

Environmental and endocrine control of adult diapause
in the brown-winged green bug,
Plautia crossota stali Scott
(Heteroptera: Pentatomidae)

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Contents

Introduction	-----1
Chapter 1	Photoperiodic control of diapause and body colour change -----10
	Materials and Methods -----14
	Results -----16
	Discussion -----23
	Summary -----25
Chapter 2	Effects of chilling on diapause development -----26
	Materials and Methods -----26
	Results -----28
	Discussion -----43
	Summary -----46
Chapter 3	Hormonal control of diapause and body colour change--Role of the
	corpus allatum -----48
	Materials and Methods -----49
	Results -----52
	Discussion -----67
	Summary -----70

Chapter 4	Juvenile hormone in heteropteran insects -----	71
	Materials and Methods -----	75
	Results -----	78
	Discussion -----	86
	Summary -----	88
Chapter 5	Bioassay for juvenile hormone activity -----	90
	Materials and Methods -----	90
	Results -----	92
	Discussion -----	100
	Summary -----	102
Chapter 6	Relationships between the JH-biosynthetic activity and volume of the corpus allatum -----	104
	Materials and Methods -----	105
	Results -----	106
	Discussion -----	115
	Summary -----	117
General Discussion	-----	119
Acknowledgements	-----	131
References	-----	133

Introduction

The brown-winged green bug, *Plautia crossota stali* Scott is one of the most important pests attacking fruit trees in Japan (Hasegawa and Umeya, 1974; Umeya, 1976; Shiga, 1980). Adults of this species and other stink bugs cause damage to young fruits including apple, persimmon, peach, pear and citrus. Trees infested heavily often drop their fruits. Even a slight attack by bugs creates spots on the surface of fruits and reduces the commercial value greatly. In 1973 and 1975, the outbreaks of these bugs occurred in a wide range of Japan. Since these outbreaks, the importance of *P. c. stali* and other stink bugs as a fruit tree-pest has been recognized (Hasegawa and Umeya, 1974; Umeya, 1976), and much effort has been made to elucidate the biology and population dynamics of these bugs (Ministry of Agriculture, Forestry and Fisheries, 1986). As a results, it has been found that *P. c. stali* reproduces mainly on coniferous trees such as the Japanese red cedar, *Cryptomeria japonica*, the hinoki cypress, *Chamaecryptaris obtusa* and the sawara cypress, *Chamaecryptaris pisifera*, usually produces two generations a year, and overwinters as adults in a state of diapause accompanied by a conspicuous body colour change from green to brown. One of remarkable characteristics for these stink bugs to attack fruit trees is that they fly into orchards during dispersal or movement between reproducing and overwintering sites, or among patchy reproducing sites (Tanaka, 1979; Uchida *et al.*, 1975; Oda, 1980; Moriya, 1995, 1996). Predicting the time when bugs invade orchards is important to protect fruits from these bugs. Because overwintering adults of *P. c. stali* are in diapause, collecting information on diapause and related phenomena is necessary to elucidate the life history of this species and to develop effective control methods for this stink bug.

However, information on diapause in *P. c. stali* is relatively poorly accumulated, in contrast to that on its population dynamics, which has been studied by Moriya (1995, 1996).

Diapause is one of physiological traits that insects have evolved to cope with unfavourable environmental conditions which they encounter during their life cycle. (Tauber *et al.*, 1986; Danks, 1987). Diapause is defined as a stage in life cycle during which morphogenesis is more or less at a standstill (Andrewartha, 1952), or as a neurohormonally mediated, dynamic state of low metabolic activity (Tauber *et al.*, 1986). Insects can undergo diapause at every life stage, but the stage that insects enter diapause is fixed species-specifically. Insects occurring in the temperate zone or areas with a dry season have to survive the adverse season when food supply is insufficient to support their life or when the ambient temperature or humidity is too low to undergo normal morphogenesis. Diapause provides insects with a way to escape from such unfavourable seasons in time ('reproduce later'), while migration gives them a way to escape from adverse seasons in space ('reproduce elsewhere') (Solbreck, 1978).

Diapause is divided into several phases; diapause induction, maintenance and termination, followed by post-diapause development (Tauber *et al.*, 1986; Danks, 1987). Insects use various environmental cues as token stimuli for timely entry to diapause. The stage sensitive to token stimuli is not necessarily immediately before diapause stage, but, in some case, long before diapause stage. When diapause-destined insects grow up to the diapause stage, their development is suppressed, in other words, diapause is induced. Most temperate zone insects enter diapause long before winter comes. The day-length is one of the most important cues used by insects as well as organisms living in

the temperate zone, and short day-length is very often a token stimulus for entering winter diapause by temperate insects. In the diapause maintenance period, to avoid untimely development, for example, caused by warm periods encountered in autumn or early winter, their diapause is maintained by token stimuli. During this stage, changes so-called diapause development occur toward timely completion of diapause. Insects in this stage apparently show no or little morphological change, but physiological change such as a decrease in sensitivity to token stimuli does take place. Some insects require a specific cue for diapause development to proceed. Once diapause development completes, insects are no longer sensitive to diapause-inducing stimuli and become ready to resume morphogenesis. In many temperate insects this occurs during winter, and subsequent morphogenesis is prevented until the temperature is above the developmental threshold. After diapause development completed and physical factors become favourable for development, post-diapause development occurs. Through these processes, the time of reproduction is synchronized within the population (Tauber *et al*, 1986; Danks, 1987).

