

Timing of diapause induction and its life-history consequences in *Nezara viridula*: is it costly to expand the distribution range?

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Abstract. 1. From the early 1960s to 2000 *Nezara viridula* (Heteroptera: Pentatomidae) expanded its range northwards in Japan and reached Osaka following climate warming recorded in the region.

2. The timing of diapause induction and its effect on life-history traits were studied under quasi-natural conditions in Osaka. Egg masses were placed outdoors in six series in July–November 1999. Developmental events were monitored until September 2000.

3. Adult diapause was induced in September–October in agreement with the photoperiodic response obtained under laboratory conditions. Induction of diapause in early October ensured the highest winter survival. Nymphs that hatched after late September died by December–April showing that the species cannot survive winter in the nymphal stage.

4. Life-history traits varied between the early (non-diapause reproduction) and late (post-diapause reproduction) series. Thus, non-diapause females produced significantly fewer egg masses than did females that reproduced only after diapause. The timing of diapause induction strongly affected overwintering success and post-diapause performance: females that became adults and entered diapause in October lived longer, had a longer period of oviposition, and produced more eggs in larger egg masses than females that attained adulthood and entered diapause in September.

5. Females from the early series reproduced until late November, although progeny from the late September eggs were destined to die during the winter. Pre-winter reproduction of adults that emerged in mid-September or later was a result of the imperfect timing of diapause induction. It is an ineffective allocation of resources and may be considered the ecological cost of range expansion.

6. To establish in the region, *N. viridula* will probably evolve a lengthening of the critical photoperiod of the diapause induction response. This will allow the species to enter diapause earlier and, thus, avoid maladaptive late-autumn reproduction but, perhaps, increase the cost of diapause because of a possible adverse impact of pre-winter high temperature conditions on overwintering.

Key words. Climate change, cost of diapause, Heteroptera, life cycle, life-history traits, overwintering, range expansion, reproduction, seasonal adaptations.

Introduction

Mean global surface temperatures have increased since the late 19th century by approximately 0.3–0.6 °C with the 1990s being the warmest decade, and further climate

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warming is predicted with an estimated increase of 1.4–5.8 °C by 2100 (Houghton *et al.*, 2001). Changes in air temperature, as well as associated changes in precipitation and the composition of the atmosphere, are likely to have both direct and indirect effects on the physiology, distribution, phenology, abundance, and adaptations of insects (Harrington & Stork, 1995; Butterfield & Coulson, 1997; Hughes, 2000; McLaughlin *et al.*, 2002). Advances in phenology and directional changes in distribution such as a northward expansion of range and poleward shifts of whole species ranges have been documented in many insect species and considered as common trends driven by climate change (Parmesan & Yohe, 2003; Root *et al.*, 2003); however, it is still unknown how range expansion caused by climate warming influences life-history traits, and physiological and life-cycle adaptations of a species in a newly colonised region. How can a species successfully occupy an area that was outside of its distribution range only several years or decades earlier? Does range expansion entail any costs in terms of fitness?

The southern green stink bug *Nezara viridula* (L.) (Heteroptera: Pentatomidae) occurs throughout tropical and subtropical regions of the world and produces up to five or six generations per year (Panizzi *et al.*, 2000). In Japan, the species has long been known from the southern part of the archipelago (Kiritani, 1971). Mochida (1991) predicted that CO₂-associated climate change would cause a northward range expansion of *N. viridula* in Japan. Recently, adults of the species were observed in Osaka, central Japan (Musolin & Numata, 2003), at least 70 km further north than the northern limit of its distribution reported in the early 1960s (Kiritani *et al.*, 1963).

The adults of *N. viridula* overwinter under litter, bark, or other shelters in a reproductive diapause (Kiritani *et al.*, 1966a; 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 diapause in temperate populations is controlled by photoperiod and associated with a seasonal polyphenism: adults change body colour from green to brown or russet (Ali & Ewies, 1977; Harris *et al.*, 1984; Musolin & Numata, 2003).

Nezara viridula is still not numerous in Osaka; however, reproductive adults of the species were continuously collected in the field from early June until late August and nymphs were recorded until late autumn. These observations suggested that at the beginning of winter, not only adults but also nymphs of different instars are present in the wild population.

The objective of this study was to examine by experimental methods how *N. viridula* is adapted to the environmental conditions of the recently colonised area, whether the species can overwinter in Osaka in the adult or nymphal stage, and how the timing of diapause induction affects winter survival and basic life-history traits of the post-diapause reproduction.

Materials and methods

Insects and the rearing procedure

Adults of *N. viridula* were collected in and around a soybean field on the campus of Osaka City University (Japan, 34.7°N; 135.5°E) in July–August 1999. These adults, and the insects in the subsequent experiment, were reared under quasi-natural conditions: metal shelves open from three sides and sheltered from rain, direct sunlight, and artificial illumination were placed on the campus of the university. Air temperature was recorded daily with a minimum/maximum thermometer on the middle shelf where the insects were kept.

