Effects of Photoperiod, Temperature and Melatonin on Nymphal Development, Polyphenism and Reproduction in Halyomorpha halys (Heteroptera: Pentatomidae)

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ABSTRACT—Influences of photoperiod, temperature and melatonin were examined on development and color patterns of nymphs, and sternum color and reproductive fate of adults in *Halyomorpha halys* (*brevis*). Short-day accelerated nymphal development, whereas long-day accelerated reproductive maturation. The two types of photoperiodic responses at different stages may help maintain the univoltinism of this species in the field, assuring the right timing for diapause and reproduction. The pronotum of fifth instar nymphs reared under LD 11:13 shows a brown-marbled color pattern darker, with less creamy-yellowish speckles, than that of nymphs reared under LD 16:8. Short-day-reared fifth instar nymphs, which are destined to diapause in adults, had shorter white stripes on the pronotum, smaller body size, less frequent feeding and more lipid accumulation than the long-day-reared. The longer the exposure to LD 13:11 at 20°C during the nymphal stage, the greater the expression of short-day associated characteristics observed in the fifth nymphal instar and adult stage. Melatonin orally administered (500 µg/ ml) to insects under LD 16:8 produced slightly reduced body size, suppressed feeding, extended nymphal period and increased accumulation of lipid compared to the untreated bugs, but did not affect the incidence of diapause in females. In males, melatonin retarded gonadal development under LD16:8. These results may suggest a possible role of melatonin for the control of seasonal polyphenism and development.

Key words: heteroptera, polyphenism, photoperiod, melatonin, diapause

INTRODUCTION

In the seasonal polyphenism, the choice of a particular phenotype out of several alternatives (discrete or graded character) depends on endocrine switch mechanisms which are turned on and off by environmental factors such as photoperiod, temperature and nutritional conditions (Hardie and Lees, 1985; Nijhout, 1994; Numata and Kobayashi, 1994). Although morphological, behavioral and developmental polyphenism may rely on the same environmental cue in some species, this relation may vary in others (Numata and Kobayashi, 1994; Kobayashi and Numata, 1995).

Halyomorpha halys (Stal, 1855) (previously called *brevis*) is a pentatomid bug that overwinters in reproductive diapause and believed to be univoltine in Japan (Watanabe, 1979). Both reproduction and pigmentation on the sternum in adults are controlled by photoperiod and temperature (Niva and Takeda, 2002). Environmental influences on the nymphal body color was suspected, since the coloration of the dorsum of fifth instar short-day-reared nymphs were apparently darker than long-day-reared nymphs and the color patterns of the former were distinct from the latter.

The present investigation attempts to elucidate whether photoperiod and temperature influence the color pattern of fifth instar nymphs of *H. halys* and also whether melatonin is involved in this determination, since it is known that melatonin promoted the appearance of short-day forms in the pea aphid (Gao and Hardie, 1997), influenced reproduction in *Pyrrhocoris apterus* (Hodkova, 1989), stimulated a PTTH release in *Periplaneta americana* (Richter *et al.*, 2000) and affected the circadian oscillation in *Acheta domesticus* (Yamano *et al.*, 2001). Furthermore, this amine was so named that it affects melanization in the skin of frog tadpole (Binkley *et al.,* 1988).

MATERIAL AND METHODS

Stock culture of insects

Adults of *H. halys* were collected in Hyogo Pref. (34°40'N; 135°15'E; late autumn – early winter, 1999–2001) and Kyoto Pref.

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(35°1.6'N; 135°47'E; late autumn-early winter, 2001), Japan, and the offspring was reared thereafter as laboratory stocks. The stock cultures were kept under LD16:8 at 25°C. Nymphs fed on peeled sunflower seeds and peanuts, drank water containing 0.05% ascorbic acid and 0.025% L-cysteine from wetted kimwipe (Kimbery-Clark, Co) or cotton.

