

Photoperiodic control of diapause termination, colour change and postdiapause reproduction in the southern green stink bug, *Nezara viridula*

DMITRY L. MUSOLIN^{1,2}, KENJI FUJISAKI² and HIDEHARU NUMATA¹

¹Department of Biology and Geosciences, Graduate School of Science, Osaka City University, Japan and ²Laboratory of Insect Ecology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan.

Abstract. The effects of day length on adult diapause development, associated with diapause body colour change as well as postdiapause reproduction are studied in *Nezara viridula* from Japan. Facultative diapause spontaneously terminates under three constant short-day and near-critical photoperiods at 25 °C without low temperature treatment. The period required for body colour change from russet to green and the precopulation and preoviposition periods differ significantly between the photoperiodic treatments, being shortest under LD 13 : 11 h, intermediate under LD 12 : 12 h and longest under LD 10 : 14 h. Photoperiodic conditions do not affect postdiapause reproductive performance: the total egg production, duration of the period of oviposition and other reproductive indices do not differ significantly between the photoperiodic conditions. The total egg production depends on the duration of the period of oviposition but not on how long females remained russet during diapause. It is concluded that diapause in *N. viridula* does not require low temperature for its successful completion and diapause duration affects winter survival but not postdiapause reproductive performance or longevity. Such independence of the postdiapause reproductive performance from the duration of diapause may have contributed to the continuous worldwide range expansion of this species into temperate zone.

Key words. Dormancy, life history trade-off, photoperiodism, polymorphism, polyphenism, seasonal adaptations.

Introduction

Both winter diapause and the subsequent quiescence, as well as the environmental conditions that induce and maintain these physiological states, can affect the subsequent life history and fitness of insects (Tauber & Tauber, 1976; Tauber *et al.*, 1986; Danks, 1994; Chang *et al.*, 1996). Thus, diapause may have effects not only on overwintering success, but also on postdiapause survivorship and reproductive performance (Ishihara & Shimada, 1995; Kroon & Veenendaal, 1998), which are an important source of natural selection (Bradshaw *et al.*, 1998).

Most research on insect diapause focuses on factors controlling induction of facultative diapause and less on the subsequent developmental steps (i.e. diapause maintenance and termination phases; Košťál, 2006) and obligatory diapause. More research is needed to reveal the whole range of natural patterns of diapause termination and also to understand the particular physical or biological stimuli required for termination of facultative and obligatory diapauses as well as whether diapause can terminate spontaneously under the same conditions as those that the insects experienced when the diapause is induced. Moreover, is longer dormancy more costly in terms of success of subsequent reproduction?

The southern green stink bug *Nezara viridula* (L.) (Heteroptera: Pentatomidae) is a polyvoltine seed-sucking species and occurs throughout tropical, subtropical and southern temperate regions of the world (Todd, 1989; Panizzi *et al.*, 2000). The adults in temperate populations overwinter

Correspondence: Dr Dmitry L. Musolin, Laboratory of Insect Ecology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan. Tel.: +81 75 7536136; fax: +81 75 7536474; e-mail: musolin@gmail.com and musolin@dm1037.spb.edu

under litter, bark or other shelters in a reproductive diapause (Kiritani *et al.*, 1966; Jones & Sullivan, 1981). The induction of diapause is controlled by photoperiod and is associated with seasonal polyphenism: adults change body colour from green to russet or brown (Ali & Ewiess, 1977; Harris *et al.*, 1984; Musolin & Numata, 2003a). In Japan, *N. viridula* has long been known in the southern part of the archipelago (Kiritani, 1971) but recently expanded its distribution range and reached Osaka (Musolin & Numata, 2003b; Musolin, 2006). Laboratory experiments reveal a variable photoperiodic response of diapause induction with a comparatively short critical day length in the Osaka population (Musolin & Numata, 2003a). Such a photoperiodic response determines the relatively late diapause induction in *N. viridula* compared with the several other seed-sucking heteropterans native to the Osaka region (Numata & Nakamura, 2002; Musolin, 2006).

The present study aims to clarify the role of photoperiod and temperature in the maintenance and termination of reproductive diapause, body colour change and any relationships between the duration of winter diapause and postdiapause reproductive performance in *N. viridula*.

Materials and methods

Insect culture

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 an LD 16 : 8 h photoperiod at 25 °C. They were supplied with dry grains of soybean *Glycine max* (L.) Merrill, shelled raw peanuts *Arachis hypogaea* L. and water containing 0.05% sodium L-ascorbate and 0.025% L-cysteine (Noda, 1991). Each egg mass laid in the stock culture was divided into two or three parts, and allotted to different experimental regimes to provide greater heterogeneity in the experimental material.

Diapause induction

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 three different constant short-day or near-critical photoperiodic conditions: (i) LD 10 : 14 h; (ii) LD 12 : 12 h; and (iii) LD 13 : 11 h at 25 ± 1 °C. Upon hatching, nymphs, and subsequently adults, were provided with the same diet as the stock culture. Food and water were replaced or renewed 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. Adult emergence was checked daily and recorded individually.

Within 24 h after adult emergence, 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 and oviposition, changes of body colour and mortality were recorded daily. Three colour grades were used: (i) green; (ii) intermediate (approximately 40–60% of the body surface is light russet) and (iii) russet (brown).

On day 60 after adult emergence, green and intermediately coloured individuals were removed from all three experimental conditions. Of the remaining (all russet) individuals, two to nine adults of each sex in each experimental condition were dissected to confirm diapause status of adults with deep russet body colour. 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 (Musolin & Numata, 2003a).

