Photoperiodic and temperature control of diapause induction and colour change in the southern green stink bug *Nezara viridula*

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Abstract. The effect of photoperiod and temperature on the duration of the nymphal period, diapause induction and colour change in adults of *Nezara viridula* (L.) (Heteroptera: Pentatomidae) from Japan was studied in the laboratory. At 20 °C, the developmental period for nymphs was significantly shorter under LD 10 : 14 h (short day) and LD 16 : 8 h (long day) than under intermediate photoperiods, whereas at 25 °C it was slightly shorter under intermediate than short- and long-day conditions. It is assumed that photoperiod-mediated acceleration of nymphal growth takes place in autumn when day-length is short and it is unlikely that nymphal development is affected by day-length under summer long-day and hot conditions. *Nezara viridula* has an adult diapause controlled by a long-day photoperiodic response. At 20 °C and 25 °C in both sexes, photoperiodic responses were similar and had thresholds close to 12.5 h, thus suggesting that the response is thermostable within this range of temperatures and day-length plays a leading role in diapause induction. Precopulation and preoviposition periods were significantly longer under near-critical regimes than under long-day ones. Short-day and near-critical photoperiods induced a gradual change of adult colour from green to brown/russet. The rate of colour change was significantly higher under LD 10 : 14 h than under LD 13 : 11 h, suggesting that the colour change is strongly associated with diapause induction. The incidences of diapause or dark colour did not vary among genetically determined colour morphs, indicating that these morphs have a similar tendency to enter diapause and change colour in response to short-day conditions.

Key words. Diapause, Heteroptera, life cycle, *Nezara viridula*, photoperiodic response, photoperiodism, polymorphism, polyphenism, seasonal adaptations, seasonality.

Introduction

To understand insect life cycle regulation, it is important to examine species-specific relationships between physiological and morphological traits involved in diapause syndrome. Owing to its environmentally controlled colour poly-

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phenism, the southern green stink bug *Nezara viridula* (L.) (Heteroptera: Pentatomidae) is suitable for studying such relationships. *Nezara viridula* occurs throughout tropical and subtropical regions of the world (Panizzi *et al.*, 2000). In Japan, the species has long been known from the southern part (Kiritani, 1971), but recently adults were observed in Osaka, central Japan (unpublished observations), at least 70 km further north than the northern limit of its distribution reported in the early 1960s (Kiritani *et al.*, 1963). *Nezara viridula* can produce up to five or six generations per year, although the number of generations in a particular

region may be limited by the availability and phenology of preferred food plants (Velasco *et al.*, 1995; Panizzi *et al.*, 2000). In Wakayama Prefecture, central Japan, *N. viridula* is reported to have three generations with a partial fourth (Kiritani *et al.*, 1963).

Similar to many other heteropteran species in the temperate zone, the adults of *N. viridula* overwinter under litter, bark or other shelter, apparently in a reproductive diapause (Kiritani *et al.*, 1966; Jones & Sullivan, 1981), although in southern Brazil the species does not enter diapause but moves to alternative host plants and eventually reproduces during the mild winter (Panizzi & Hirose, 1995). The induction of the reproductive diapause in the temperate populations is controlled by photoperiod (Ali & Ewiess, 1977; Harris *et al.*, 1984; Seymour & Bowman, 1994).

Nezara viridula has attracted much attention because of its conspicuous colour polymorphism. Ten genetically determined colour morphs of adults are recognized and four of them are dominant in Japan (Yukawa & Kiritani, 1965; Hokkanen, 1986; Ohno & Alam, 1992). This species also shows a pronounced seasonal polyphenism: during the warm months adults are green, whereas in autumn and winter they are typically brown or russet (Harris *et al.*, 1984; Seymour & Bowman, 1994).

Reports on adult diapause and seasonal colour polyphenism in *N. viridula* are sometimes contradictory. Thus, it has been claimed that the males overwinter in a state of quiescence rather than diapause (Kiritani, 1963; Kiritani *et al.*, 1966), that one of the morphs is controlled by an environmental rather than a genetic factor (Takizawa, 1994) and that diapause is primarily an adaptation for regulating the life cycle rather than a mechanism to survive the cold season (Elsey, 1993). Also, it has been disputed whether the russet colour is a reliable indicator of diapause or not (Harris *et al.*, 1984; Seymour & Bowman, 1994; Jones & Westcot, 2002).

The objective of this study was to examine the role of daylength and temperature in the induction of adult diapause and colour changes in both sexes of *N. viridula*. In addition, the effect of day-length was examined on the duration of nymphal period, precopulation and preoviposition periods, dynamics of colour change, parameters of reproduction, as well as differences between colour morphs of *N. viridula* in relation to diapause induction and colour changes.

Materials and methods

Insects and the rearing procedure

More than 40 adults of *N. viridula* were collected in and around an experimental soybean field on the campus of Osaka City University (Japan, 34.7°N; 135.5°E) from August 1998 to September 2000. Adults were transferred to the laboratory to establish a stock culture and reared in transparent plastic containers (diameter 150 mm, depth 90 mm; lids with openings 60 mm in diameter for aeration covered with mesh) under LD 16 : 8 h at 25 °C. They were supplied with dry soybean *Glycine max* (L.) Merrill grains,

shelled raw peanuts *Arachis hypogaea* L. and water containing 0.05% sodium L-ascorbate and 0.025% L-cystein (Noda, 1991). Each egg mass laid in the stock culture was divided into two to three parts, each of which was used in subsequent experiments in different regimes to provide a higher heterogeneity of the experimental material.

The eggs were transferred to smaller transparent plastic containers (diameter 90 mm, depth 45 mm; lids with openings 45 mm in diameter for aeration covered with mesh) under different constant photoperiodic conditions ranging from LD 10 : 14 h to LD 18 : 6 h at 20 and 25 ± 1 °C. Upon hatching, nymphs, and subsequently adults, were provided with the same diet as the stock culture. Food and water were replaced every other day. The density of nymphs was kept at 40–80 per container for the first and second instars and was then gradually reduced to five to six per container by the final (fifth) nymphal instar. Nymphal ecdysis to the fifth instar and adult emergence were checked daily and recorded individually.

After adult emergence, colour morph was recorded in each individual. Female-male pairs were transferred to a new container (diameter 90 mm, depth 45 mm) and kept as described above for a further 60 days under the same photoperiodic and temperature conditions. Start of copulation, days of oviposition, size of egg masses, changes of colour and mortality were recorded daily. Three colour grades were used: green, intermediate (approximately 40–60% of the body surface is light russet) and russet.

Diapause status was judged by the criteria used for some other pentatomids (Nakamura & Numata, 1997). Adults were dissected 60 days after emergence and females without mature eggs or vitellogenic oocytes in the ovarioles, and males without secretory fluids in the ectodermal sacs of the accessory glands were considered to be in diapause.