Environmental influence

Color patterns of fifth instar nymphs and reproductive status of adults (Hyogo culture) were observed under LD16:8 both at 25°C and 20°C and LD11:13 at 20°C. Comparisons were made for parameters described in the following section (Fig. 1e). The adults grown under these conditions were dissected 15–16 days after eclosion and the percentage of diapausing individuals was computed. Unlike reproductively active adults grown under long-days, adults grown under short-days are characterized by undeveloped oocytes in females and not noticeable ectodermal sac in males. Diapause was recognized by undeveloped gonads upon dissection at day 15–16 after emergence (Niva and Takeda, 2002).

Environmental influence was also investigated during the nymphal period by transferring nymphs from LD16:8 at 25°C to LD11:13 at 20°C at ecdysis to the second, fourth or fifth instars. Last (fifth) instar nymphs were photographed as described below. Upon adult emergence, they were kept at LD16:8 at 25°C until days 15–16 when their reproductive status was checked by dissection.

Pigmentation and the digital images

Bugs were photographed with a digital microscope (VH-6300–
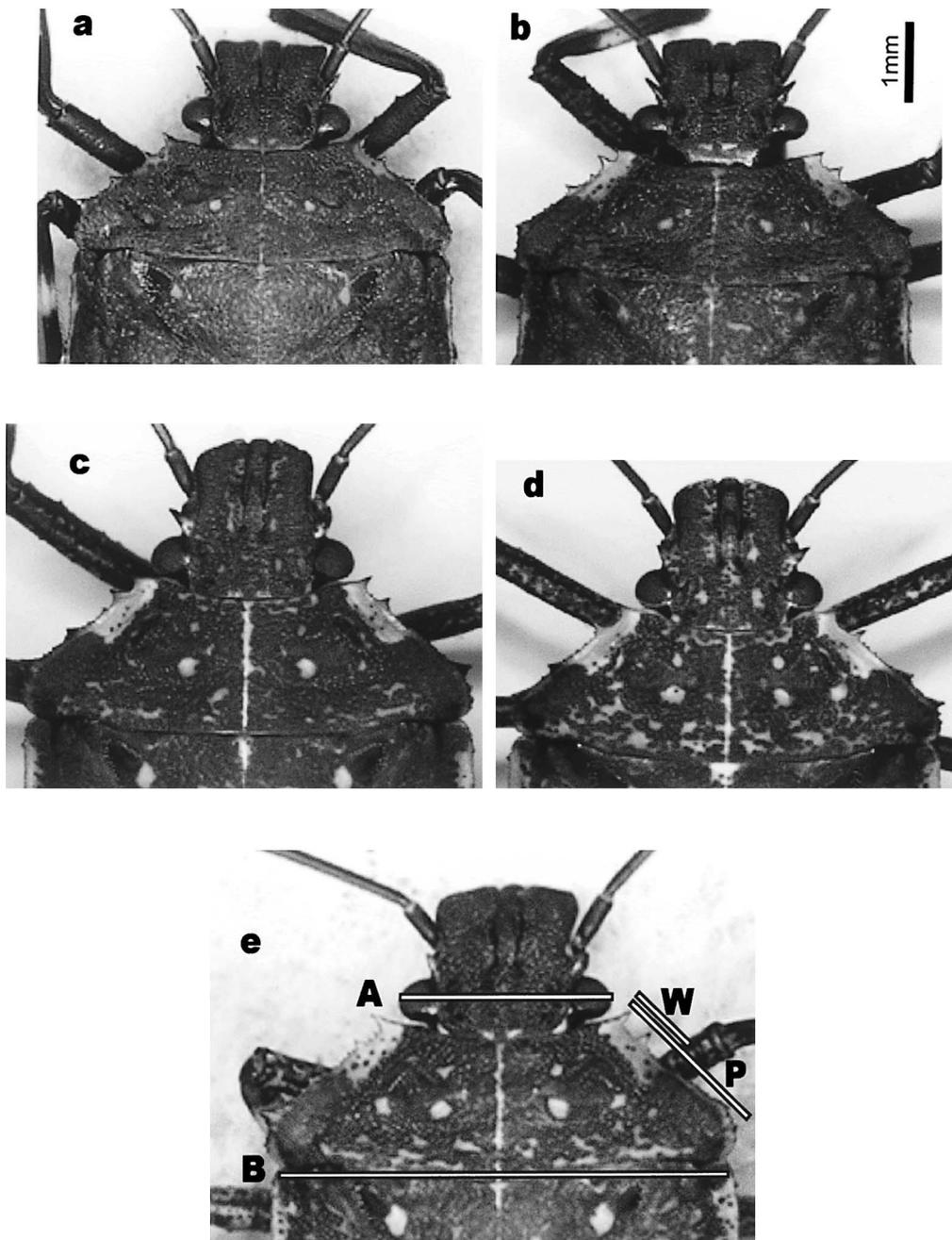
Fig. 1. Dorsal aspects of fifth instar nymphs of *H. halys* showing different patterns (a–d) of pronotum color pattern. Abbreviations of body size parameters (e): W, length of white stripe; P, length of lateral border pronotum; A, head width; B, thorax width.