Diapause maintenance and termination

The remaining female–male pairs (25 in LD 10 : 14 h, 42 in LD 12 : 12 h and 19 in LD 13 : 11 h; all deep russet) were considered to be in diapause and left under the same conditions. These adults were reared further as described above. If a male in a pair died, it was replaced by a male of the same colour grade from the same photoperiodic regime. Changes in body colour and all developmental events (start of copulation and oviposition, appearance of all further egg masses and mortality) were recorded daily. The experiment was terminated when the last female died at the adult age of 351 days (Fig. 1).

Patterns of diapause-associated adult body colour changes and postdiapause reproduction were compared between the photoperiodic conditions to examine whether photoperiodically induced diapause in *N. viridula* can spontaneously terminate and how temporal pattern of this diapause termination and further postdiapause reproduction are affected by photoperiodic conditions. Reproductive parameters were calculated for assessment of reproductive performance: (i) the number of egg masses produced by each female; (ii) the number of eggs in each egg mass; (iii) the total number of eggs produced by each female; and (iv) the period of oviposition (i.e. the period between the days when the first and the last egg masses were laid by a female).

Statistical analysis

For statistical analysis of different parameters of reproduction, the Kruskal–Wallis and Steel–Dwass tests were used (EXCEL TOKEI, version 5.0; Esumi Ltd, Japan). To compare proportions of copulating and ovipositing females, the Turkey-type multiple comparison test for proportions was

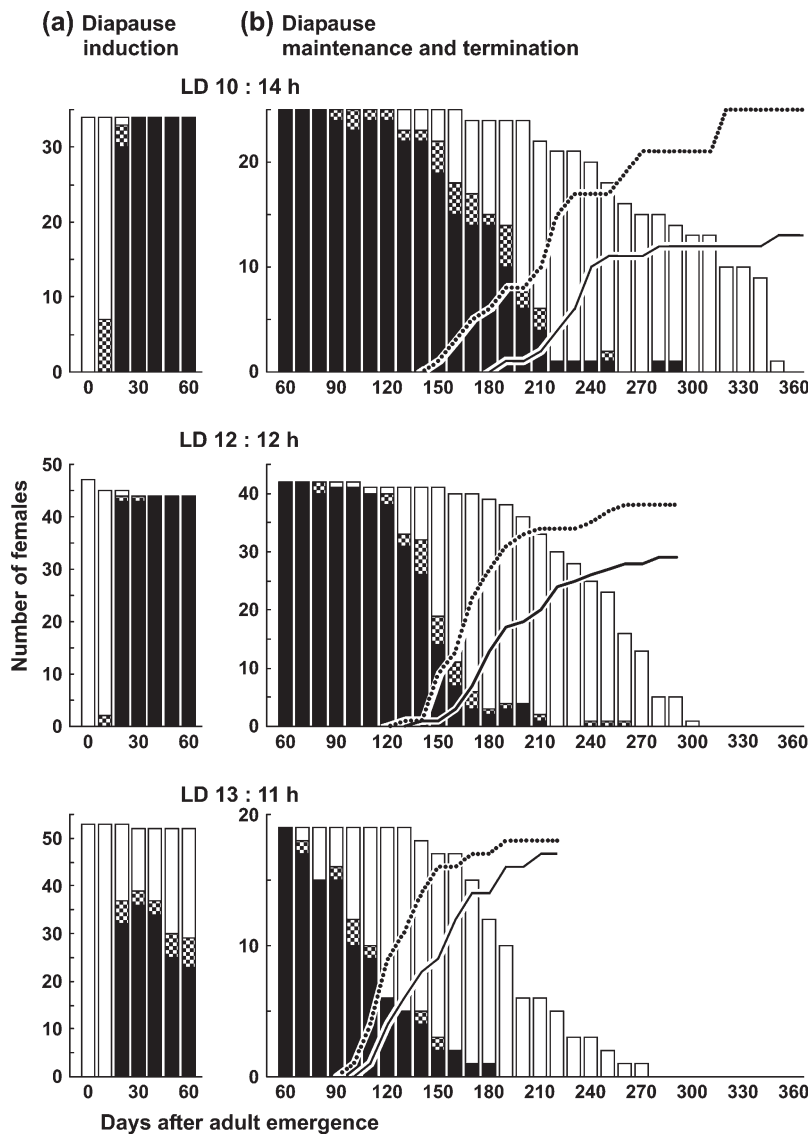


Fig. 1. Effect of day length on (a) diapause induction and (b) diapause maintenance and termination in females of *Nezara viridula* at 25 °C. On day 60 after adult emergence, green and intermediately coloured individuals were removed and only russet ones were left for further exposure (see text for details). Open, shaded and black sections of the bars indicate the proportion of adults with green, intermediately coloured and russet bodies, respectively. The dotted line shows cumulative copulation and the solid line cumulative oviposition.

used (Zar, 1999). For analysis of the relationship between the life history traits, the Spearman's rank correlation procedure was applied (Yanai, 1998).

Results

Changes of body colour

All females changed body colour from green to russet within the first 60 days after the final moult under LD 10 : 14 h and LD 12 : 12 h and almost 70% of females did so under LD 13 : 11 h (Fig. 1a; data on males are similar but not presented here). After day 60, when only russet adults were left for further exposure under the three photoperiodic conditions, females remained russet for varying periods but then

spontaneously started to change colour to the intermediate grade and green (Fig. 1b). A few females, mostly those kept under LD 13 : 11 h, went through a cycle of colour change (i.e. from green to intermediate and russet and then back to intermediate and green) more than once. The shorter the day length, the longer the females remained russet and the longer the period needed to reach the final stable green colour grade (medians were significantly different between the photoperiodic conditions; Table 1).