As token stimuli, the day-length is one of most important cues. One of reasons for its importance as a signal for arrival of adverse seasons is that day-length is more reliable than other factors including temperature, humidity and food, which tend to fluctuate greatly.

In *P. c. stali*, adult diapause is under the control of day-length and its photoperiodic response for diapause induction is long-day type (Tanaka, 1979; MAFF, 1986; Numata and Kobayashi, 1994). However, information thus far available is still not enough to explain life cycle regulation in this bug, e. g., whether a specific conditions

are required for its completion of diapause development is not known. Some cases of invasion into orchards by this bug may be related to diapause because movement from reproducing sites to overwintering sites and *vice versa* provide an opportunity to invade into orchards (Oda, 1980; Moriya, 1995, 1996). However, not only the relationship between diapause and flight behaviour or flight activity but also the role of environmental factors in control of diapause is not fully elucidated. As a first step to approach these problems, photoperiodic transfer from long-day conditions to short-day conditions and *vice versa* is examined to ask whether adults of this species are sensitive to diapause-inducing or diapause-averting photoperiods and how diapause development proceeds during diapause period. In Chapter 1 of this thesis, if insects are sensitive to a change in photoperiod during diapause is examined. Because overwintering, diapause adults do not aggregate in hibernacula (Oda, 1980), and it is not easy to collect such adults from the field enough to conduct experiments, in Chapter 1 and following chapters, bugs raised under controlled conditions in the laboratory are used for experiments.

Even if the day-length is one of the most important cues as a token stimulus for many temperate insects, some other environmental conditions such as temperature, humidity and food often modify their response to the main determinant for diapause or course of diapause development (Tauber *et al*, 1986; Danks, 1987). An exposure to low temperatures or chilling has often been considered to be a prerequisite for completion of diapause, or to affect the course of diapause development in insects entering diapause in winter, though this is not always true (Tauber *et al.*, 1986; Danks, 1987; Hodek and Hodková, 1988). To test if chilling exerts its effect on the course of diapause

development in *P. c. stali*, in Chapter 2, diapause bugs are transferred to several temperature regimes, followed by a photoperiodic transfer to long-day conditions or maintained under short-day conditions.

When a token stimulus predicting arrival of adverse season is perceived by insects during their sensitive period, this information is stored in the central nervous system until the insect attain species-specific diapause stage. Towards and/or during the diapause stage, a variety of physiological changes that characterize diapause take place within insect body. In this process, two major insect hormones, *i.e.* moulting hormone and juvenile hormone, play important roles (*e. g.*, Denlinger, 1985). However, any hormone specific to diapause is not known except for diapause hormone identified in *Bombyx mori*, which undergoes egg diapause. In pupae of this insect destined to produce diapause eggs, diapause hormone is released from the suboesophageal ganglion and this hormone, in turn, stimulates biochemical or physiological change such as accumulation of carbohydrates and a precursor of diapause-specific pigment in eggs (For a review, Yamashita, 1996). In most other species with egg diapause, hormonal mechanism of diapause control has not been elucidated yet. Only in *Antheraea yamamai*, whose pharate first instar larvae enter diapause within their egg shell, "repressive factor" and "maturation factor" originating from mesothorax and abdomen, respectively, are indicated to play a role in control of diapause in conjunction with each other (Suzuki *et al.*, 1990).

Hormonal control of larval diapause is multifaceted because insects can enter diapause at various stages of larval period, such as feeding stage in early, mid or last instars, prepupal stage, even pharate first larval stage. The last case is, however, usually

classified to a type of egg diapause as *A. yamamai*. In some species, larvae stay without moulting and some undergo ecdyses with or without an increase in body size. Hormonally, in many insects high titres of juvenile hormone (JH) in the haemolymph and/or suppression of moulting hormone release, which is, in turn, caused by suppression of prothoracicotropic hormone (PTTH) release are involved. For example, a high JH titre is responsible for diapause in last instar larvae of *Chilo suppressalis* and *Diatraea grandiosella*, but PHHT release is not inhibited in these larvae because they undergo supernumerary larval ecdysis during diapause. The occurrence of supernumerary larval ecdysis indicates that JH titres are high in those insects (Yagi and Fukaya, 1974; Yin and Chippendale, 1973). In contrast, in *Ostrinia nubilalis* and *Laspeyresia pomonella*, a high JH titre exerts to induce diapause, but JH plays no role in diapause maintenance (Chippendale and Yin, 1979; Sieber and Benz, 1977). In contrast to these examples, diapause in *Nosania vitripennis* is attributed to a shutdown of PHHT-moulting hormone system (de Loof *et al.*, 1979).