Keyence) 5 or 6 days after moulting, when the sclerotization and melanization processes were complete and the color and texture of the cuticle were stabilized. The magnification (200×) and light intensity were fixed at a constant value for each picture. Two parameters for the color pattern were used for evaluation at the fifth instar (the Kyoto culture). The length of white stripe (W) along the lateral edge of pronotum and the length of the edge itself (P) were measured at the base of laterally projecting spines. The ratios W/P were compared between treatments (Fig. 1e). Creamy-yellowish speckles were spread over the dark-brownish pronotum in a marbled pattern. Both, white stripes and yellowish speckles were observed through the transparent cuticle. The dark-brown pigmentation was present in other areas of the cuticle. A relative amount of these yellowish speckles was measured as a digital image by using Adobe Photoshop 6.0 (software). The digital images had 752×480 pixels of size, 72 pixels/ inch of resolution and 24 bit color of luminous intensity level. A binary image of the pronotal region was then obtained and the thresholding value decided by referring a histogram of luminous intensity in the region. An average value of the luminous intensity (LI) inside the binary image, that is automatically calculated and displayed, was considered to express the amount of yellowish speckles on the pronotum marbled pattern and thus used for comparisons. The marbled pattern was analyzed only in insects used for the melatonin experiment.

Effects of melatonin treatment

First instar nymphs of Kyoto culture reared under LD16:8 or 12:12 at 25°C were orally administered with melatonin. Preliminary study using 200 μg/ml did not show significant difference in reproduction between the treated and untreated groups reared under LD16:8 at 25°C. Also, as Yamano *et al.* (2001) and Gao and Hardie (1997) showed that orally administered melatonin exerted stronger influence at higher concentrations and as in humans also higher doses were required for melatonin therapy (DeMuro *et al.*, 2000), we decided to use 500 μg/ml melatonin. Ethanol at a final concentration of less than 0.5% was used to dissolve melatonin. Control groups were provided with no melatonin or ethanol. Nymphs reaching the fifth instar were separated and photographed as described above and the resulting adults were dissected 16–17 days after adult emergence to examine the development of the gonads.

The effects of melatonin on the body size, duration of nymphal stage, mortality and feeding frequency were also observed. Parameters of body size are based on the maximum measurement of head width (including the compound eyes), the maximum span of the base of the pronotum (Fig. 1e) and the area of pronotum that were calculated from the digital pictures. Feeding frequency was observed by exposing them to half-peeled peanut to fifth instar nymphs for two consecutive days after moulting. The stylet sheaths left on half-peeled peanut were stained by immersing the peanut in 5% fuchsin solution for one hour (Bowling, 1979; Panizzi *et al.,* 1995). Under a fluorescent microscope with a filter G (Leica MZF1III) the stylet sheaths glowed and their number was easily determined. The lipid content of whole body including gonads was measured according to Morita *et al.*(1999).