Body colour change from russet and intermediate to green preceded the start of reproduction. Almost 94% of females were already green on the day of the first copulation (all photoperiods combined); only one russet female copulated with a green male (Fig. 1; males showed a similar trend; not shown). By the day of the first oviposition, 100% of females were green (Fig. 1).

Table 1. Effect of day length on life history traits in females of *Nezara viridula* under different constant photoperiodic conditions at 25 °C.

Indices	Photoperiodic conditions		
	LD 10 : 14 h	LD 12 : 12 h	LD 13 : 11 h
Body colour changes			
Duration of stable russet colour period (days), <i>n</i>	154 (74–206) ^a 25	126 (61–199) ^b 41	84 (34–169) ^c 19
Period from adult emergence to stable green colour (days), <i>n</i>	197 (133–299) ^a 25	150 (81–263) ^b 41	116 (74–182) ^c 19
Temporal parameters of reproduction and female life span			
Period from the intermediate-to-green colour change to the first copulation (days), <i>n</i>	18.5 (0–178) ^a 22	12.5 (0–109) ^a 36	10 (0–37) ^a 14
Period from the intermediate-to-green colour change to the first oviposition (days), <i>n</i>	42 (9–201) ^a 13	29 (6–111) ^a 29	24 (14–58) ^a 17
Adult life of females (days), <i>n</i>	312 (165–351) ^a 25	253 (106–299) ^b 42	191 (139–279) ^c 19
Adult life of females minus stable russet colour period (days), <i>n</i>	145 (28–236) ^a 25	120 (26–194) ^a 41	108 (43–212) ^a 19
Adult life of females minus precopulation period (days), <i>n</i>	69 (4–151) ^a 25	80 (5–148) ^a 38	61.5 (25–137) ^a 18
Adult life of females minus preoviposition period (days), <i>n</i>	71 (6–129) ^a 13	66 (4–139) ^a 29	52 (1–124) ^a 17
Reproductive performance			
Number of copulating females (the same as percentage of females that remained in the regime beyond day 60 after adult emergence, %)	25 100.0 ^a	38 90.5 ^b	18 94.7 ^{a,b}
Precopulation period (days), <i>n</i>	217 (145–317) ^a 25	166.5 (123–259) ^b 38	122 (98–184) ^c 18
Number of ovipositing females (the same as percentage of females that remained in the regime beyond day 60 after adult emergence, %)	13 52.0 ^a	29 69.0 ^b	17 89.5 ^c
Preoviposition period (days), <i>n</i>	237 (184–342) ^a 13	184 (135–271) ^b 29	144 (101–207) ^c 17
Copulation to oviposition period (days), <i>n</i>	26 (9–95) ^a 11	18 (7–91) ^a 29	18.5 (8–36) ^a 16
Period of oviposition (days), <i>n</i>	63 (5–119) ^a 11	62 (4–108) ^a 28	52 (9–136) ^a 15
Eggs per reproductive female (= the total egg production), <i>n</i>	477 (18–1437) ^a 12	482 (68–1291) ^a 29	395 (45–1496) ^a 17
Egg masses per reproductive female, <i>n</i>	8 (1–19) ^a 13	8 (1–19) ^a 29	5 (1–17) ^a 17
Egg mass size (eggs per egg mass), <i>n</i>	76 (5–129) ^a 99	74 (2–97) ^a 230	78 (1–102) ^a 110

Medians (with range) followed by the same superscript letters within a row are not significantly different ($P > 0.01$; Kruskal–Wallis test followed by the Steel–Dwass test). *n*, number of females, except for ‘Egg mass size’, where it refers to number of egg masses (not all females are included in some analyses because of reasons specific to particular indices). Percentages of copulating and ovipositing females followed by the same superscript letters within a row are not significantly different ($P > 0.01$; Tukey-type multiple comparison test for proportions).

Postdiapause reproduction

After 98–317 days from emergence, adults started to copulate under all three photoperiodic conditions (Fig. 1b). Of the females further exposed to each photoperiodic condition beyond day 60, 90.5–100% survived until copulation (Table 1). The shorter the day length, the longer the precopulation period in females (medians were significantly different between the photoperiodic conditions; Table 1).

After another 7–95 days, females began to deposit eggs (Fig. 1b), although only 52.0–89.5% of the females further

exposed to each photoperiodic condition beyond day 60 survived until oviposition. The shorter the day length, the smaller the percentage surviving and ovipositing females, and the longer the preoviposition period (medians were significantly different between the photoperiodic conditions; Table 1).

The period from the intermediate-to-green colour change to the first copulation or the first oviposition, the period from the first copulation to the first oviposition and the duration of the oviposition period did not differ significantly between the photoperiodic conditions (Table 1). The total adult life span of females differed significantly between the treatments: the shorter

the day length, the longer the females lived (Table 1). However, when either the stable russet period, precopulation or preoviposition periods were subtracted from the total adult life span, the difference between the medians in different photoperiodic conditions became statistically insignificant (Table 1).

No evidence was found indicating that photoperiodic conditions affect reproductive performance after the start of the postdiapause reproduction. Thus, the total life-time egg production (i.e. the number of eggs per reproductive female), the number of egg masses per reproductive female, the egg mass size, the duration of the period from the first copulation to the first oviposition or the duration of the period of oviposition itself did not differ significantly between the photoperiodic conditions (Table 1).

However, the total egg production depended on the duration of the period of oviposition under all three photoperiodic conditions and the correlation was positive and significant (Spearman's rank correlation: $r_s = 0.95$, $n = 11$, $P = 2.7 \times 10^{-3}$ under LD 10 : 14 h; $r_s = 0.7$, $n = 28$, $P = 2.7 \times 10^{-4}$ under LD 12 : 12 h; and $r_s = 0.92$, $n = 15$, $P = 6.1 \times 10^{-4}$ under LD 13 : 11 h). When data for all females from the three photoperiodic conditions were pooled, the overall trend remained the same (Fig. 2a; Spearman's rank correlation $r_s = 0.81$, $n = 54$, $P = 3.8 \times 10^{-9}$).