The field-collected adults were reared in transparent plastic containers (diameter 150 mm, depth 90 mm; lids with openings for aeration covered with mesh) and supplied with dry soybean *Glycine max* (L.) Merrill seeds and shelled raw peanuts *Arachis hypogaea* L. and water containing 0.05% sodium L-ascorbate and 0.025% L-cysteine (Noda, 1991). At intervals, from July until November 1999, fresh egg masses produced by these adults were transferred to smaller transparent plastic containers (diameter 90 mm, depth 45 mm; lids with openings for aeration covered with mesh) and reared under the same quasi-natural conditions, thus setting six series of the experiment (Fig. 1). To provide a higher heterogeneity of the experimental material, in each series the progeny of two to four females was used. Upon hatching, nymphs, and subsequently adults were provided with the same diet as the field-collected adults. Food and water were replaced every other day. The density of nymphs was kept at 40–60 per container for the first and second instars and was then gradually reduced to five to six per container by the final (fifth) instar. The emergence of adults was checked daily.

After adult emergence, bugs were sexed, and each female–male pair was transferred to a new container (diameter 90 mm, depth 45 mm) and reared as described above. If the male in a pair died, it was replaced with another from the same series. The start of copulation, dates of oviposition, changes of body colour, and mortality were recorded daily until December 1999, every other day during the winter (December–February), and then again daily from March until the end of September 2000 when only two females remained alive and the experiment was terminated. In addition, the size of egg masses deposited after overwintering was recorded by counting all eggs in each egg mass. To record changes in the body colour of adults, the following grades were used: *green*, *intermediate* (approximately 40–60% of the body surface is light russet), and *russet* (Musolin & Numata, 2003).

On 1 December 1999, in each series of the experiment, russet individuals were transferred into group transparent plastic containers (diameter 150 mm, depth 90 mm) at a density of 8–10 pairs per container and provided with food and water as described above. Several leaves of deciduous trees were also added to provide shelter for overwintering. Adults of two other colour grades (mostly in series 2) were

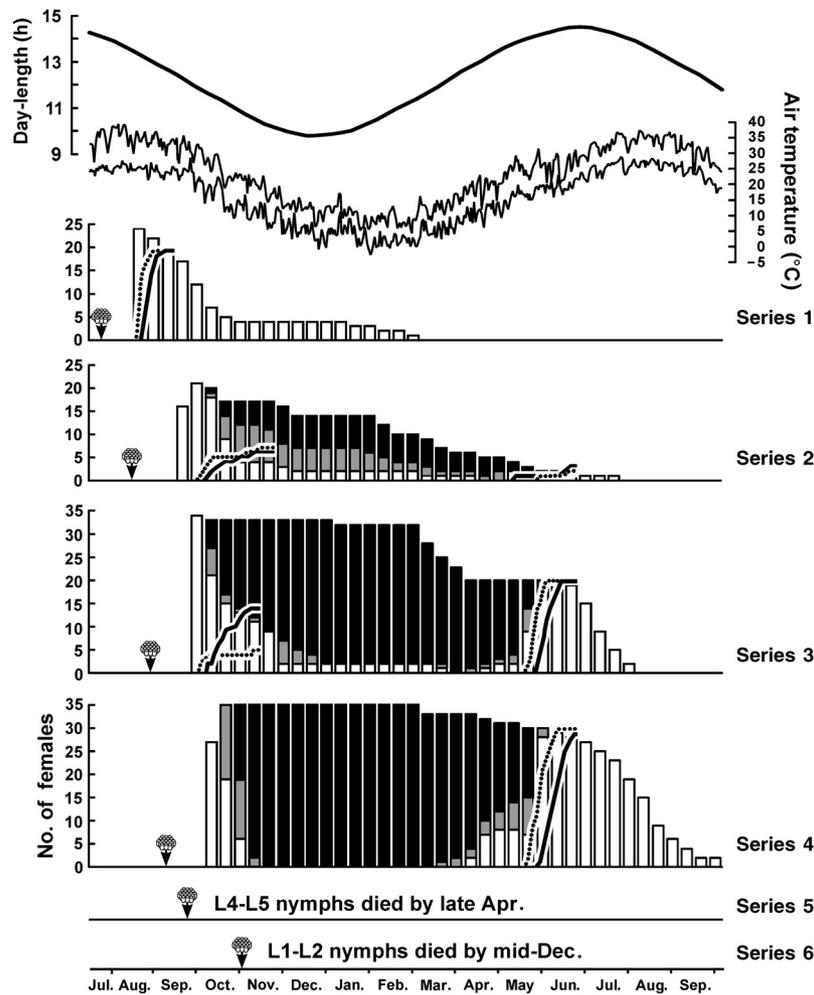


Fig. 1. Seasonal development of *Nezara viridula* under quasi-natural conditions in six series of the experiment. The symbol for egg mass shows the date when the series was initiated. Histograms show the adult female emergence timing, survival, and relative abundance of colour grades of the females: *green* (white bars), *intermediate* (grey bars), *russet* (black bars). The dotted line shows cumulative copulation and the solid line cumulative oviposition. L1–L5 refer to nymphal instars. Environmental conditions (at the top of the figure): thick line, natural day-length; thin lines, daily maximum and minimum air temperature at the experimental shelves.