RESULTS

Effects of photoperiod and temperature on the white stripes of nymphal pronotum, adult pigmentation and reproduction

The results from three temperature/ photoperiod combinations produced a positive correlation between W/P ratio in fifth instar nymphs and percentages of diapause in adult stage (Table 1). The average W/P ratio was smaller in nymphs reared under LD11:13 at 20°C than those reared under LD16:8 at 25°C or LD16:8 at 20°C. Different temperatures, 25°C and 20°C, also produced a significant difference. More diapause adults were obtained under LD 11:13 than 16:8. Photoperiod and temperature decisively affected the size of white stripe of the pronotum in nymphs and percentage of diapause in adults.

The effect of changes in environmental conditions during the nymphal stage on W/P ratio was also evaluated. Table 2 shows that nymphs exposed to short-day-low-temperature conditions at earlier developmental stages tended

Table 1. Correlation between the ratio of white stripe to length of pronotum lateral edge (W/P) of fifth instar nymphs and percentage of diapause in groups of *H. halys* reared under LD16:8 at 25°C, LD16:8 at 20°C and LD11:13 at 20°C.

Attributes	Treatments		
	LD16:8 at 25°C	LD16:8 at 20°C	LD11:13 at 20°C
Ratio W/ P	0.54±0.05 (20) a*	0.43±0.07 (28) b	0.35±0.04 (28) c
Range of W/ P	0.4–0.7	0.3–0.6	0.2–0.4
Diapausing adults	5%	38%	100%

* Different letters show significant difference between treatments (ANOVA, Fisher's PLSD; $p < 0.0001$)

Table 2. Correlation between the ratio of white stripe to length of pronotum lateral edge (W/P) of fifth instar nymphs and percentage of diapause in groups of *H. halys* nymphs transferred from LD16:8 at 25°C to LD11:13 at 20°C at specific instars.

Attributes	Incubation period under 20°C and LD11:13		
	2nd–5th instars	4th–5th instars	5th instar
Ratio W/P	0.43±0.06 (16) a*	0.49±0.05 (44) b	0.52±0.04 (39) c
Range of W/P	0.3–0.5	0.4–0.6	0.4–0.6
Diapausing adults	100%	82%	85%

* Different letters show significant difference between treatments (ANOVA, Fisher's PLSD; $p < 0.024$)
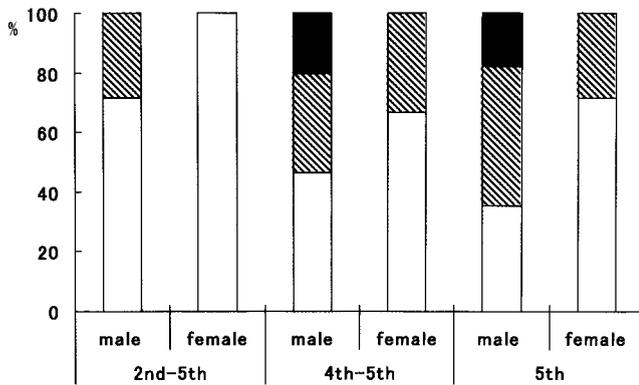
Fig. 2. Frequency of color types in the sternum of adults (%) exposed to LD16:8 at 25°C and then to LD11:13 at 20°C. Abscissa shows the nymphal instars exposed to LD11:13 at 20°C. Open bars: ivory-faintly-red sternum; Shadowed bars: light red sternum; Filled bars: red sternum. Difference between treatments in male (Contingency table test; chi-square= 13, df= 4, p<0.05) was significant but not in female (Contingency table test; chi-square= 9.49, df=4, p>0.05).

to have a smaller W/P ratio and had a greater probability to enter diapause. The percentages of diapausing adults were similar between nymphs transferred to LD11:13 at 20°C at the fourth instar and those transferred at the fifth instar, but 100% of those transferred at the second instar entered diapause.