In hormonal control of pupal diapause, the importance of inactive brain in maintaining diapause is first demonstrated in *Hyalophora cecropia* (Williams, 1946). In many species with pupal diapause, release of PTTH is inhibited during diapause, *i. e.*, the brain is inactive, and at the end of diapause, reactivated brain releases PTTH to induce biosynthesis of moulting hormone by the prothoracic gland, which then leads insects to resumption of morphogenesis, finally to adult emergence.

Since pioneer work by Wigglesworth (1936, 1985), it is well established that, in insects, the gonadotropin, hormone stimulating development of reproductive organs, is the juvenile hormone (JH). Because adult diapause is characterized by arrested

development of reproductive organs, the deficiency of gonadotropin has been anticipated to be responsible for inducing adult diapause. This was first demonstrated for *Leptinotarsa decemlineata* (de Wilde and de Boer, 1961), and a large body of evidence supporting this hypothesis has been accumulated in many species. In heteropteran insects, evidence along this line has also been collected in several species including *Pyrrhocoris apterus* (Sláma, 1964), *Dolycoris baccarum* (Conradi-Larsen and Sømme, 1978), *Oncopeltus fasciatus* (Rankin and Riddiford, 1977, 1978), *Riptortus clavatus* (Numata and Hidaka, 1984; Morita and Numata, 1997) and *Menida scotti* (Koshiyama *et al.*, 1994).

In the last decade, groups of neuropeptides, allatotropins and allatostatins, have been identified in several insects (For reviews, Stay and Woodhead, 1993; Gäde *et al.*, 1997). Because these neuropeptides exerts stimulatory and inhibitory effects on the JH biosynthesis by CA, they may play a role in control of adult diapause. Although in diapause adults of *L. decemlineata* and *P. apterus*, the CA activity is inhibited via nervous pathway (Khan, 1988; Hodková, 1976, 1977a, 1979), whether allatostatins involve in this process has not been fully elucidated yet. In the latter insect, on the contrary, presence of a brain factor stimulating the CA activity is reported recently (Hodková *et al.*, 1996). If peptides such as allatotropins or allatostatins play a role in control of diapause, disrupting or manipulating diapause regulation mechanism by these peptides or their analogue may provide a new way to control pest insects. This may also be useful for mass-production of natural enemies or other beneficial insects as a method for long-term storage or for obtaining their progeny at any desirable time. To develop such methods for a particular insect, it is necessary to elucidate control mechanism of

diapause in that insect. In Chapters 3-6 of this thesis, I focused on the endocrine mechanism controlling adult diapause and diapause related-body colour change in *P. c. stali*.

In Chapter 3, the hypothesis that the deficiency of JH or low titres of JH in the haemolymph induces diapause in *P. c. stali* adults was examined by means of surgical operations. The corpus allatum (CA), an endocrine gland located posterior to the brain is the site of biosynthesis and release of JH (Cassier, 1979, 1990). Effects of CA removal from reproductively active adults and effects of CA implantation into diapause adults were determined. How the activity of CA is controlled by upstream regulatory centre, i. e. the brain, was also examined in this chapter.

To test the above hypothesis further, it is necessary to quantify amounts of JH in relation to presence or absence of diapause. The JH is a group of substances with biological activity to inhibit insect metamorphosis and to serve as insect gonadotropin. Thus far, several forms (homologues) of JH have been identified from several orders (For reviews, Schooley and Baker, 1985; Riddiford, 1994). Which homologue(s) occurs in a particular insect is, therefore, indispensable information for quantification of JH. In Chapter 4, which form of JH the CA of *P. c. stali* produce was determined by *in vitro* incubation technique using a radiolabelled precursor. Because results obtained in this chapter implied that JH in *P. c. stali* was chemically different from any known forms of JH and JH-related compounds, a bioassay for testing JH activity in *P. c. stali* was established and biological activity in the presumptive JH fraction was examined by this bioassay in Chapter 5.

In Chapter 6, rates of biosynthesis of JH-active fraction by the CA were

determined adopting the *in vitro* incubation technique as in Chapter 4, which is so-called radiochemical assay (RCA) for JH biosynthesis (Pratt and Tobe, 1974). At the same time, the status of reproductive organs and size of CA were also determined to analyse relationships between these variables and rate of JH biosynthesis.

This thesis was written to submit to The United Graduate School of Agricultural Sciences, Iwate University based on papers already published (Kotaki and Yagi, 1987, 1989; Kotaki, 1993, 1996, 1998a, b) and unpublished results.

Chapter 1 Photoperiodic control of diapause and body colour change

P. c. stali enters diapause as an adult. In the autumn, adults undergo diapause and move to overwintering sites in the field (Uchida *et al.*, 1975; Tanaka, 1979; Oda, 1980; Moriya, 1995). While reproductively active adults show a green body colour with brown-coloured wings, overwintering adults are reddish-brown (Fig. 1). Before leaving the overwintering sites in the spring, the body colour turns green again. Photoperiodic response for diapause induction in the laboratory is indicated to be long-day type (Tanaka, 1979; Yanagi and Hagihara, 1980; Numata and Kobayashi, 1994). Yanagi and Hagihara (1980) have also reported that the period sensitive to photoperiod for diapause induction is the fifth (last) nymphal stage.