Because of a large variation of photoperiodic response under near-critical photoperiods at 25 °C (see below), the whole experiment under these conditions was performed in three replicates and the results were combined.

Statistical analysis

The duration of the nymphal period was statistically examined by the *t*-test and Tukey multiple comparison test (Zar, 1999); parameters of reproduction and colour by the Kruskal–Wallis test (Zar, 1999) followed by the Steel–Dwass test (Excel Tokei, version 5.0, Esumi Ltd, Tokyo, Japan); and comparison of proportions by the chi-square test (Zar, 1999).

Results

Effect of day-length on duration of nymphal period

When compared within each regime, the duration of the total nymphal period did not differ significantly between the sexes under most conditions (Table 1). However, different

Table 1. Duration of nymphal period in *Nezara viridula* under laboratory conditions.

Conditions	Nymphal instars (20 °C)				Nymphal instars (25 °C)			
	n	1st to 4th	5th	Total	n	1st to 4th	5th	Total
Females								
LD 10:14 h	60	33.0 ± 3.4^a	15.1 ± 1.3^a	48.1 ± 4.3^a	41	19.7 ± 1.6^{ab}	7.4 ± 0.6^a	27.2 ± 2.0^a
LD 12:12 h	29	39.3 ± 2.9^b	15.2 ± 0.8^{ab}	54.5 ± 3.5^{bc}	151	20.0 ± 2.0^b	8.4 ± 0.7^b	28.3 ± 2.2^b
LD 13:11 h	41	39.3 ± 3.5^b	16.7 ± 1.1^c	56.0 ± 3.9^b	209	18.2 ± 2.1^{cd}	8.3 ± 1.0^b	26.5 ± 2.3^a
LD 14:10 h	63	38.1 ± 2.8^b	15.8 ± 1.2^b	$54.0 \pm 3.5^{bc*}$	67	17.8 ± 1.7^d	7.6 ± 0.7^a	25.4 ± 1.8^c
LD 15:9 h	34	37.4 ± 3.0^b	15.0 ± 1.1^a	52.4 ± 3.5^c	81	18.9 ± 2.0^{ac}	7.6 ± 0.7^a	$26.5 \pm 2.2^{a*}$
LD 16:8 h	48	30.7 ± 2.3^c	13.0 ± 0.7^d	43.7 ± 2.8^d	53	19.3 ± 1.9^{ab}	7.7 ± 0.7^a	$27.0 \pm 2.0^{a*}$
LD 18:6 h					46	21.3 ± 1.8^e	7.8 ± 0.6^a	29.1 ± 2.0^b
Males								
LD 10:14 h	57	33.9 ± 3.9^a	15.7 ± 2.6^a	49.6 ± 5.9^a	44	19.2 ± 1.1^{ab}	7.4 ± 0.6^{ad}	26.6 ± 1.5^a
LD 12:12 h	42	38.9 ± 3.4^{bc}	15.5 ± 1.2^{ab}	54.4 ± 4.3^{bc}	167	20.0 ± 1.9^a	8.4 ± 1.2^b	28.3 ± 2.4^b
LD 13:11 h	50	39.0 ± 3.8^{bc}	16.2 ± 1.0^a	55.2 ± 4.2^b	221	18.2 ± 2.3^{cd}	8.1 ± 0.9^{bc}	26.4 ± 2.6^a
LD 14:10 h	58	40.3 ± 4.0^b	15.8 ± 1.9^a	56.0 ± 4.8^b	72	17.7 ± 1.4^c	7.8 ± 0.9^{cd}	25.5 ± 1.9^a
LD 15:9 h	49	37.5 ± 2.4^c	14.7 ± 1.1^b	52.2 ± 2.5^c	93	18.3 ± 1.5^{bc}	7.3 ± 0.6^a	25.6 ± 1.8^a
LD 16:8 h	42	31.0 ± 2.6^d	12.5 ± 1.1^c	43.5 ± 3.1^d	67	19.0 ± 1.5^{bd}	7.3 ± 0.6^{ae}	26.3 ± 1.7^a
LD 18:6 h					66	21.2 ± 2.0^e	7.7 ± 1.0^{de}	29.0 ± 2.6^b

Means in days ($\pm$ SD) followed by the same superscript letters within each column and sex are not significantly different ($P > 0.05$, Tukey multiple comparison test). Totals for the nymphal period in females followed by asterisks are significantly different from the values for males under the same conditions ($P < 0.05$, *t*-test).

patterns of influence of day-length on the duration of the nymphal period were observed. At 20 °C, the nymphal period was significantly shorter under short-day (LD 10:14 h) and long-day (LD 16:8 h) conditions than under intermediate photoperiods and the difference in mean values amounted to 12 days. In contrast, at 25 °C, the total nymphal period was shorter under the intermediate photoperiodic conditions than under the short- and long-day conditions, but the difference was significant only for females.

Precopulation and preoviposition periods

Under LD 10:14 h at both temperatures, bugs neither copulated nor laid eggs for 60 days after adult ecdysis. Under the long-day conditions, almost all bugs started to copulate and then laid eggs shortly after the adult moult, but reproduction was delayed under intermediate photoperiods of LD 12:12 h and LD 13:11 h. At both temperatures, the precopulation and preoviposition periods were significantly shorter under long-days than intermediate photoperiods (Fig. 1). Photoperiodic effects on the precopulation and preoviposition periods were more pronounced at the lower temperature (20 °C versus 25 °C).

Diapause induction

Diapause induction in *N. viridula* is controlled by a long-day photoperiodic response: almost all bugs were reproductive under the long-day conditions, whereas those under the short-day conditions were in diapause when dissected 60 days after adult ecdysis (Fig. 2). At both temperatures,

the photoperiodic response curves were similar and the critical day-length for diapause induction fell into a narrow range close to 12.5 h, suggesting that the response is thermo-stable within this range of temperatures and that day-length is a dominant factor in diapause induction. Under near-critical photoperiods (LD 12:12 h and LD 13:11 h), a marked variation in the incidence of diapause was recorded (Fig. 2). Both sexes showed photoperiodic response curves of similar shape, but females exhibited a higher incidence of diapause in most regimes than did males, and this difference was significant at 20 °C under LD 14:10 h and at 25 °C under LD 12:12 h and LD 13:11 h (chi-square test; $P < 0.05$).

Effect of day-length on adult body colour

Adults in all treatments emerged green (Fig. 3). Under long-day conditions, almost all of them remained green during the 60-day observation period. Under the near-critical and short-day photoperiods, adults started to change their body colour soon after ecdysis. The colour change began earlier at 25 °C than at 20 °C and the dynamics of colour change were similar in both sexes. Insects of the intermediate colour grade were always present in the near-critical regimes. Also under these conditions, some adults changed colour more than once (e.g. from green to russet and then back to intermediate or green).