The color of adult sternum was also affected by photoperiod and temperature during the nymphal period. Nymphs exposed to LD11:13 at 20°C during fourth-fifth and fifth instars, respectively, and otherwise kept at LD 16:8 at 25°C

produced similar proportions of red adults and ivory adults, whereas a longer exposure to the same conditions from the second to the fifth instars reduced the proportion of red adults, both in males and females, with a significant reduction detected only in males (Fig. 2). The exposure to LD11:13 at 20°C only after the adult emergence produced a lower proportion (27%) of diapause insects than the groups exposed to short-days (80–100%) at nymphal stage also. Exposure to short-day/ low temperature conditions during the adult stage only was not enough to induce a maximum rate of reproductive diapause.

Effects of photoperiod and melatonin on color pattern on the pronotum and body size of fifth instar nymphs

Nymphs reared under LD12:12 at 25°C had a luminous intensity value (LI) significantly smaller than the value for those reared under LD16:8 at 25°C (Table 3), i.e., the pronotum of short-day nymphs had a marbled pattern with significantly less or smaller proportion of yellowish speckles than that of long-day nymphs. The size of white stripe in the fifth instar nymphs also differed significantly between these treatments (Table 3). However, melatonin drinking did not significantly change the size of the white stripe in LI of the marbled pattern (Table 3).

The head width, thorax width and area of pronotum, showed parallel changes (Table 4). Short-day reduced these parameters significantly compared to long-day. Melatonin treatment under long-day conditions, however, slightly reduced the nymphal body size to levels similar to short-day insects (with or without melatonin) in all parameters considered, though the differences were not always statistically

Table 3. Length of white stripe (W), lateral span of the pronotum (P), ratio W/P and luminous intensity value (LI) of 5th instar pronotum in *H. halys* reared under LD16:8 and LD12:12 at 25°C with or without melatonin in drinking water.

Attributes	Control		Melatonin (500 µg/ml)	
	LD16:8 (N)	LD12:12	LD16:8	LD12:12
W (mm)	0.90±0.09 (97) a*	0.73±0.07 (109) b	0.87±0.08 (39) a	0.72±0.11 (33) b
P (mm)	1.82±0.13 (97) a	1.64±0.11 (109) b	1.75±0.12 (39) ac	1.63±0.15 (33) bc
W/P ratio	0.49±0.04 (97) a	0.44±0.04 (109) b	0.50±0.03 (39) a	0.44±0.04 (33) b
LI value	34.50±6.73 (97) a	25.60±4.54 (109) b	34.40±8.07 (39) a	23.00±5.72 (33) b

* Different letters show significant difference between treatments (ANOVA, Fisher's PLSD; p<0.0001)

Table 4. Head width, thorax width and pronotum area of 5th instar nymphs in *H. halys* reared under LD16:8 and LD12:12 at 25°C with or without melatonin in drinking water.

Attributes	Control		Melatonin (500 µg/ml)	
	LD16:8 (N)	LD12:12	LD16:8	LD12:12
Head width (mm)	2.67±0.13 (97) a*	2.51±0.14 (109) bc	2.60±0.12 (39) ac	2.45±0.17 (33) b
Thorax width (mm)	5.58±0.36 (97) a	5.12±0.28 (109) b	5.37±0.35 (39) ac	5.14±0.42 (33) bc
Pronotum area (mm^2)	6.98±1.37 (97) a	5.73±0.48 (109) b	6.17±0.78 (39) ac	5.64±0.94 (33) bc

* Different letters show significant difference between treatments (ANOVA, Fisher's PLSD; p<0.0001).

significant.

Effects of photoperiod and melatonin on the duration of nymphal development, feeding frequency and mortality

The total duration of nymphal development was significantly shorter in control insects reared under LD12:12 than under LD16:8 at 25°C, showing a response of short-day type. Melatonin treatment prolonged nymphal development by ca. 7 days compared to control under LD16:8 at 25°C. Duration of nymphal development under short-day conditions did not differ between the treated and control groups (Table 5).