Diapause is a dynamic rather than static state during which the physiological conditions in the insects change (Tauber *et al.*, 1986; Danks, 1987; Hodek and Hodková, 1988). The rate of physiological changes is determined or influenced by the environmental factors such as photoperiod and temperature. Some insects require a specific stimulus for these changes to occur. In *P. c. stali*, whether photoperiods exert diapause-maintaining and/or diapause-terminating effect on diapause adults, and whether a specific stimulus is required for the progress of diapause development have not been examined. In this and the following chapter, to determine whether diapause adults are sensitive to a photoperiodic change and how diapause development proceeds in the laboratory, bugs reared under short-day conditions were transferred to long-day conditions without exposure to low temperatures, chilling (this chapter) or after chilling (Chapter 2). In these chapters, females starting oviposition were judged as those that have terminated diapause, and the progress of diapause development, in other words,

change in diapause intensity or diapause depth, was assessed by measuring the time required for the first oviposition after the transfer from short-day to long-day conditions. Here, diapause intensity is defined as the difference between the pre-oviposition period for bugs treated as above and that for reproductively active bugs that were continuously reared under long-day conditions.

In *P. c. stali*, the diapause status and body colour change appear to be closely related, because adults reared under short-day conditions at or below 20°C and those collected from the field during the winter show a brown body colour. However, the relationship between the progress of diapause development and the change in body colour has not been examined in detail. In the southern green stink bug, *Nezara viridula*, Kiritani and Hokyo (1970) have reported that adults both with reddish-brown body colour and with green colour are observed in the overwintering site. Harris *et al.* (1984) has reported the possibility of use of body colour as an indicator of diapause in this stink bug. Understanding the relationship between diapause and body colour change in *P. c. stali* may provide insight into the usefulness of body colour as an indicator of diapause in this species. Short day-lengths induce adult diapause in many other species of Heteropteran insects, including *P. apterus*, *Aelia accuminata* (For a review for these two species, see Hodek, 1983), *O. fasciatus* (Dingle, 1974), *Dolycoris baccarum* (Conradi-Larsen and Sømme, 1978), *R. clavatus* (Kidokoro, 1978; Numata and Hidaka, 1982) and *Halyomorpha mista* (Watanabe *et al.*, 1979), *Aelia fieberi* (Nakamura and Numata, 1995). However, none of these species show any conspicuous colour change associated with diapause. It may be possible, therefore, to uncover an other aspect of diapause that is masked in species showing no body colour change by following body

colour change. For this reason, change in body colour, as well as the time of oviposition, was recorded in Chapters 1 and 2.

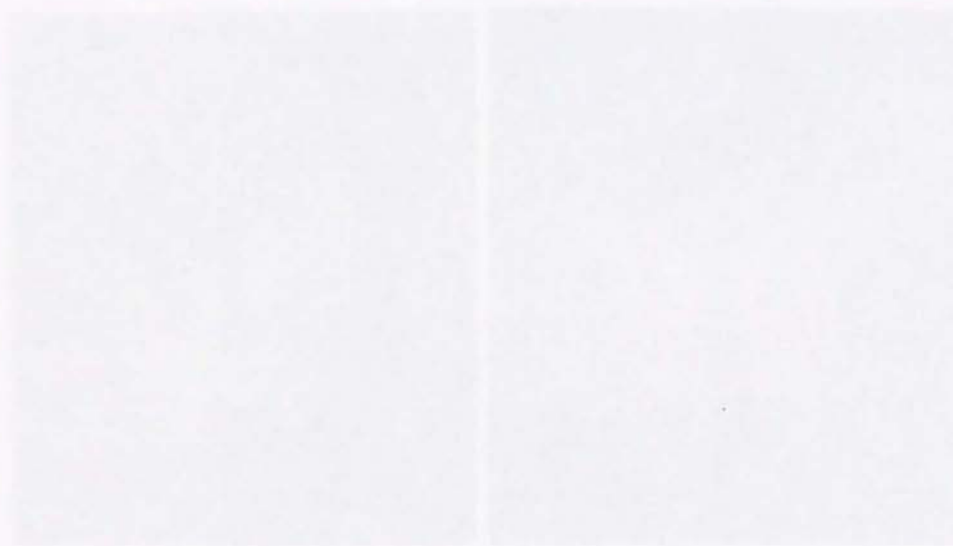


Fig. 1. Dorsal (left) and ventral (right) views of the butterfly, *Pieris* sp. (1955). The dorsal view shows the dark markings on the wings, and the ventral view shows the dark markings on the underside of the wings. The body is light-colored with dark markings.