To determine the rates of colour change under different photoperiodic conditions, the period required to reach an intermediate colour grade and then the russet one were calculated (Table 2). Only those adults that finally reached the russet colour grade were used in this analysis.
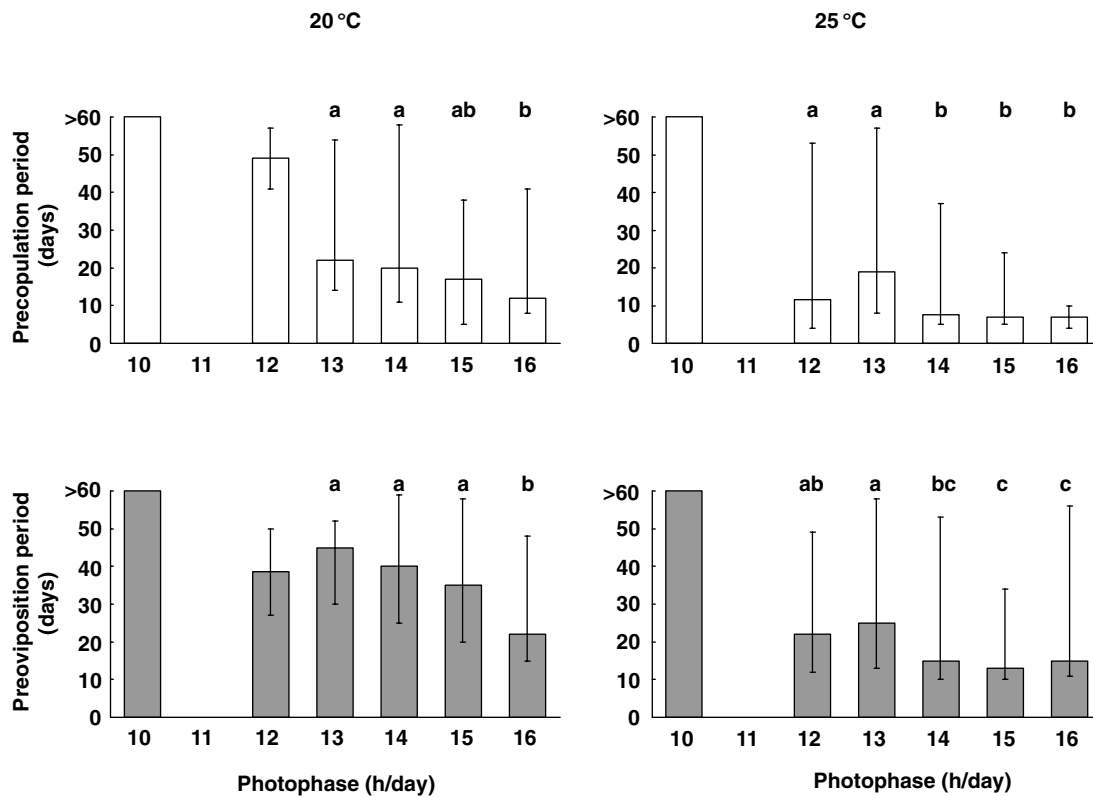
20 °C   **25 °C**

Fig. 1. Effect of day-length on precopulation and preoviposition periods in *Nezara viridula* at 20 °C and 25 °C. Medians (with range) followed by the same letters within each histogram are not significantly different (*P* > 0.05 by the Kruskal–Wallis test followed by the Steel–Dwass test). *n* = 7–36 (at LD 12 : 12 h and 20 °C, only two females copulated and oviposited; data not included in analysis).
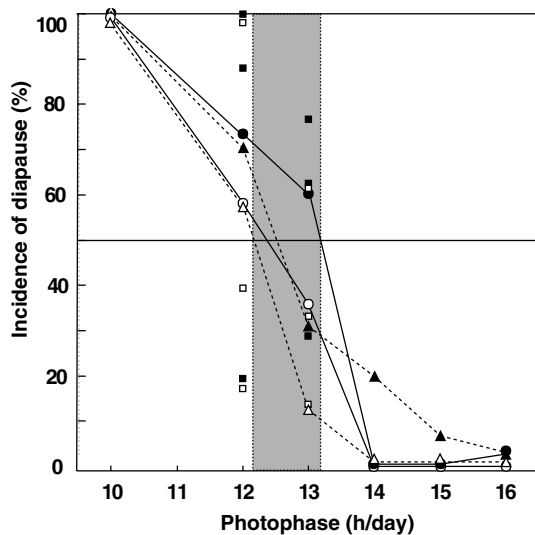

Fig. 2. Photoperiodic response curves for diapause induction in females and males of *Nezara viridula*. Solid line and closed circles, females at 25 °C; solid line and open circles, males at 25 °C; dashed line and closed triangles, females at 20 °C; dashed line and open triangles, males at 20 °C (for LD 12 : 12 h and LD 13 : 11 h at 25 °C, the results of three replications are also shown as closed squares, females; open squares, males). Shaded area shows a range of critical day-length inducing diapause in 50% of individuals. *n* = 27–128.

Adults normally did not change colour under long-day conditions of LD 14 : 10 h to LD 16 : 8 h (Fig. 3). Under the near-critical and short-day conditions where bugs changed colour, the rate of the colour change was variable (Table 2). Although there were slight differences between the two temperatures and sexes, the shorter the photophase, the faster the rate of colour change. These results suggest that the colour change is controlled by photoperiod not only qualitatively (russet versus green colour), but also quantitatively (different rate of colour change under different photoperiods).