The total egg production of individual females did not depend on how long females remained russet. The correlation between the total number of eggs produced and the duration of the preceding russet period was positive and significant under LD 10 : 14 h (Spearman's rank correlation: $r_s = 0.64$, $n = 11$, $P = 0.044$), but negative and insignificant under the two other conditions ($r_s = -0.29$, $n = 28$, $P = 0.126$ under LD 12 : 12 h; and $r_s = -0.34$, $n = 15$, $P = 0.2$ under LD 13 : 11 h). When the data for all females from the three conditions were pooled, the resulting overall trend was not significant (Fig. 2b; Spearman's rank correlation $r_s = -0.003$, $n = 54$, $P = 0.98$).

Discussion

Spontaneous termination of diapause

It has long been assumed that low temperature is necessary for termination of insect diapause. Although Andrewartha (1952) suggested that chilling is not required in all species for diapause development (= physiogenesis) and subsequent termination of diapause, the importance of low temperature

exposure has been generalized and overestimated (see Hodek, 1983, 2002; Hodek & Hodková, 1988).

Summer diapause apparently does not require chilling for its termination (Noda, 1984; Musolin & Saulich, 2000), although some decrease of temperature can accelerate it (Masaki, 1980). The requirements for termination of the winter diapause are not well understood (Hodek, 1996, 2002; Košťál, 2006). All temperate zone insects occupying open habitats experience a period of low temperature during the winter and some of them require chilling for termination of diapause (e.g. the mirid bug *Leptopterna dolabrata*; Braune, 1973; weevil *Exechesops leucopis*; Matsuo, 2006). However, the experimental data obtained under laboratory conditions show that, in many species, winter diapause can terminate simply in response to increase of either day length or temperature, or both, without preceding low temperature treatment. For example, in Heteroptera, this is demonstrated in *Dolycoris baccarum* (day length; Babrakzai & Hodek, 1987), *Riptortus clavatus* (day length; Numata, 1987), *Cavelerius saccharivorus* (temperature; Hokyo *et al.*, 1983), *Orius strigicollis* (temperature; Cho *et al.*, 2005), *Oncopeltus fasciatus* (day length and temperature; Dingle, 1974) and *Orius insidiosus* (day length and temperature; van den Meiracker, 1994). Furthermore, diapause in some species can terminate spontaneously without any change in environmental conditions (i.e. when insects continue to experience the conditions that had induced the diapause). For example, in Heteroptera, this is shown in *Carbula humerigera* (Kiritani, 1985), *Plautia crossota stali* (Kotaki, 1998) and *Nysius huttoni* (He *et al.* 2004). Finally, numerous experiments involving transfer of insects from outdoors to the laboratory at different stages of overwintering further supported the idea that chilling is not necessary for termination of winter diapause in many species (Hodek, 1975, 1978; Kingsley & Harrington, 1982), although such a transfer always represents change of conditions and makes it difficult to interpret the results unambiguously.

The results of the present study show that adult diapause in *N. viridula* can terminate spontaneously under short-day conditions without any preceding low temperature treatment (Fig. 1). This species is believed to be of southern origin and it occurs in an ever-increasing range through tropical, subtropical and warm temperate regions (Hokkanen, 1986; Jones, 1988; Kavar *et al.*, 2006; Musolin, 2006). In areas where winter is mild and long-term cold does not occur regularly, it may be advantageous for an insect species to be able to terminate winter diapause within a few months without requiring

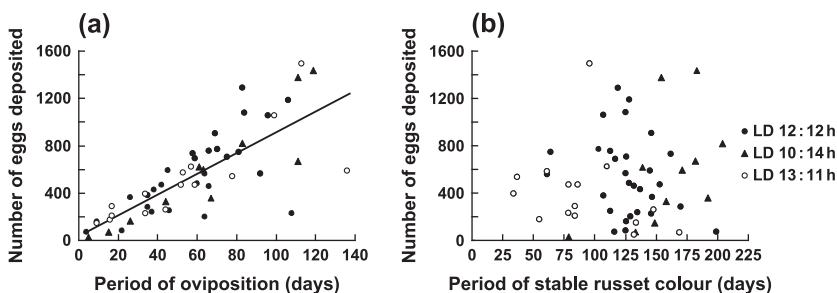


Fig. 2. The relationship between (a) duration of the period of oviposition and total egg production after diapause termination and (b) the period of stable russet colour and total egg production after diapause termination in *Nezara viridula* under different photoperiodic conditions at 25 °C (see text for results of the statistical analysis).

any low temperature exposure. Even though the overwintering physiology in the more southern populations of *N. viridula* has never been studied in detail, it is known that diapause in this species lasts only 2 months in Australia (29°S; Coombs, 2004). Closer to the equator, *N. viridula* may have no pronounced diapause at all and low-intensity reproduction is observed during the colder weeks or months in India (23°N; Singh, 1973) and southern Brazil (23°S; Panizzi & Hirose, 1995; Panizzi, pers. comm.). The expansion of *N. viridula* towards the temperate zone is probably associated with the evolution of a more intensive diapause, which appears to be necessary for survival of severe winters, although termination of this diapause still does not require low temperature.