The number of stylet sheaths left on peanuts during the first two days of the fifth instar showed a large individual variation ranging from 0 to 40. A significantly higher number of stylet sheaths was observed under LD16:8 at 25°C than under LD12:12 at 25°C in control groups. Although melatonin tended to suppress feeding under LD16:8 at 25°C, no significant difference was observed between the melatonin-treated and the control under both photoperiods (Table 5).

The mortality during nymphal stage was very high in melatonin-treated groups, reaching almost 80% under LD12:12 at 25°C, suggesting a toxic effect of the used dosage (Table 5). Mortality under long-day and short-day controls did not reach 40% differing only by ca.10%.

Effect of photoperiod and melatonin on the development of gonads, adult pigmentation, size and fat content

As Niva and Takeda (2002) previously observed, adults of *H. halys* showed a long-day type photoperiodic response for development of gonads, where insects reared under LD16:8 at 25°C continued reproduction while the ones reared under LD12:12 at 25°C entered reproductive diapause (Table 6). Melatonin treatment affected gonad development under LD16:8 at 25°C significantly only in males (Table 6). The ectodermal sac showed some degrees of development, significantly smaller in the melatonin-treated than in the control under LD16:8 at 25°C, though, it was wider than that of diapausing males reared under LD12:12 at 25°C. In females, on the other hand, melatonin treatment

Table 5. Feeding frequency, duration of nymphal period (egg-adult) and mortality of 5th instar nymphs in *H. halys* reared under LD16:8 and LD12:12 at 25°C with or without melatonin in drinking water.

Attributes	Control		Melatonin (500 µg/ml)	
	LD16:8 (N)	LD12:12	LD16:8	LD12:12
Stylet sheaths*	8.89±5.5 (114) a**	7.3 ±5.3 (111) b	6.88±5.3 (41) ab	7.2 ±7.1 (44) ab
Nymphal duration (days)	43.40±3.0 (97) a	38.30±2.2 (109) b	49.80±3.4 (39) c	36.10±3.8 (33) b
Mortality	35.4% (206)	26.5% (185)	62.9% (143)	77.6% (161)

* Number of stylet sheaths deposited on peanuts on the first two days of fifth instar
** Different letters show significant difference between treatments (ANOVA, Fisher's PLSD; p<0.0001)

Table 6. Length of ovariole and width of the ectodermal sac of *H. halys* adults reared under LD16:8 and LD12:12 at 25°C with or without melatonin in drinking water 15–16 days after adult emergence.

Attributes	Control		Melatonin (500 µg/ml)	
	LD16:8(N)	LD12:12	LD16:8	LD12:12
Ovariole length (mm)	4.98±1.05 (27) a*	0.55±0.04 (24) b	4.79±1.29 (21) a	0.57±0.05 (13) b
Ectodermal sac width (mm)	4.08±0.54 (34) a**	0.55±0.55 (31) c	3.37±1.23 (18) b	0.56±0.04 (15) c

* different letters show significant difference between treatments (ANOVA, Fisher's PLSD; p<0.0001)
** different letters show significant difference between treatments (ANOVA, Fisher's PLSD; p<0.0002)

Table 7. Head width, pronotum width and total lipid content of *H. halys* adults reared under LD16:8 and LD12:12 at 25°C with or without melatonin in drinking water.

Attributes	Sex**	Control		Melatonin (500 µg/ml)	
		25°C/LD16:8(N)	25°C/LD12:12	25°C/LD16:8	25°C/LD12:12
Head width (mm)	M	2.94 ±0.1 (13) a*	2.81 ±0.1 (20) b	2.83 ±0.2 (21) b	2.74 ±0.1 (12) b
	F	3.14 ±0.1 (14) a	2.96 ±0.1 (22) b	3.08 ±0.1 (20) ac	3.03 ±0.1 (12) bc
Pronotum width (mm)	M	7.60 ±0.2 a	7.30 ±0.2 b	7.57 ±0.3 a	7.19 ±0.5 b
	F	8.65 ±0.3 a	7.97 ±0.4 b	8.54 ±0.4 a	8.13 ±0.4 bc
Lipid content (mg)/ g dry weight	M	0.231±0.1 (5) a	0.406±0.01 (4) d	0.321±0.03 (5) b	0.390±0.00 (3) cd
	F	0.203±0.1 (4) a	0.379±0.02 (4) b	0.317±0.1 (3) b	0.396±0.02 (3) b