The relationship between adult colour and diapause status

On day 60 after adult emergence under short-day conditions (LD 10 : 14 h), all individuals were in diapause and deeply russet at both temperatures whereas almost all adults were not in diapause and green under long-day conditions (LD 14 : 10 h to LD 16 : 8 h) (Figs 2 and 3). Under the near-critical conditions of LD 12 : 12 h and LD 13 : 11 h, the incidence of dark coloured individuals exceeded that of diapause individuals. This difference in incidence was significant in the males at both temperatures and in the females at 20 °C under LD 13 : 11 h (chi-square test; *P* < 0.05). Under the near-critical conditions, all combinations of diapause status and body colour were present (Fig. 4).
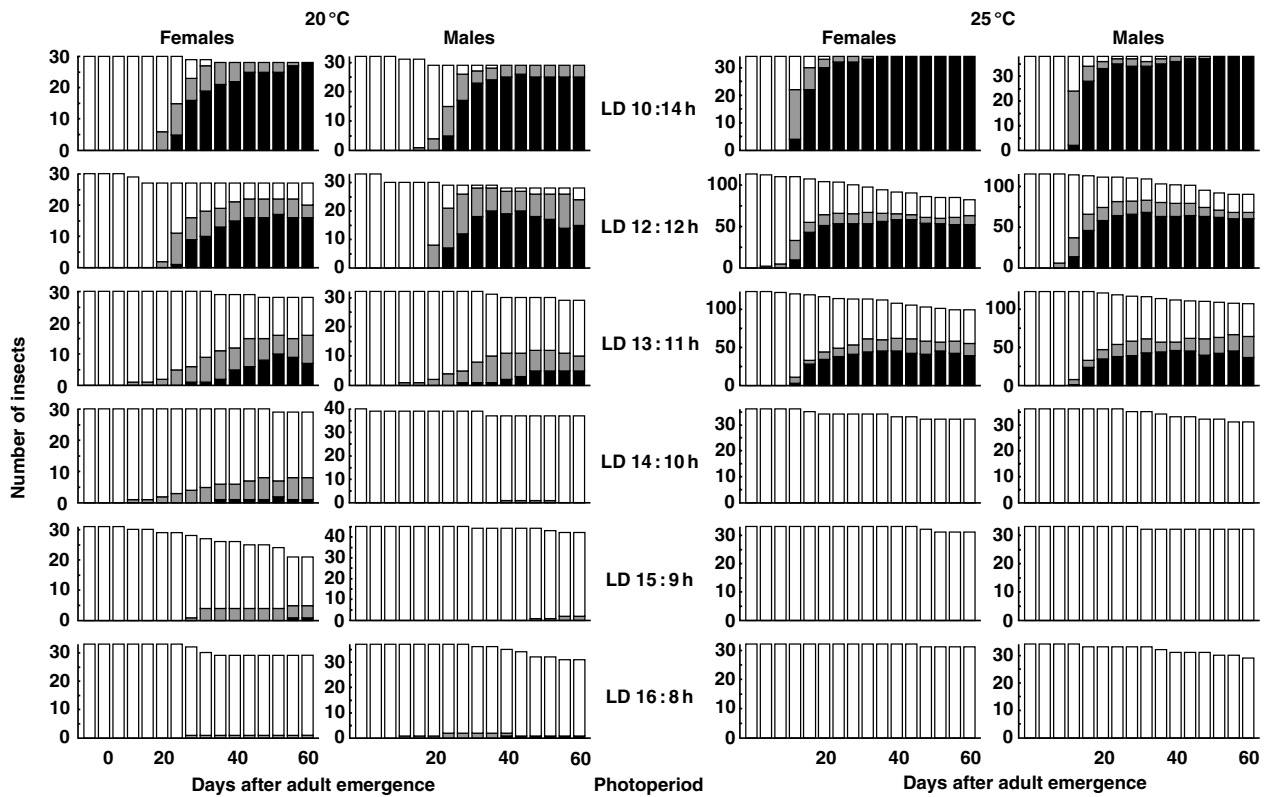
Fig. 3. Effect of day-length on adult body colour in *Nezara viridula* at 20 °C and 25 °C. Open, shaded, and black sections of the bars indicate the proportion of bugs with green, intermediately coloured, and russet bodies, respectively.

In total, 226 pairs copulated within 60 days in all regimes. In 91.2% of the cases when the first copulation was recorded, both sexes were green, and one or both adult(s) had another colour grade in 8.8%. If only females are considered, 96% of individuals were green, 3.5% intermediate and only one female (0.5%) was russet on the day of the first copulation. On the day the first egg mass was laid, 97.3% of females were green and 2.7% were intermediately coloured.

Parameters of reproduction under different photoperiodic conditions

To estimate how photoperiodic conditions influenced reproduction, several parameters of reproduction were recorded in reproductive females under different near-critical and long-day photoperiodic conditions (Table 3). At 20 °C, females tended to produce more eggs in larger egg masses at LD 16:8 h than at LD 14:10 h and LD 15:9 h, but

Table 2. Effect of day-length on duration of colour grades in adults of *Nezara viridula* under laboratory conditions.

	Duration of colour grades (20 °C)					Duration of colour grades (25 °C)				
Conditions	*N*	*n*	Green	Intermediate	Total to russet	*N*	*n*	Green	Intermediate	Total to russet
Females										
LD 10:14 h	29	29	24 (17–36)[a]	5 (3–21)[a]	28 (22–57)[a]	34	34	12 (9–21)[a]	3 (2–9)[a]	15 (12–30)[a]
LD 12:12 h	27	20	23.5 (19–38)[ab]	8 (3–22)[ab]	32.5 (23–58)[ab]	82	63	13 (4–33)[a]	2 (1–16)[b]	15 (11–43)[a]
LD 13:11 h	28	12	32 (20–44)[b]	11.5 (5–16)[b]	43.5 (25–52)[b]	99	66	17 (10–51)[b]	2 (1–15)[ab]	18 (12–59)[b]
Males										
LD 10:14 h	29	28	24 (16–34)[a]	4 (2–19)[a]	27 (18–53)[a]	38	38	12 (9–40)[a]	2.5 (1–13)[ab]	15 (11–44)[a]
LD 12:12 h	28	24	22 (18–28)[a]	6 (2–15)[b]	28 (23–43)[a]	90	77	13 (7–38)[b]	1 (1–16)[a]	15 (10–48)[a]
LD 13:11 h	29	5	28 (22–34)[a]	15 (6–16)[c]	44 (28–47)[b]	107	58	16 (8–51)[c]	2 (1–26)[b]	19.5 (11–57)[b]

Median periods in days (with range) followed by the same superscript letters within each column and sex are not significantly different (*P* > 0.05, Kruskal–Wallis test followed by the Steel-Dwass test). *N* adults survived until day 60 and *n* adults became russet (samples with *n* > 2 were used in the analysis).
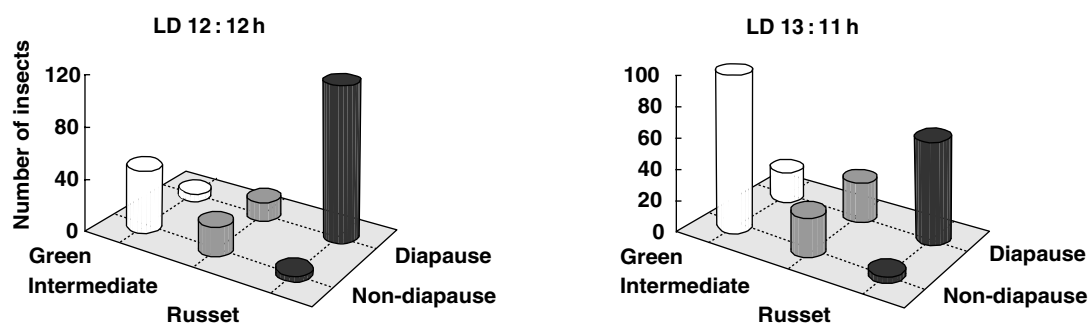
Fig. 4. Diapause status and colour of adults of *Nezara viridula* at day 60 after emergence at LD 12:12 h and LD 13:11 h and 25 °C (the sexes are combined).

the difference was not significant between LD 16:8 h and LD 13:11 h. Also, the rate of egg production as defined by total number of eggs laid during 60 days after adult ecdysis/median interval between successive egg masses (=11 days) did not differ significantly between LD 13:11 h, LD 15:9 h, and LD 16:8 h. At 25 °C, reproduction seemed to be suppressed at LD 13:11 h and no clear effect on egg production was observed under other photoperiodic conditions.