However, the observed spontaneous termination of diapause without chilling and start of reproduction in *N. viridula* is poorly synchronized: the range of the first oviposition dates is 106–158 days under different short-day conditions at 25 °C (Fig. 3a–c), whereas it is only 43 days under non-diapause long-day conditions at the same temperature (Fig. 3d) and even less (23 days) in adults that overwinter and start reproduction outdoors (Musolin & Numata, 2003b, 2004).

The actual moment of the end of insect diapause (= the end of the termination phase, according to Košťál, 2006) is very difficult, if not impossible, to detect precisely, and externally observed indirect markers vary in different species (Hodek, 1996). The spontaneous start of copulation and oviposition apparently indicates that the diapause is over and postdiapause development has started. In *N. viridula*, almost

all adults change body colour back to green before they start copulation and all females are green on the day of their first oviposition (Fig. 1). Together with a similar trend recorded earlier under outdoor conditions (Musolin & Numata, 2003b), this observation suggests that the brightening of the body colour (i.e. change from russet to intermediate and then green colour) is the closest and most easily observed symptom of the end of the diapause in this species.

Photoperiodic conditions strongly affect the timing of diapause termination in *N. viridula*, even though all the photoperiods tested in the experiment are short enough to induce diapause in many (LD 13 : 11 h) or all females (LD 10 : 14 h and LD 12 : 12 h) (Fig. 4). The shorter the photophase, the longer adults remain russet and the later they start to reproduce; thus, the longer the diapause. The preoviposition period is 10–15-fold longer under the conditions tested in the present study than under the long-day conditions (e.g. LD 14 : 10 h) at the same temperature in a parallel laboratory experiment (Fig. 3; Musolin & Numata, 2003a).

Comparison of diapause incidence 60 days after adult emergence (i.e. the diapause induction response curve) with that on days 180, 210 and 240 after adult emergence further demonstrates that the short-day and near-critical photoperiods used in this experiment differ in their effects on maintaining diapause: the shorter the photoperiod, the longer the diapause is maintained (Fig. 4). It still remains to be investigated whether the duration of diapause in *N. viridula* is determined during the diapause induction phase (as an effect of the day

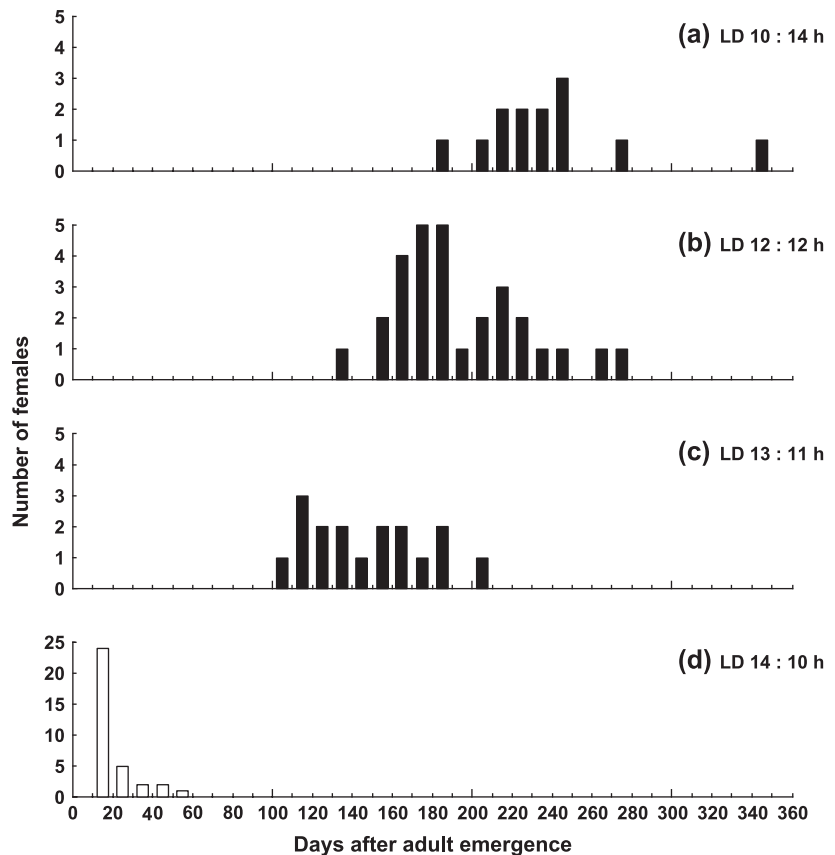


Fig. 3. Preoviposition period in females of *Nezara viridula* under different photoperiodic conditions at 25 °C. Distribution under LD 14 : 10 h (d) is shown for comparison (Musolin & Numata, 2003a).

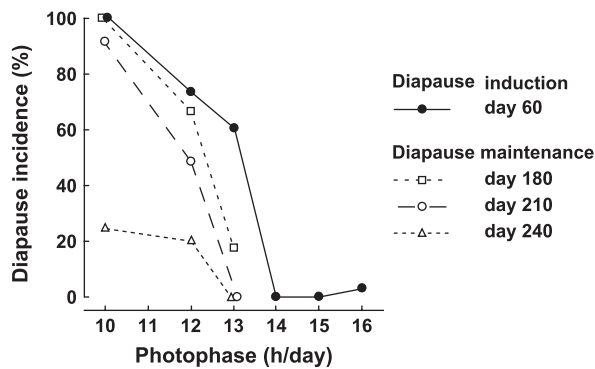


Fig. 4. Effect of day length on diapause induction (judged upon dissection on day 60 after adult emergence; Musolin & Numata, 2003a) and diapause maintenance (on days 180, 210 and 240 after adult emergence; non-ovipositing deep russet females were considered to be in diapause) in *Nezara viridula* at 25 °C.

length experienced; Danks, 1987; Nakamura & Numata, 2000) or the critical photoperiod for termination of diapause decreases during the further process of diapause development (= physiogenesis) (Numata & Hidaka, 1984; Danks, 1987). It is also possible that both processes are involved.