* different letters show significant difference between treatments (ANOVA, Fisher's PLSD; p<0.0322)
** M: males; F: females

under LD16:8 at 25°C did not significantly affect the length of ovariole compared to the untreated controls. Under LD12:12, females and males were all in diapause and no difference was observed in the development of gonads between the melatonin-treated and the control (Table 6). Though the diapause state was not clearly affected by the melatonin treatment, the frequency of red bugs, which is characteristically higher in non-diapause adults than in diapause ones (Niva and Takeda, 2002), was reduced by melatonin treatment (Fig. 3).

The head width and pronotum width of diapause adults reared under LD12:12 at 25°C were smaller than non-diapausing ones. Melatonin treatment slightly reduced the head width when compared to the controls reared under long-day conditions, but the head width were never smaller than the ones of insects reared under short-day (Table 7). Fat content was significantly higher in short-day-reared insects. Melatonin treatment under long-day significantly elevated lipid accumulation, but did not surpass the levels of short-day treatments (Table 7).

DISCUSSION

Pigmentation, body size, feeding frequency, developmental rate and lipid accumulation at nymphal stage of *H. halys* are under photoperiodic control. Short-day-reared diapause-destined fifth instar nymphs had shorter stripes of the pronotum, smaller LI value of the pronotum, smaller body size, less feeding frequency and shorter nymphal period than long-day-reared ones. Effects of photoperiod and temperature on seasonal polyphenism and diapause have been reported for other heteropterans. In *Euschistus tristigmus*, for example, photoperiod influenced the body shape and color (MacPherson, 1974). Nymphs of *Plautia stali* and adults of *Riptortus clavatus* were darker when reared under short-days compared to those reared under long-days (Numata and Kobayashi, 1994; Kobayashi and Numata, 1995). Furthermore, in *R. clavatus*, the coloration of lateral metathorax was influenced by photoperiod and ventral metathorax by temperature (Kobayashi and Numata, 1995). In *Podisus maculiventris* and *Perillus bioculatus,* melanization in adults was strongly influenced by temperature condition during the fifth instar (Aldrich, 1986; Knight, 1924). Although the present data cannot conclude which specific instar(s) is the most important for the determination of color pattern of fifth instar nymphs or adults, it appears that the longer the exposure was to short-day/ low temperature, the smaller the size of white stripe in fifth instar nymphs and the frequency of adults with a red sternum became (Fig. 2).

The effect of photoperiod on reproduction in *H. halys* has already been investigated (Watanabe, 1979), but short-day treatment was not given at low temperature (LD 12:12 at 25°C) as in the present report. Bugs exposed to low-temperature with short-days (LD11:13 at 20°C) from the second through the fifth instars and otherwise kept at high temperature/ long-days had a higher percentage of diapausing adults (100 versus 75%) than Watanabe's experiment (Fig. 3). On the other hand, the exposure to low temperature/ short days only during adult stage resulted in a smaller percentage diapause (17 versus 43%). Both studies show that photoperiodic condition during the nymphal stage, especially on the last two nymphal instars, is crucial for determination of the reproductive status in adults. Low temperature, especially on the fifth instar, seems to have a strong synergistic effect on the induction of diapause.
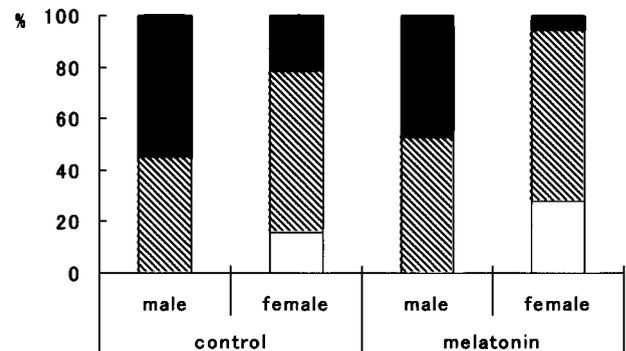
Fig. 3. Frequency of color types in the sternum of adults (%) reared under LD16:8 at 25°C with or without melatonin treatment (500 μg/ ml). Open bars: ivory-faintly-red sternum; Shadowed bars: light red sternum; Black bars: red sternum. Difference was significant between treatments for each sex (Contingency table test; chi-square= 4, df=2, p<0.05).