Diapause and colour changes in genetically determined colour morphs

The insects used in our experiments represented three of the four most common genetically controlled colour morphs (Table 4). The incidences of diapause and dark-coloured individuals (russet and intermediate colour grades combined) were compared between the morphs. In some regimes, the incidence of diapause and/or dark colour was somewhat higher in one morph than in the others. However,

there was no pronounced difference in the tendency to enter diapause and change body colour between the morphs.

Discussion

Effect of day-length on duration of nymphal period

Day-length has been shown to induce nymphal diapause in some true bugs (Kiritani, 1985; Tanaka *et al.*, 2002), whereas in some others, day-length controls the duration of the nymphal period without induction of nymphal diapause (Hori, 1988; Musolin & Saulich, 1997, 1999). It is sometimes difficult to distinguish the photoperiod-mediated retardation of nymphal period as an adaptation evolved for a more precise timing of seasonal development from the photoperiodically induced nymphal diapause which is primarily an adaptation for survival of adverse seasons (Danks, 2000). In such cases, other characteristics of diapause (i.e. food consumption, behaviour, the seasonal incidence) should be taken into consideration.

Table 3. Parameters of reproduction of *Nezara viridula* under different temperature and photoperiodic conditions within 60 days after emergence of adults.

Temperature and Indices	Photoperiodic conditions				
	LD 12:12 h	LD 13:11 h	LD 14:10 h	LD 15:9 h	LD 16:8 h
20 °C					
Number of reproductive females	1	5	8	11	29
Eggs per female	76	150.0 (114–273)[ab]	74.5 (14–214)[a]	137 (31–294)[a]	335 (15–539)[b]
Egg masses per female	1	2 (2–3)[ab]	1 (1–3)[a]	2 (1–4)[a]	4 (1–6)[b]
Egg mass size (eggs per egg mass)	76	69.5 (21–103)[ab]	68 (14–80)[a]	60 (2–98)[a]	90 (11–122)[b]
Eggs per female per 11 days*	–	48.5 (36.9–88.3)[ab]	26.4 (5.0–75.9)[a]	58 (13.1–124.4)[ab]	89.9 (4.0–144.6)[b]
25 °C					
Number of reproductive females	18	23	33	32	29
Eggs per female	448.5 (136–827)[a]	164 (40–588)[b]	519 (86–1035)[ac]	637.5 (255–969)[c]	496 (83–802)[a]
Egg masses per female	5.5 (2–10)[a]	3 (1–6)[b]	6 (1–9)[a]	6.5 (3–9)[a]	5 (1–8)[a]
Egg mass size (eggs per egg mass)	84.5 (7–117)[a]	61 (17–147)[b]	90 (8–157)[c]	99 (66–184)[d]	95 (11–128)[c]
Eggs per female per 8 days†	78 (23.7–143.8)[ac]	42.3 (10.3–151.7)[a]	83.9 (14.0–169.0)[bc]	102 (40.8–155.0)[b]	79.4 (13.3–128.3)[c]

Medians (with range) followed by the same superscript letters within a row are not significantly different ($P > 0.05$; Kruskal–Wallis single-factor analysis of variance by ranks test followed by the Steel-Dwass test). Samples with $n > 2$ only were used in the analysis.

*, †, 11 and 8 days are median intervals between successive egg masses at 20 °C and 25 °C, respectively. 'Eggs per female per 11 (or 8) days' indices were calculated as total number of eggs laid during 60 days after adult ecdysis/median interval between successive egg masses (i.e. 8 or 11 days).

Table 4. Incidence of diapause and dark-coloured individuals in different genetically determined colour morphs of *Nezara viridula*.

Conditions	Incidence of diapause in colour morphs,% (*n*)			Incidence of dark-coloured grades in colour morphs,% (*n*)		
	G	O	F	G	O	F
20 °C						
LD 10:14 h	98.1 (52)	100.0 (7)	–	100.0 (52)	100.0 (7)	–
LD 12:12 h	64.0 (50)	50.0 (4)	50.0 (2)	80.0 (49)	100.0 (4)	100.0 (2)
LD 13:11 h	20.0 (50)	100.0 (2)	14.3 (14)	52.0 (50)	100.0 (2)	30.8 (13)
LD 14:10 h	7.3 (55)	0.0 (3)	28.6 (7)	12.7 (55)	33.3 (3)	0.0 (7)
LD 15:9 h	2.9 (68)	0.0 (2)	0.0 (1)	7.7 (65)	0.0 (2)	–
LD 16:8 h	0.0 (47)	5.3 (19)	–	2.1 (48)	6.6 (15)	–
25 °C						
LD 10:14 h	100.0 (43)	100.0 (29)	–	100.0 (43)	100.0 (29)	–
LD 12:12 h	63.7 (193)	80.0 (25)	–	74.0 (181)	78.9 (19)	–
LD 13:11 h	47.0 (183)	47.1 (51)	100.0 (3)	50.6 (174)	52.1 (48)	100.0 (3)
LD 14:10 h	0.0 (69)	0.0 (3)	–	0.0 (69)	0.0 (3)	–
LD 15:9 h	0.0 (68)	0.0 (1)	–	0.0 (68)	0.0 (1)	–
LD 16:8 h	1.7 (60)	0.0 (7)	0.0 (1)	1.7 (60)	0.0 (7)	0.0 (1)

n refers to the total number of adults (the sexes are combined) in each colour morph.
Nomenclature of the colour morphs follows Yukawa & Kiritani (1965): G, *N. viridula* f. *smaragdula* Fabr. (entirely green); O, *N. viridula* f. *torquata* Fabr. (mostly green, median and lateral lobes, and the anterior margin of pronotum are yellow); F (as O, but connexivum is yellow).