Postdiapause reproduction

Life-history traits such as life-time reproductive success (measured as the total number of offspring produced), lifespan and survival to reproduction are linked and variation in these traits is constrained by trade-offs (Roff, 1992; Zera & Harshman, 2001). Dormancy as a specific prolonged physiological state affects not only winter survival, but also postdiapause reproduction and such relationships may differ in different species (Leather *et al.*, 1993; Ellers & van Alphen, 2002). Thus, the reproductive potential may be higher in a non-diapause than in a postdiapause generation in some species (Sullivan & Wallace, 1967; Fujiie, 1980; Bradshaw *et al.*, 1998; Ito, 2004) or vice versa in others (Spence, 1989; Blanckenhorn, 1994; Chang *et al.*, 1996).

Many insects, especially those in the cooler climate zones, spend up to nine or more months in diapause and postdiapause quiescence and some species have prolonged diapause lasting more than 1 year. Not only diapause itself, but also its duration may affect the reproductive performance after diapause, although, again, the patterns of relationships may differ among different species. Thus, the duration of diapause is negatively correlated with postdiapause egg production in the European pine sawfly *Neodiprion sertifer* (Sullivan & Wallace, 1967), the bruchid *Kytorhinus sharpianus* (Ishihara & Shimada, 1995) and in the spider mite *Tetranychus urticae* (Kroon & Veenendaal, 1998), but such a relationship is not evident in the bumblebee *Bombus terrestris* (Beekman *et al.*, 1998; Beekman & van Stratum, 2000) or the spider mite *Tetranychus kanzawai* (Ito, 2004). Moreover, fecundity of females of the sawfly *Neodiprion swainei* is higher after a

normal (9-month-long) diapause than after prolonged diapause (i.e. after an additional 1 year; Lyons, 1970). However, in the chestnut weevil *Curculio elephas*, fecundity is similar in adults that emerge after a normal (1-year-long) and prolonged (2-year-long) diapause (Menu & Debouzie, 1993). Finally, the results obtained for the cabbage beetle *Colaphellus bowringi* are even more complicated. Short diapause has negative or no effect on reproduction compared with direct development, whereas long diapause has positive effects (Wang *et al.*, 2006). In most of these cases, the experimental protocols used do not allow the separation of the effect of the duration of diapause from those of chilling, or other factors such as the complex genetic structure of the population or difference in body size. In the present experiment with *N. viridula*, the adults are not chilled but experience true diapause, which is exhibited as both the arrest of reproduction and an associated body colour change. The results clearly suggest that postdiapause reproductive performance in *N. viridula* depends on the duration of the period of oviposition but not the duration of the preceding diapause (Fig. 2) or photoperiodic conditions after diapause (Table 1). The duration of diapause apparently affects survival of dormant adults (Fig. 1; Table 1). Fecundity and longevity after diapause appear to be determined by feeding after diapause but not by the duration of the preceding diapause (Table 1).

Comparison of the present data with those obtained earlier under outdoor conditions shows that the mild natural chilling during winter does not reduce the postdiapause reproductive output. Thus, females overwintered outdoors produce even more eggs than do females after diapause proceeded at 25 °C: in the two outdoor experiments, reproductive females produce 614 and 704 eggs (medians; Musolin & Numata, 2003b, 2004), whereas each female produced only 385–482 eggs (medians) under different photoperiods in the present experiment (Table 1). Note that, in both cases, the preoviposition periods are similar (ranged 222–255 days in the females overwintered outdoors; Musolin & Numata, 2003b, 2004; for the data from the present experiment, see Fig. 3).

In summary, the present study suggests that adult diapause in a southern species such as *N. viridula* may not require low temperature treatment for its successful completion and the duration of diapause affects winter survival but not postdiapause reproductive performance or longevity. Such independence of the postdiapause reproductive performance from the duration of diapause may have contributed to the continuous worldwide range expansion of this species into the temperate zone (Panizzi *et al.*, 2000; Musolin, 2006).

Acknowledgements

The research was supported by the Ministry of Education, Culture, Science, Sports and Technology of Japan (Grants-in-Aid for JSPS Fellows no. 98116 and the 21st Century COE Program *Innovative Food and Environmental Studies Pioneered by Entomomimetic Sciences* at Kyoto University, Japan).