Fig. 1. Adults of *P. c. stali* with green body colour, body colour grade 1 (left), which are typically found in reproductively active adults, and with brown body colour, body colour grade 5 (right), typical of diapause adults. Scale bar: 5 mm.

Materials and Methods

Insects

Throughout this thesis the scientific name, *Plautia crossota stali* will be used for the brown-winged green bug according to Tomokuni *et al.* (1993), although the scientific name, *Plautia stali* Scott has been used before. A laboratory colony was established from adults collected in Koganei, Tokyo in 1982 and 1983. Their offsprings were reared by the methods of Kotaki *et al.* (1983) under a photoperiod of LD15:9 h at 20°C. A group of 14 newly hatched nymphs was held in a plastic cup (9 cm diameter, 4.5 cm height) and provided with raw peanuts and a piece of water-soaked cotton. Food and water were replaced once a week. Newly emerged adults were maintained as above in a group less than 5 individuals, usually two pairs of males and females. Reproductively active adults were randomly sampled from these adults with known age, and they were, hereafter, referred to as long-day insects. Insects kept under a short-day photoperiod of LD 12:12 h at 20°C after hatching were referred to as short-day insects.

Photoperiodic transfer

Pairs of female and male adults were either kept under the same photoperiod throughout their whole life (Experiment I), or transferred to other photoperiodic conditions at a selected age of adult life. The regimes in which bugs were kept at long-day and short-day photoperiods are referred to as LL and SS regimes, respectively, and those in which insects were transferred from long-day to short-day conditions (Experiment II) and *vice versa* (Experiment III) as LS- and SL- regimes, respectively, followed by numbers indicating the adult age at the photoperiodic transfer. In SL

regimes, insects were transferred from short to long-day conditions on days 0, 4, 10, 20, 30, 45-47, 60-65 and 90-100. The last three regimes will be mentioned as SL-45, SL-60 and SL-90, respectively. Bugs were observed daily for their survival, oviposition and body colour for 60 days in LL, SL and LS regimes or for 90 days in SS regime. Body colour was determined by visual observation and classified into 5 grades: green body colour typically found among long-day, reproductively active adults was grade 1 (Fig. 1, left) and brown bugs found under diapause inducing conditions short-day were designated to grade 5 (Fig. 1, right). Grades 2-4 were classified according to the darkness of the body colour. Adults in grades 1 and 2 were referred to as 'green' and those in grade 3, and grades 4 and 5 'intermediate' and 'brown', respectively. Although some newly emerged adults under short-day conditions showed reddish green body colour, they were classified as grade 1.

Statistical analysis

Statistical analyses were mostly conducted using a computer software, either SYSTAT or STATISTICA throughout experiments in this study. To test the significance of correlation between pre-oviposition period and age at photoperiodic transfer in Experiment III, Kendall's coefficient of rank correlation, τ was calculated (Sokal and Rohlf, 1995), because not negligible fraction of females survived until the end of observation period (60 days after transfer) without oviposition, and normality of data distribution required for parametric analysis was not supported in this experiment. A value of 61 days was arbitrarily given for such females as their pre-oviposition period, not to exclude these females from analysis.

Results

Experiment I: Effects of stationary photoperiods

In the long-day insects, females started laying eggs relatively rapidly after adult emergence. The first oviposition was observed on 16 days after emergence, and the proportion of ovipositing females increased thereafter (Fig. 2, upper). Out of 27 females in this regime, 21 laid eggs with a pre-oviposition period of 26.9 ± 8.6 days (average \pm SD) after adult emergence, 6 died without oviposition, and 1 deposited no egg but lived until the end of experiment. All bugs showed green colour throughout experimental period (Fig. 2, upper). During handling of these bugs, mating pairs were sometimes observed, and most eggs obtained in this regime were fertilized (data not shown). These results indicated that adults of *P. c. stali* reared under long-day conditions were reproductively active.

In the SS regime, females did not lay any eggs during the experimental period (Fig. 2, lower). Instead, a remarkable change in body colour from green to brown was observed. The first appearance of brown bugs with a body colour grade 5 or 4 was about 16 days after adult emergence. The percentage of brown insects increased gradually thereafter to attain a maximum of 83 % 40 days after emergence. Brown insects then turned green again and the percentage of brown ones declined slowly until the end of observation period of 90 days. Bugs with body colour grade 3, intermediate body colour between green and brown, first appeared on the 8th day of adult life. The proportion of those adults increased up to 40 % on day 20. After this age, the value remained relatively unchanged around 10-20 %. Brown bugs appeared relatively inactive,

compared with green, long-day bugs, in moving as well as in feeding. These results showed that adults reared under a photoperiod of LD 12:12 h entered diapause in the laboratory.