Photoperiod is known to control nymphal development in insects. In most cases, long-day induces fast development and short-day, slow development (Danilevskii, 1965). However, some insects are known to have an opposite photoperiodic reaction (Masaki and Ohmachi, 1967; Masaki and Sugahara, 1992; Musolin and Saulich, 1997). This type of photoperiodic response of development, observed also in nymphs in the present investigation, is termed short-day type (Danilevskii, 1965) and considered to be an adaptation for the maintenance of univoltinism (Masaki and Ohmachi, 1967). Physiological significances of this type of photoperiodic response in *H. halys* may be to reinforce univoltinism. Long-days would prevent early adult emergence which may lead to a precocious oviposition in early fall. This bug has a long-day type of photoperiodic response for reproduction. Therefore, short days prevailing in the fall will help suppress the reproductive activity in late fall. The high sensitivity during the last nymphal stage may be to guarantee the success of this type of life cycle.

Melatonin is synthesized during the dark phase of LD cycle in vertebrates having seasonal reproductive cycles and it is involved in the measurement of the night length (see Arendt, 1998 for references). Melatonin treatment is, thus, expected to mimic dark conditions and produce short-day effects. Although melatonin is known to be present in insects (Vivien-Roels *et al.*, 1984; Itoh *et al.*, 1994) and playing some physiological roles (Hodkova, 1989; Gao and Hardie, 1997; Richter *et al.*, 2000; Yamano *et al.*, 2001), the

short-day mimicking effect was observed only in aphids (Gao and Hardie, 1997). In *P. apterus* the onset of oviposition was delayed by melatonin treatment (Hodkova, 1989). In males of *H. halys*, melatonin treatment caused an apparent delay in the development of ectodermal sacs. Also, fewer individuals with red colored sternum were observed under long-day condition when treated with melatonin, also suggesting a slight delay in maturation caused by melatonin. The current study failed to show melatonin influence in female reproduction. One possible explanation is that maybe melatonin does not affect the development of female gonads, or the timing of the dissections was not appropriate to detect differences. Another possible explanation is that melatonin affects physiological routes not directly involved in the reproduction of *H. halys* females.

The high lipid content, typical of diapause adults (Morita *et al.*, 1999), long nymphal development and slightly decreased feeding activity found in melatonin-treated *H. halys* even under long-day conditions suggest that melatonin may be involved in several physiological processes. However, a toxic effect of melatonin given by the high mortality in the treated groups, cannot be denied. Considering that lipid accumulation in *R. clavatus* is not mediated by juvenile hormone (Morita *et al.*, 1999), melatonin could be a likely candidate involved in the control of these diapause-associated characteristics, via still unknown mechanisms. Interestingly, Hodkova (1994), using surgical approaches, reported that a factor produced in the brain not acting through the corpora allata controlled male reproductive characteristics. The fact that melatonin reduced the rate of nymphal development under LD 16:8 suggests that melatonin may play a role also in photoperiodic responses not linked to diapause. In the brain and gonads of *H. halys*, melatonin and its key enzyme, *N*-acetyltransferase were detected by radioimmunossay and positive immunoreactivity for anti-melatonin sera was found in many cells of the brain (unpublished data). These data support the notion that melatonin might be involved in reproductive and/ or developmental functions.

ACKNOWLEDGEMENTS

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