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.
- Andrewartha, H.G. (1952) Diapause in the relation to the ecology of insects. *Biological Reviews*, **27**, 50–107.
- Babrákzai, Z.H. & Hodek, I. (1987) Diapause induction and termination in a population of *Dolycoris baccarum* (Heteroptera, Pentatomoidea) from central Bohemia. *Věstník Československé Společnosti Zoologické*, **51**, 85–88.
- Beekman, M. & van Stratum, P. (2000) Does the diapause experience of bumblebee queens *Bombus terrestris* affect colony characteristics? *Ecological Entomology*, **25**, 1–6.
- Beekman, M., van Stratum, P. & Lingeman, R. (1998) Diapause survival and post-diapause performance in bumblebee queens (*Bombus terrestris*). *Entomologia Experimentalis et Applicata*, **89**, 207–214.
- Blanckenhorn, W.U. (1994) Fitness consequences of alternative life histories in water striders, *Aquarius remigis* (Heteroptera: Gerridae). *Oecologia*, **97**, 354–365.
- Bradshaw, W.E., Armbruster, P.A. & Holzapfel, C.M. (1998) Fitness consequences of hibernal diapause in the pitcher-plant mosquito, *Wyeomyia smithii*. *Ecology*, **79**, 1458–1462.
- Braune, H.J. (1973) The role of temperature in controlling obligatory diapause. *Effects of Temperature on Ectothermic Organisms* (ed. by W. Weiser), pp. 233–238. Springer-Verlag, Germany.
- Chang, Y.-F., Tauber, M.J. & Tauber, C.A. (1996) Reproduction and quality of F-1 offspring in *Chrysoperla carnea*: differential influence of quiescence, artificially-induced diapause, and natural diapause. *Journal of Insect Physiology*, **42**, 521–528.
- Cho, J.R., Kim, J.H., Lee, M. & Kim, H.S. (2005) Induction and termination of the reproductive diapause in the minute pirate bug *Orius strigicollis* Poppius (Hemiptera: Anthocoridae). *Journal of Asia-Pacific Entomology*, **8**, 167–174.
- Coombs, M. (2004) Overwintering survival, starvation resistance, and post-diapause reproductive performance of *Nezara viridula* (L.) (Hemiptera: Pentatomidae) and its parasitoid *Trichopoda giacomellii* Blanchard (Diptera: Tachinidae). *Biological Control*, **30**, 141–148.
- Danks, H.V., (1987) *Insect Dormancy: An Ecological Perspective*, Monograph Series N1. Biological Survey of Canada, Canada.
- Danks, H.V. (1994) Diversity and integration of life-cycle control in insects. *Insect Life-Cycle Polymorphism: Theory, Evolution and Ecological Consequences for Seasonality and Diapause Control*, (ed. by H.V. Danks) *Series Entomologica*, Vol. **52**, pp. 5–40. Kluwer Academic Publishers, Germany.
- Dingle, H. (1974) Diapause in a migrant insect, the milkweed bug *Oncopeltus fasciatus* (Dallas) (Hemiptera: Lygaeidae). *Oecologia*, **17**, 1–10.
- Ellers, J. & van Alphen, J.J.M. (2002) A trade-off between diapause duration and fitness in female parasitoids. *Ecological Entomology*, **27**, 279–284.
- Fujii, A. (1980) Ecological studies on the population of the pear leaf miner, *Bucculatrix pyrivorella* Kuroko (Lepidoptera: Lyonetiidae). III. Fecundity fluctuation from generation to generation within a year. *Applied Entomology and Zoology*, **15**, 1–9.
- 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.
- He, X.Z., Wang, Q. & Carpenter, A. (2004) Effect of day length on development and reproductive diapause in *Nysius huttoni* White (Heteroptera, Lygaeidae). *Journal of Applied Entomology*, **128**, 528–532.
- Hodek, I. (1975) Diapause development in *Aelia acuminata* females (Heteroptera). *Věstník Československé Společnosti Zoologické*, **39**, 178–194.
- Hodek, I. (1978) Role of temperature in diapause of *Pyrrhocoris apterus* (Heteroptera). *Věstník Československé Společnosti Zoologické*, **42**, 172–187.
- Hodek, I. (1983) Role of environmental factors and endogenous mechanisms in the seasonality of reproduction in insects diapausing as adults. *Diapause and Life Cycle Strategies in Insects*, (ed. by V.K. Brown and I. Hodek) *Series Entomologica*, Vol. **23**, pp. 9–33. Dr W. Junk Publishers, The Netherlands.
- Hodek, I. (1996) Diapause development, diapause termination and the end of diapause. *European Journal of Entomology*, **93**, 475–487.
- Hodek, I. (2002) Controversal aspects of diapause development. *European Journal of Entomology*, **99**, 163–173.
- Hodek, I. & Hodková, M. (1988) Multiple role of temperature during insect diapause: a review. *Entomologia Experimentalis et Applicata*, **49**, 153–165.
- Hokkanen, H. (1986) Polymorphism, parasites, and the native area of *Nezara viridula* (Hemiptera, Pentatomidae). *Annales Entomologici Fennici*, **52**, 28–31.
- Hokyo, N., Suzuki, H. & Murai, M. (1983) Egg diapause in the oriental chinch bug, *Cavelerius saccharivorus* Okajima (Heteroptera: Lygaeidae). I. Incidence and intensity. *Applied Entomology and Zoology*, **18**, 382–391.
- Ishihara, M. & Shimada, M. (1995) Trade-off in allocation of metabolic reserves: effects of diapause on egg production and adult longevity in a multivoltine bruchid, *Kytorhinus sharpianus*. *Functional Ecology*, **9**, 618–624.
- Ito, K. (2004) Deteriorating effects of diapause duration on postdiapause life history traits in the Kanzawa spider mite. *Physiological Entomology*, **29**, 453–457.
- Jones, W.A. (1988) World review of the parasitoids of the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae). *Annals of the Entomological Society of America*, **81**, 262–273.
- 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.
- Kavar, T., Pavlovič, P., Sušnik, S. et al. (2006) Genetic differentiation of geographically separated populations of the southern green stink bug *Nezara viridula* (Hemiptera: Pentatomidae). *Bulletin of Entomological Research*, **96**, 117–128.
- Kingsley, P.C. & Harrington, B.J. (1982) Factors influencing termination of reproductive diapause in *Orius insidiosus* (Hemiptera: Anthocoridae). *Environmental Entomology*, **11**, 461–462.
- 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, Japan.
- Kiritani, Y. (1985) Effect of stationary and changing photoperiods on nymphal development in *Carbula humerigera* (Heteroptera: Pentatomidae). *Applied Entomology and Zoology*, **20**, 257–263.
- 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)*, **2**, 199–207.
- Košťál, V. (2006) Eco-physiological phases of insect diapause. *Journal of Insect Physiology*, **52**, 113–127.
- Kotaki, T. (1998) Effects of low temperature on diapause termination and body colour change in adults of a stink bug, *Plautia stali*. *Physiological Entomology*, **23**, 53–61.