Ali & Ewiess (1977) showed that the nymphal development of *N. viridula* in Cairo, Egypt, was accelerated by long days and delayed by short days and inferred that a long photophase provided a long feeding period and thus accelerated development. However, adults of *N. viridula* feed during scotophase rather than photophase (Shearer & Jones, 1996) and, perhaps, this might be true also for nymphs.

In insect species with a pronounced photoperiodic control of duration of the larval period, the greatest photoperiod-mediated acceleration or retardation of growth was shown to occur during the final larval instar (e.g. Hori, 1988; Leimar, 1996; Musolin & Saulich, 1997). In *N. viridula*, however, this photoperiodic effect does not seem to be restricted to the last nymphal instar. In general, at 20 °C, the nymphal period was significantly shorter under short-day and long-day conditions than under the intermediate photoperiods, although the difference was not pronounced at 25 °C (Table 1). Under the natural conditions in Osaka, the day-length ranges from 9 h 49 min to 14 h 30 min (without civil twilight; from Beck, 1980). Thus, it is possible that some photoperiod-mediated acceleration of nymphal growth takes place in autumn when the temperature is moderate and the day-length is getting shorter and the nymphal development would not be affected by day-length under summer long-day conditions when the temperature is high. This response would promote an earlier and more-synchronized emergence of adults of the last generation in autumn as is seen in some other insect species (Danks, 1987; Nylin & Gotthard, 1998).

Effect of day-length and temperature on the precopulation and preoviposition periods

At both 20 °C and 25 °C, the precopulation and preoviposition periods were significantly shorter under long-day conditions than under short-day and intermediate conditions (Fig. 1). Although the photoperiodic control of the precopulation period in true bugs is reported here for the first time, a similar control of preoviposition period has been observed in some heteropterans. The differences in duration of the preoviposition period under different photoperiodic conditions were reported to be associated with different wing morphs (Harada, 1992) or with production of diapause/nondiapause eggs (Musolin *et al.*, 1999). A marked photoperiod-related difference in the age at first reproduction was found in the milkweed bug *Oncopeltus fasciatus* and explained by the extreme variability of the diapause response under uncertain environmental conditions (Evans, 1982; cited by Dingle, 1986). Ruberson *et al.* (1991) suggested that in *Orius insidiosus* the difference in the duration of the preoviposition period under different photoperiods might be the result of a low-intensity diapause, which can be terminated within a short period. However, in the bean bug *Riptortus clavatus*, the preoviposition period varied with photoperiod even within the diapause-preventing range, being significantly longer under LD 14:10 h than the longer photoperiods (Musolin *et al.*, 2001). The results in *N. viridula* (Fig. 1) suggest that the species responds to photoperiod not only in a qualitative manner (diapause versus reproduction), but also in a quantitative one: the duration of photophase controls a graded response in the commencement of copulation and oviposition. Similar to other examples (Musolin & Saulich, 1997), the response is more pronounced at lower temperatures.

Effect of day-length and temperature on diapause induction

The photoperiodic response curves were similar for both sexes (Fig. 2), although the incidence of diapause was

somewhat higher in the females than in the males in most regimes, especially under the near-critical photoperiods as in another pentatomid *Aelia fieberi* (Nakamura & Numata, 1997). Under natural conditions, males would probably enter diapause a little later than females.

Lower temperature (20 °C versus 25 °C) did not increase the critical day-length, as was reported in the heteropterans *Pyrrhocoris apterus* (Numata *et al.*, 1993), *Graphosoma lineatum* (Musolin & Saulich, 1995) and some other insects (Danilevsky, 1961; Danks, 1987). The photoperiodic response of diapause induction in *N. viridula* is thermostable within the range of tested temperatures similar to two other heteropterans, *Riptortus clavatus* (Kobayashi & Numata, 1995) and *Arma custos* (Volkovich & Saulich, 1994). The critical day-length for induction of diapause fell within a narrow range close to 12.5 h. This value is a little shorter than the critical day-length in some other heteropterans studied to date in central Japan (Numata & Nakamura, 2002). As previously mentioned, *N. viridula* expanded its distribution range in Japan and reached the Osaka region only recently. Perhaps, the shorter critical day-length along with a marked variation of photoperiodic response within the near-critical range (Fig. 2) reflect a southern origin of the species as well as its ongoing adaptation to new environment.

Effect of day-length and temperature on colour change in adults

Adults of *N. viridula* are green during the warmer months of the year, whereas in autumn and winter russet or brown adults are commonly found (Harris *et al.*, 1984; Seymour & Bowman, 1994). This seasonal polyphenism has been reported in many insect species including heteropterans (e.g. the mirid *Notostira elongata*, the pentatomids *Plautia crossota stali* and *Euschistus servus*) (Dolling, 1973; Kotaki & Yagi, 1987; Borges *et al.*, 2001; for a review, see Musolin & Saulich, 1999). The present experiments show that changes of adult colour are gradual and controlled by photoperiodic conditions: adults remain green under long-day conditions and turn russet under short-day conditions. Temperature plays a secondary role in this response (Fig. 3). The colour change proceeds at different rates under different photoperiodic conditions: the shorter the day-length, the more rapidly bugs changed colour and became russet (Table 2), suggesting a quantitative nature of the photoperiodic response is involved. Under natural conditions, this response will accelerate adaptive change of body colour in autumn when foliage changes colour and day-length is declining.

The relationship between adult colour and diapause state of gonads

The association between reproductive diapause and colour changes found in *N. viridula* suggests that both responses are symptoms of one diapause syndrome (Tauber *et al.*, 1986). Harris *et al.* (1984) proposed that this colour change might serve as an indicator of adult diapause, but Seymour & Bowman (1994) and Jones & Westcot (2002) questioned this notion. In the present study, under most conditions, but particularly under very short- and very long-day photoperiods, the proportions of russet individuals completely coincided with or slightly exceeded the incidence of diapause, thus suggesting a close association between diapause state and russet body colour. However, under near-critical conditions, the incidence of dark-coloured individuals often exceeded incidence of diapause (Figs 2 and 3) and all combinations of diapause status and colour were present (Fig. 4).

In some swallowtail butterflies, diapause status and the colour of pupae are associated, but loosely coupled (Hazel & West, 1983). This finding led Tauber *et al.* (1986) to suggest that the development of each of these diapause symptoms may be controlled by a slightly different critical photoperiod. This may be true for *N. viridula* too, although the possibility of the same photoperiodic response controlling both diapause and colour cannot be rejected. In the present experiments, the partial discrepancy between the state of gonads and body colour of *N. viridula* adults under the near-critical conditions on day 60 more likely reflects the dynamic nature of the diapause syndrome and/or uncertainty of conditions around the critical photoperiod. Under these conditions, diapause is not deep and dormant insects can relatively easily terminate diapause and start reproduction, whereas reproductive adults can cease reproduction and enter diapause. This interpretation of results is supported by an observation that, under the near-critical conditions, some adults changed colour more than once, although such repeated colour changes very seldom happened under short- or long-day conditions. It also is possible that there is a time gap between the changes in colour and the state of gonads. Thus, the colour of adults may serve as a good but not absolute indicator of reproductive status.