Experiment II: Effects of a transfer from long-day to short-day

Females transferred from long-day to short-day conditions at adult emergence (LS-0 regime) laid no egg (Fig. 3). In this regime, all bugs were green during first 20 days. They started changing their colour on day 24, and by day 40 about 50 % of bugs turned brown. At the end of observation period, more than 75 % of bugs were brown. In this regime, the change in body colour from green to brown occurred later and the maximum percentage of brown bugs was lower than that in the SS-regime. In the LS-30 regime, bugs were ovipositing actively at the time of the photoperiodic transfer, *e. g.*, almost all females in this regime laid eggs in first several days after the transfer. The percentage of ovipositing females then decreased, and all females ceased egg laying within 28 days after transfer. The day of the last oviposition after the transfer was 14.7 days after the transfer on average. In this regime, the bugs started turning brown later than those in the LS-0 regime, and the proportion of those bugs was lower than of the LS-0 regime. These results indicated that long-day adults remained sensitive to diapause inducing, short-day photoperiods at least until 30th day of adult life.

Experiment III: Effects of a transfer from short-day to long-day

In every regimes where short-day bugs were transferred to long-day conditions, females started laying eggs, although the percentage of ovipositing females varied

among regimes from 24 % to 86 % (Fig. 4). Short-day adults seemed sensitive to a long-day photoperiod after adult emergence except for those in SL-0 regime. In this regime, 18 out of 29 females survived until the end of experiment, but 11 of them did not lay any eggs. In the other SL regimes, the percentage of such females was relatively low (< 17 %). Therefore, bugs at or shortly after adult emergence may be refractory to a change in photoperiods. The pre-oviposition period, the time required for egg laying after the transfer, of ovipositing females tended to decrease with an increase in the period during which bugs were kept under short-day conditions (Fig. 4, Kendall's $\tau = -0.175$, $n = 143$, $p < 0.05$). The differences in the pre-oviposition period between the LL and SL-90 regimes was not statistically significant (Mann-Whitney U-test). The difference between the LL and remaining SL regimes was statistically significant (Mann-Whitney U-test, $p < 0.05$ for SL-60 vs. LL regimes, $p < 0.01$ for the others vs. LL regimes). These results suggested that the diapause intensity decreased with an increase in the period kept under short-day conditions, and diapause was terminated until day 90 under the conditions used in this experiment.

In this series of experiments, adults changed their body colour after photoperiodic transfer (Fig. 4). The patterns of changes in proportion of green and brown bugs varied greatly depending on the time of the transfer from short-day to long-day conditions. These patterns could be divided into three types: 1) the proportion of brown insects was relatively low throughout the observation period, e. g. SL-0; 2) the proportion of brown bugs once increased after a transfer to long-day conditions, but decreased later and almost all bugs turned green, SL-4, 10 and 20; and 3) most individuals showed brown colour at the beginning of the experiment, and then they

turned green, SL-30, 45, 60 and 90. On individual basis, body colour change occurred relatively rapidly. It usually took less than 10 days for brown bugs to turn green. Regardless of the patterns females followed, oviposition was observed only in females with green body colour.

In SL regimes, there was no distinct relationship between the body colour at the photoperiodic transfer and the pre-oviposition period after the transfer. There was a large variation in the survival rate at the end of observation as well as the percentage of ovipositing females. No remarkable relationship was detected between these variables and the age of the transfer except that the survival rate tended to decrease with an increase in the age of transfer. In all regimes, female and male adults followed almost the same pattern in body colour change and any notable sexual difference in colour change was not observed.