- Kroon, A. & Veenendaal, R.L. (1998) Trade-off between diapause and other life-history traits in the spider mite *Tetranychus urticae*. *Ecological Entomology*, **23**, 298–304.
- Leather, S.R., Walters, K.F.A. & Bale, J.S. (1993) *The Ecology of Insect Overwintering*. Cambridge University Press, U.K.
- Lyons, L.A. (1970) Some population features of reproductive capacity in *Neodiprion swaini* (Hymenoptera: Diprionidae). *The Canadian Entomologist*, **102**, 68–84.
- Masaki, S. (1980) Summer diapause. *Annual Review of Entomology*, **25**, 1–25.
- Matsuo, Y. (2006) Cost of prolonged diapause and its relationship to body size in a seed predator. *Functional Ecology*, **20**, 300–306.
- van den Meiracker, R.A.F. (1994) Induction and termination of diapause in *Orius* predatory bugs. *Entomologia Experimentalis et Applicata*, **73**, 127–137.
- Menu, F. & Debouzie, D. (1993) Coin-flipping plasticity and prolonged diapause in insects: example of the chestnut weevil *Curculio elephas* (Coleoptera: Curculionidae). *Oecologia*, **93**, 367–373.
- Musolin, D.L. (2006) Insects in a warmer world. Ecological, physiological and life-history responses of Heteroptera to climate change. *Global Change Biology*, **12**, (in press).
- Musolin, D.L. & Numata, H. (2003a) Photoperiodic and temperature control of diapause induction and colour change in the southern green stink bug *Nezara viridula*. *Physiological Entomology*, **28**, 65–74.
- Musolin, D.L. & Numata, H. (2003b) Timing of diapause induction and its life-history consequences in *Nezara viridula*: is it costly to expand the distribution range? *Ecological Entomology*, **28**, 694–703.
- Musolin, D.L. & Numata, H. (2004) Late-season induction of diapause in *Nezara viridula* and its effect on adult coloration and post-diapause reproductive performance. *Entomologia Experimentalis et Applicata*, **11**, 1–6.
- Musolin, D.L. & Saulich, A.H. (2000) Summer dormancy ensures univoltinism in the predatory bug *Picromerus bidens*. *Entomologia Experimentalis et Applicata*, **95**, 259–267.
- Nakamura, K. & Numata, H. (2000) Photoperiodic control of the intensity of diapause development in the bean bug, *Riptortus clavatus* (Heteroptera: Alydidae). *European Journal of Entomology*, **97**, 19–23.
- Noda, T. (1984) Short day photoperiod accelerates the oviposition in the oriental green stink bug, *Nezara antennata* Scott (Heteroptera: Pentatomidae). *Applied Entomology and Zoology*, **19**, 119–120.
- 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, Japan. [In Japanese]
- Numata, H. (1987) Photoperiodic sensitivity after diapause termination in the bean bug, *Riptortus clavatus* Thunberg (Heteroptera: Coreidae). *Applied Entomology and Zoology*, **22**, 352–357.
- Numata, H. & Hidaka, T. (1984) Photoperiodic control of adult diapause in the bean bug, *Riptortus clavatus* Thunberg (Heteroptera: Coreidae). III. Diapause development and temperature. *Applied Entomology and Zoology*, **19**, 356–360.
- 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.
- 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. et al. (2000) Stink bugs (Pentatomidae). *Heteroptera of Economic Importance* (ed. by C.W. Schaefer and A.R. Panizzi), pp. 421–474. CRC Press, Boca Raton, FL.
- Roff, D.A. (1992) *The Evolution of Life Histories: Theory and Analysis*. Chapman & Hall, New York, NY.
- Singh, Z. (1973) *Southern Green Stink Bug and its Relationship to Soybeans: Bionomics of the Southern Green Stink Bug Nezara viridula (Hemiptera: Pentatomidae) in Central India*. Metropolitan Book Co. (PVT) Ltd, India.
- Spence, J.R. (1989) The habitat templet and life history strategies of pond skaters (Heteroptera: Gerridae): reproductive potential, phenology, and wing dimorphism. *The Canadian Journal of Zoology*, **67**, 2432–2447.
- Sullivan, C.R. & Wallace, D.R. (1967) Interaction of temperature and photoperiod in the induction of prolonged diapause in *Neodiprion sertifer*. *The Canadian Entomologist*, **99**, 834–850.
- Tauber, M.J. & Tauber, C.A. (1976) Insect seasonality: Diapause maintenance, termination, and postdiapause development. *Annual Review of Entomology*, **21**, 81–107.
- Tauber, M.J., Tauber, C.A. & Masaki, S. (1986) *Seasonal Adaptations of Insects*. Oxford University Press, New York, NY.
- Todd, J.W. (1989) Ecology and behavior of *Nezara viridula*. *Annual Review of Entomology*, **34**, 273–292.
- Wang, X.-P., Xue, F.-S., Hua, A. & Ge, F. (2006) Effects of diapause duration on future reproduction in the cabbage beetle, *Colaphellus bowringi*: positive or negative? *Physiological Entomology*, **31**, 190–196.
- Yanai, H. (1998) *Four Steps in Excel Statistics*. OMS Ltd, Japan. [In Japanese]
- Zar, J.H. (1999) *Biostatistical Analysis*, 4th edn. Prentice Hall, Inc., Upper Saddle River, NJ.
- Zera, A. & Harshman, L.G. (2001) The physiology of life history trade-offs in animals. *Annual Review of Ecology and Systematics*, **32**, 95–126.

Accepted 21 July 2006

First published online 19 October 2006