Reproduction under different photoperiodic conditions

Parameters of reproduction may be controlled not only by temperature and food, but also by day-length. Thus, the parasitoid wasp *Ooencyrtus nezarae*, the phytophagous bug *Euschistus heros* and the weevil *Otiorhynchus ovatus* produce fewer eggs under short-day than under long-day conditions (Numata, 1993; Mourão & Panizzi, 2002; Umble & Fisher, 2002). In the present study, similar trends were observed under some but not all conditions and the difference in reproductive indices between treatments was not always significant (Table 3). Perhaps, this inconsistency is partly due to the high individual variability in reproductive potential.

Seasonal development and voltinism in Osaka

Adults of *N. viridula* were collected in the field from mid-June onward showing that the species has expanded its distribution range in Japan and reached Osaka.

The adults collected in June probably belong to the first summer generation produced by adults that overwintered either nearby or further south (for example, in Wakayama Prefecture) or to the overwintered generation of migrants. A pronounced variation in the incidence of diapause recorded under laboratory conditions (Fig. 2) may be explained by the heterogeneity of the local population caused by either recent colonization or by annual immigration. The critical day-length for induction of diapause fell within a narrow range close to 12.5 h and was little influenced by temperature within the tested range (20 °C and 25 °C) (Fig. 2), suggesting that the species can enter diapause in Osaka in late September after producing three generations provided that food is available throughout the season.

The present study shows that day-length has multiple influences on the life cycle of *N. viridula* and both types of photoperiodic responses were found: nymphs respond to photoperiod in a quantitative manner (the control of duration of nymphal period) whereas adults do so in both qualitative (diapause induction and colour change) and quantitative (control of duration of precopulation and preoviposition periods, and rates of colour change) manners.

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References

Ali, M. & Ewiess, M.A. (1977) Photoperiodic and temperature effects on rate of development and diapause in the green stink bug, *Nezara viridula* L. (Heteroptera: Pentatomidae). *Zeitschrift für Angewandte Entomologie*, **84**, 256–264.

Beck, S.D. (1980) *Insect Photoperiodism*, 2nd edn. Academic Press, New York.

Borges, M., Zhang, A., Camp, M.J. & Aldrich, J.R. (2001) Adult diapause morphs of the brown stink bug, *Euschistus servus* (Say) (Heteroptera: Pentatomidae). *Neotropical Entomology*, **30**, 179–182.

Danilevsky, A.S. (1961) *Photoperiodism and Seasonal Development of Insects*. Leningrad State University Press, Leningrad [in Russian] [Translated into English by J. Johnson assisted by N. Waloff, Oliver & Boyd, Edinburgh, 1965].

Danks, H.V. (1987) *Insect Dormancy: An Ecological Perspective*, Monograph Series No. 1. Biological Survey of Canada, Ottawa.

Danks, H.V. (2000) Measuring and reporting life-cycle duration in insects and arachnids. *European Journal of Entomology*, **97**, 285–303.

Dingle, H. (1986) The evolution of insect life cycle syndrome. *The Evolution of Insect Life Cycles* (ed. by F. Taylor and R. Karban), pp. 187–203. Springer, New York.

Dolling, W.R. (1973) Photoperiodically determined phase production and diapause termination in *Notostira elongata* (Geoffroy) (Hemiptera: Miridae). *Entomological Gazzette*, **24**, 75–79.

Elsey, K.D. (1993) Cold tolerance of the southern green stink bug (Heteroptera: Pentatomidae). *Environmental Entomology*, **22**, 567–570.

Evans, K.E. (1982) *Migration and Reproduction in Northern California Populations of the Milkweed Bug* Oncopeltus fasciatus. PhD Thesis, University of California, Berkeley.

Harada, T. (1992) The oviposition process in two direct breeding generations in a water strider, *Aquarius paludum* (Fabricius). *Journal of Insect Physiology*, **38**, 687–692.

Harris, V.E., Todd, J.W. & Mullinix, B.G. (1984) Color change as an indicator of adult diapause in the southern green stink bug, *Nezara viridula. Journal of Agricultural Entomology*, **1**, 82–91.

Hazel, W.N. & West, D.A. (1983) The effect of larval photoperiod on pupal colour and diapause in swallowtail butterflies. *Ecological Entomology*, **8**, 37–42.

Hokkanen, H. (1986) Polymorphism, parasites, and the native area of *Nezara viridula* (Hemiptera, Pentatomidae). *Annales Entomologici Fennici*, **52**, 28–31.

Hori, K. (1988) Effects of stationary photoperiod on nymphal growth, feeding and digestive physiology of *Palomena angulosa* Motschulsky (Hemiptera: Pentatomidae). *Applied Entomology and Zoology*, **23**, 401–406.

Jones, V.P. & Westcot, D. (2002) The effect of seasonal changes on *Nezara viridula* (L.) (Hemiptera: Pentatomidae) and *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) in Hawaii. *Biological Control*, **23**, 115–120.

Jones, W.A. & Sullivan, M.J. (1981) Overwintering habitats, spring emergence patterns, and winter mortality of some South Carolina Hemiptera. *Environmental Entomology*, **10**, 409–414.

Kiritani, K. (1963) The change in reproductive system of the southern green stink bug, *Nezara viridula*, and its application to forecasting of the seasonal history. *Japanese Journal of Applied Entomology and Zoology*, **7**, 327–337.

Kiritani, K. (1971) Distribution and abundance of the southern green stink bug, *Nezara viridula. Proceedings of the Symposium on Rice Insects*, pp. 235–248. Tropical Agricultural Research Center, Tokyo.

Kiritani, K., Hokyo, N. & Kimura, K. (1966) Factors affecting the winter mortality in the southern green stink bug, *Nezara viridula* L. *Annales de la Société Entomologique de France, Nouvelle Série (Sunn Pest Memoirs 9)*, **2**, 199–207.

Kiritani, K., Hokyo, N. & Yukawa, J. (1963) Co-existence of the two related stink bugs *Nezara viridula* and *N. antennata* under natural conditions. *Researches on Population Ecology*, **5**, 11–22.

Kiritani, Y. (1985) Effect of stationary and changing photoperiods on nymphal development in *Carbula humerigera* (Heteroptera: Pentatomidae). *Applied Entomology and Zoology*, **20**, 257–263.

Kobayashi, S. & Numata, H. (1995) Effects of temperature and photoperiod on the induction of diapause and the determination of body coloration in the bean bug, *Riptortus clavatus*. *Zoological Science*, **12**, 343–348.