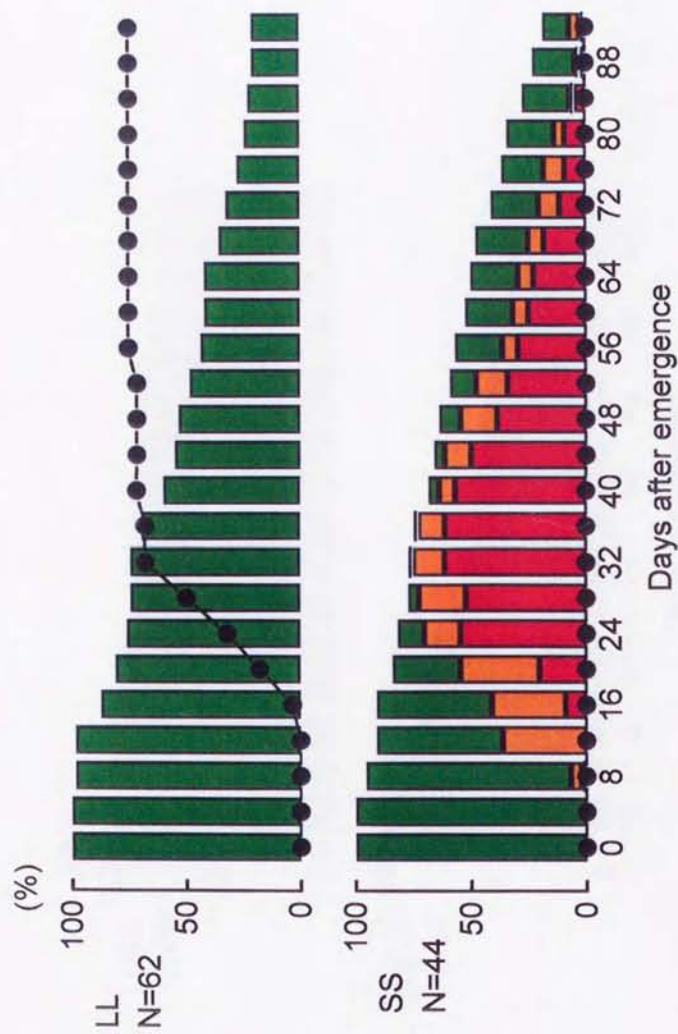


Fig. 2. Beginning of oviposition by females and body colour changes in *P. c. stali* reared under long-day (upper) and short-day conditions (lower) at 20°C. Green, orange and red areas indicate the proportion of insects showing green, intermediate and brown body colour, respectively. Closed circles indicate the cumulative percentage of ovipositing females.

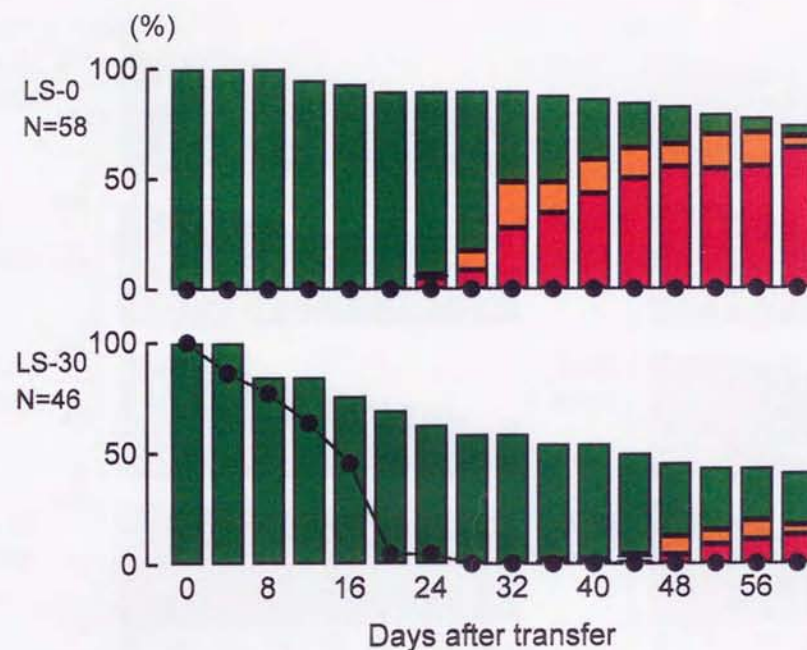


Fig. 3. Cessation of oviposition in LS-30 regime and body colour changes in *P. c. stali* adults transferred from long-day to short-day conditions at 20°C. Numbers following LS- indicate the adult age at transfer. Green, orange and red areas indicate the proportion of insects showing green, intermediate and brown body colour, respectively. Closed circles indicate the percentage of females which laid eggs in each 4-day period.

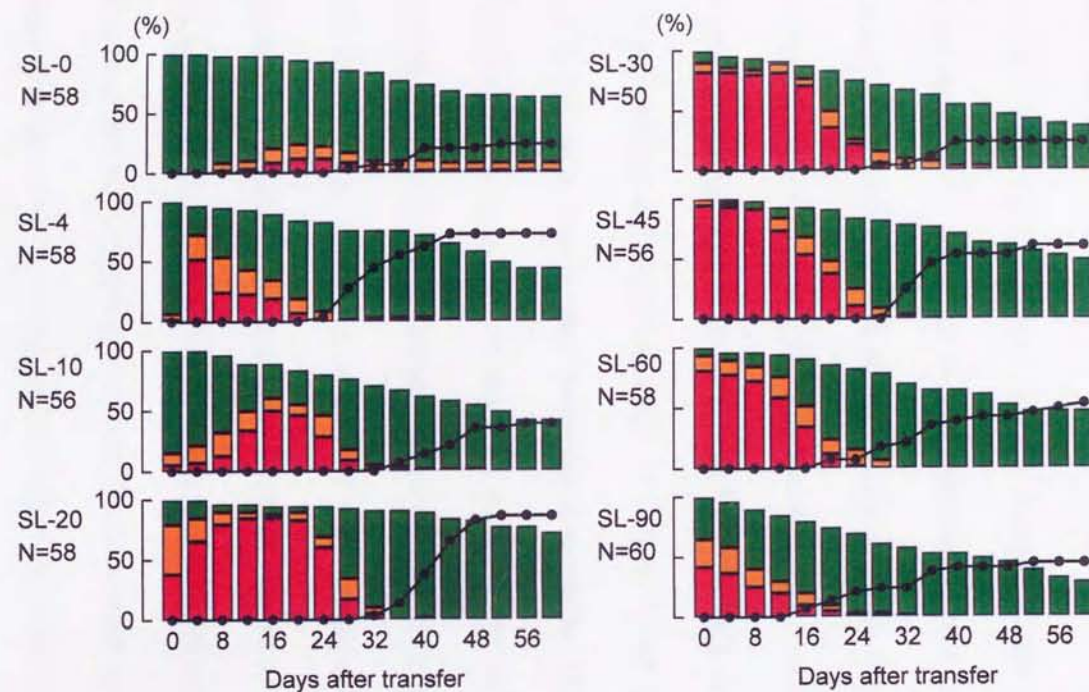


Fig. 4. Beginning of oviposition and body colour changes in *P. c. stali* adults transferred from short-day to long-day conditions. Numbers following SL- indicate the adult age at transfer at 20°C. Green, orange and red areas indicate the proportion of insects showing green, intermediate and brown body colour, respectively. Closed circles indicate the cumulative percentage of ovipositing females.

