, injury of the integument increases the rate of oxygen consumption rapidly (Harvey and Williams 1961). It is possible that such an effect causes the acceleration of tachytelic process and/or post-diapause development in *R. clavatus*.

Photoreceptors for photoperiodism

Truman (1972) proposed to divide animal clocks into two categories based on the difference in the mode of action of light. Type I clocks are stopped in continuous light and thus must have a "dark process". Type II clocks can free-run in continuous light. This ability of the latter is due to the fact that photoreceptors are external to the clock mechanism. In insects, the com-

pound eyes or other "organized" photoreceptors are not involved in type I clocks, associated with developmental rhythms such as of eclosion, hatching or brain hormone release, the photoperiodic receptors lying in the brain itself. In type II clocks, such as those controlling locomotor activity rhythm, the compound eyes are the principal and sometimes the only photoreceptors involved and the light information is transmitted synaptically to the clock. He suggested that photoperiodic clocks be classed as type I.

This suggestion has been supported by many studies (Saunders 1982). Both the photoperiodic clock and the receptor were localized to a small region of the protocerebral lobes of the brain in *Antheraea pernyi* (Williams and Adkisson 1964, Williams 1969) and *Megoura viciae* (Lees 1964, Steel and Lees 1977).

Nevertheless, the present results demonstrated first an example of developmental photoperiodism in which the compound eyes are the principal photoreceptors. The role of compound eyes as photoreceptors in insect developmental photoperiodism should be more carefully examined.

The location of the photoreceptors involved in entrainment of the circadian activity rhythm is in the compound eyes and the location of the driving clock is within the optic lobes in *Leucophaea maderae* (Nishiitsutsuji-Uwo and Pittendrigh 1968a, 1968b) and *Teleogryllus commodus* (Loher 1972). It is interesting to examine the location of the photoperiodic clock in *R. clavatus*, because the optic lobe clock has not been reported to be involved in developmental photoperiodism (Saunders 1982). Such a study will contribute to the elucidation of the relationship between the photoperiodic clock and the circadian clock, and the reconsideration of the classification of insect clocks.

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