Kotaki, T. & Yagi, S. (1987) Relationship between diapause and coloration change in the brown-winged green bug, *Plautia stali*

Scott (Heteroptera: Pentatomidae). *Japanese Journal of Applied Entomology and Zoology*, **31**, 285–290 [in Japanese].

Leimar, O. (1996) Life history plasticity: Influence of photoperiod on growth and development in the common blue butterfly. *Oikos*, **76**, 228–234.

Mourão, A.P.M. & Panizzi, A.R. (2002) Photophase influence on the reproductive diapause, seasonal morphs, and feeding activity of *Euschistus heros* (Fabr., 1798) (Hemiptera: Pentatomidae). *Brazilian Journal of Biology*, **62**, 231–238.

Musolin, D.L., Maysov, A.V. & Saulich, A.H. (1999) Egg diapause in *Alydus calcaratus* (L.) (Heteroptera: Alydidae): Photoperiodic induction under natural temperature conditions. *Applied Entomology and Zoology*, **34**, 469–473.

Musolin, D.L., Numata, H. & Saulich, A.H. (2001) Timing of diapause induction outside the natural distribution range of a species: An outdoor experiment with the bean bug *Riptortus clavatus*. *Entomologia Experimentalis et Applicata*, **100**, 211–219.

Musolin, D.L. & Saulich, A.H. (1995) Factorial regulation of the seasonal cycle of the stink bug *Graphosoma lineatum* L. (Heteroptera, Pentatomidae). 1. Temperature and photoperiodic responses. *Entomologicheskoe Obozrenie*, **74**, 736–743 [in Russian] [translation in *Entomological Review* (1996), **75**, 84–93].

Musolin, D.L. & Saulich, A.H. (1997) Photoperiodic control of nymphal growth in bugs (Heteroptera). *Zoologicheskii Zhurnal*, **76**, 530–542 [in Russian] [translation in *Entomological Review* (1997), **77**, 768–780].

Musolin, D.L. & Saulich, A.H. (1999) Diversity of seasonal adaptations in terrestrial true bugs (Heteroptera) from the Temperate Zone. *Entomological Science*, **2**, 623–639.

Nakamura, K. & Numata, H. (1997) Seasonal life cycle of *Aelia fieberi* (Hemiptera: Pentatomidae) in relation to the phenology of its host plants. *Annals of the Entomological Society of America*, **90**, 625–630.

Noda, T. (1991) *Nezara antennata* and *N. viridula*. *Rearing Methods of Insects* (ed. by K. Yushima, S. Kamano and Y. Tamaki), pp. 33–35. Japanese Society for Plant Protection, Tokyo [in Japanese].

Numata, H. (1993) Induction of adult diapause and of low and high reproductive states in a parasitoid wasp, *Ooencyrtus nezarae*, by photoperiod and temperature. *Entomologia Experimentalis et Applicata*, **66**, 127–134.

Numata, H. & Nakamura, K. (2002) Photoperiodism and seasonal adaptations in some seed-sucking bugs (Heteroptera) in central Japan. *European Journal of Entomology*, **99**, 155–161.

Numata, H., Saulich, A.H. & Volkovich, T.A. (1993) Photoperiodic responses of the linden bug, *Pyrrhocoris apterus*, under conditions of constant temperature and under thermoperiodic conditions. *Zoological Science*, **10**, 521–527.

Nylin, S. & Gotthard, K. (1998) Plasticity in life-history traits. *Annual Review of Entomology*, **43**, 63–83.

Ohno, K. & Alam, M.Z. (1992) Hereditary basis of adult color polymorphism in the southern green stink bug, *Nezara viridula* Linné (Heteroptera: Pentatomidae). *Applied Entomology and Zoology*, **27**, 133–139.

Panizzi, A.R. & Hirose, E. (1995) Seasonal body weight, lipid content, and impact of starvation and water stress on adult survivorship and longevity of *Nezara viridula* and *Euschistus heros*. *Entomologia Experimentalis et Applicata*, **76**, 247–253.

Panizzi, A.R., McPherson, J.E., James, D.G., Javahery, M. & McPherson, R.M. (2000) Stink bugs (Pentatomidae). *Heteroptera of Economic Importance* (ed. by C. W. Schaefer and A. R. Panizzi), pp. 421–474. CRC Press, Boca Raton.

Ruberson, J.R., Bush, L. & Kring, T.J. (1991) Photoperiodic effect on diapause induction and development in the predator *Orius insidiosus* (Heteroptera: Anthocoridae). *Environmental Entomology*, **20**, 786–789.

Seymour, J.E. & Bowman, G.J. (1994) Russet coloration in *Nezara viridula* (Hemiptera: Pentatomidae): an unreliable indicator of diapause. *Environmental Entomology*, **23**, 860–863.

Shearer, P.W. & Jones, V.P. (1996) Diel feeding pattern of adult female southern green stink bug (Hemiptera: Pentatomidae). *Environmental Entomology*, **25**, 599–602.

Takizawa, H. (1994) Effect of temperature and photoperiod on the induction of brown color type (forma *aurantica* Costa) adult of the southern green stink bug, *Nezara viridula* L. (Heteroptera: Pentatomidae). *Bulletin of National Institute of Sericultural and Entomological Science*, **11**, 27–35 [in Japanese].

Tanaka, S.I., Imai, C. & Numata, H. (2002) Ecological significance of adult summer diapause after nymphal winter diapause in *Poecilocoris lewisi* (Distant) (Heteroptera: Scutelleridae). *Applied Entomology and Zoology*, **37**, 469–475.

Tauber, M.J., Tauber, C.A. & Masaki, S. (1986) *Seasonal Adaptations of Insects*. Oxford University Press, New York.

Umble, J.R. & Fisher, J.R. (2002) Influence of temperature and photoperiod on preoviposition duration and oviposition of *Otiorynchus ovatus* (Coleoptera: Curculionidae). *Annals of the Entomological Society of America*, **95**, 231–235.

Velasco, L.R.I., Walter, G.H. & Harris, V.E. (1995) Voltinism and host plant use by *Nezara viridula* (L.) (Hemiptera: Pentatomidae) in Southeastern Queensland. *Journal of the Australian Entomological Society*, **34**, 193–203.

Volkovich, T.A. & Saulich, A.H. (1994) The predatory bug *Arma custos*: Photoperiodic and temperature control of diapause and coloration. *Zoologicheskii Zhurnal*, **73**, 26–37 [in Russian].

Yukawa, J. & Kiritani, K. (1965) Polymorphism in the southern green stink bug. *Pacific Insects*, **7**, 639–642.

Zar, J.H. (1999) *Biostatistical Analysis*, 4th edn. Prentice Hall, New Jersey.