Discussion

In *P. c. stali*, photoperiodic response for diapause induction is reported to be long-day type (Yanagi and Hagihara, 1980; Numata and Kobayashi, 1994). This is confirmed by the results in the present study, *i. g.*, bugs reared under long-day photoperiods reproduce shortly after adult emergence while those exposed to short-day photoperiods enter adult diapause (Fig. 2). Results in this chapter show that *P. c. stali* adults are sensitive to a change in photoperiods, so that their diapause is induced and terminated by the photoperiodic transfer from long-day to short-day conditions and *vice versa*, respectively (Figs. 3 and 4). Only exceptional is results in the SL-0 regime, where newly emerged short-day adults were transferred to long-day conditions. In this regime, notable proportion of females (11 out of 29) survived until the end of experiment without laying eggs, that is, they remained in diapause. On the contrary, when females were transferred to long-days on day 4 of adult life or later, most terminated diapause and started oviposition, and relatively small portion of females (at most 16 %) survived without oviposition. Therefore, a part of very young females may be refractory to photoperiodic change, or diapause may be most intense at the time of adult emergence. Indeed, in the SL regimes, effects of photoperiodic transfer to long-day conditions on the time required for oviposition after the transfer were age-dependent, and there was a tendency that the pre-oviposition period decreased with an increase in the age at which bugs were transferred from short-day to long-day photoperiods. This suggests that in this bug diapause development proceeds and diapause become less intense gradually under short-day conditions without any specific stimuli such as an exposure to low temperatures. In the SL-90 regime, the time required to lay eggs after the transfer is not

statistically different from the oviposition period in long-day females. This suggests that, under 20°C and short-day conditions, diapause development is almost completed during the 90 days of short-day conditions. *P. apterus*, *A. accuminata*, *R. clavatus* and *D. baccarum* are also known to remain sensitive to changes in photoperiods in the adult stage (Hodek, 1968, 1979; Numata and Hidaka, 1982; Hodková *et al.*, 1989). In those species, diapause development proceeds under short-day conditions and no chilling is required.

Short-day adults alter their body colour reversibly from green to brown and *vice versa* in the laboratory (Fig. 4). This occurs without chilling. On individual basis, the time required to change body colour was relatively short, and it took about 10 days to turn from green to brown and *vice versa*, respectively. For this reason, the proportion of bugs showing intermediate colour between green and brown was not so high. Patterns of body colour changes in SL regimes are age-dependent. When newly emerged short-day adults were transferred to long day conditions, only a few turned brown within 60 days after the transfer. However, if the transfer occurred later, *e. g.*, on days 4, 10 and 20, substantial portion of bugs turned brown after the transfer. Therefore, bugs may be destined to alter their body colour from green to brown if they were exposed to short-day conditions for a certain period after adult emergence. After transfer to long-day conditions, females took various lengths of time to start oviposition, depending on the age at which the bugs are transferred to long-day conditions. In all cases, females laid eggs when they were green in colour, and no oviposition was observed before the body colour changed from brown to green. Therefore, it is likely that this change in body colour is closely associated with diapause termination, and brown body colour

seems one of symptoms of diapause syndrome in *P. c. stali*.

In experiments in this chapter, bugs were kept at a constant temperature of 20°C. Since low temperatures are known as one of factors affecting diapause development in many insects (for reviews, Tauber *et al.*, 1986; Danks, 1987; Hodek and Hodková 1988), it is important to examine effect of different temperatures on diapause development. This will be tested in the following chapter.

Summary

Bugs reared under short-day conditions (LD 12:12 h) at 20°C entered diapause and turned brown in the adult stage while those reared under long-day conditions (LD 15:9 h) started reproduction shortly after adult emergence and remained green throughout the adult stage. A transfer of adults from long-day to short-day conditions suppressed their oviposition and changed their body colour from green to brown. Adults transferred from short-day to long-day conditions started laying eggs and their body colour turned green. Females maintained under short-day conditions for a longer period of time tended to oviposit earlier after a transfer to long-day conditions. These results indicate that *P. c. stali* is sensitive to photoperiod even after adult emergence, and that an exposure to a low temperature is not a prerequisite for diapause termination. The results also suggest that diapause development proceeds under short-day conditions spontaneously. It is also suggested that body colour is closely associated with diapause and brown body colour seems one of symptoms of diapause syndrome.