kept in pairs as before or in groups (separately for each colour grade and series), but again leaves were provided for shelter. When in spring adults started to change colour, they were transferred to the corresponding colour grade container. When copulation was observed, the pair was transferred into a smaller container (diameter 90 mm, depth 45 mm).

Survivorship, patterns of non- and post-diapause reproduction, and diapause-associated adult body colour changes were then compared between series of the experiment to examine whether *N. viridula* can overwinter in Osaka in the adult or nymphal stage and how timing of diapause induction affects survivorship and basic life-history traits. For assessment of reproductive performance, the following reproductive parameters were calculated: number of egg masses produced by each female, number of eggs in each

egg mass, total number of eggs produced by each female, and 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 the statistical analysis of different parameters of reproduction the Steel–Dwass test was used (Excel Tokei, version 5.0, Esumi Ltd, Tokyo, Japan, 2001). For analysis of the relationship between period of oviposition and total number of eggs produced the Spearman's rank correlation procedure was applied (Yanai, 1998). Other tests were conducted according to Zar (1999) and are specified in the figure legends and in the text.

Results

Seasonal development

In series 1, eggs were deposited on 22 July, nymphs developed quickly and adults emerged in mid-August (Fig. 1; data on males are similar and not presented). In 9.1 ± 2.6 days (mean \pm SD) after the emergence of the females, all adults copulated and in another 5.9 ± 2.6 days, all females produced their first egg masses (Fig. 1). Most of the adults had died by late October and all were dead by early spring of the next year.

In series 2, eggs were deposited on 14 August and adults emerged in mid-September. Out of 21 females, only seven copulated 24.0 ± 15.6 days after emergence and only six females started oviposition before the winter. Most of the adults changed body colour from green to russet starting from early October. Less than 50% of adults of both sexes survived until early spring of the next year. Mortality was high in spring and only three females survived till oviposition.

In series 3, eggs were deposited on 29 August and adults emerged in late September. Only five out of 34 females copulated, but 14 females oviposited before winter and produced a small number of mostly non-fertilised egg masses. Almost all adults changed body colour from green to russet during October–December and survived winter with low mortality obviously in a state of reproductive diapause. The rates of mortality increased sharply in spring. All 20 surviving females changed body colour back to green in early June, copulated and laid eggs. Apparently, 14 females that reproduced before winter died by spring.

In series 4, eggs were deposited on 9 September. No females copulated or produced eggs before winter. All adults changed colour to russet and survived winter with zero mortality. In spring, mortality in this series was also significantly lower than in the earlier series (Tukey-type multiple comparison test for proportions, $P < 0.05$). All females changed body colour to green, copulated, and produced eggs.

In the two final series, eggs were deposited on 24 September and 2 November, respectively. Upon hatching, nymphs suffered high mortality and gradually all died by mid-December in the first or second instar (series 6) or by late April in the fourth or fifth instar (series 5).

Late-season behaviour and reproduction

Soon after their transfer into group containers on 1 December 1999, russet-coloured adults discontinued active feeding and started gathering into groups inside the leaves of deciduous trees provided as shelter. The lowest daily air temperature dropped below 0°C only during two nights in December (-1.0 and -0.3°C), four nights in January (-2.7 to -0.2°C) and two nights in February (-0.6 and -0.3°C) (Fig. 1), but in daytime the highest temperature sometimes was as high as $+15.8^\circ\text{C}$ (December)

or $+17.2^\circ\text{C}$ (January). During such warm hours, many adults left their shelter, drank water, and probably fed on peanuts and soybeans.

Green-coloured adults and sometimes those in the intermediate colour grade continued to copulate until 5 December (series 1 and 2) or 24 November (series 3). The last egg masses before winter were produced on 13 November (series 1), 23 November (series 2), and 25 November (series 3) (Fig. 2). About 35% of all egg masses in series 1 were produced after 24 September, the date of commencement of series 5, in which all nymphs died during winter. Thus, this portion of the late-season reproduction was destined to die during winter too. In the next two series, all pre-winter egg masses were produced after 24 September and these egg masses amounted to 59.1% (series 2) and 22.0% (series 3) of the total number of egg masses produced both before and after winter in these series.

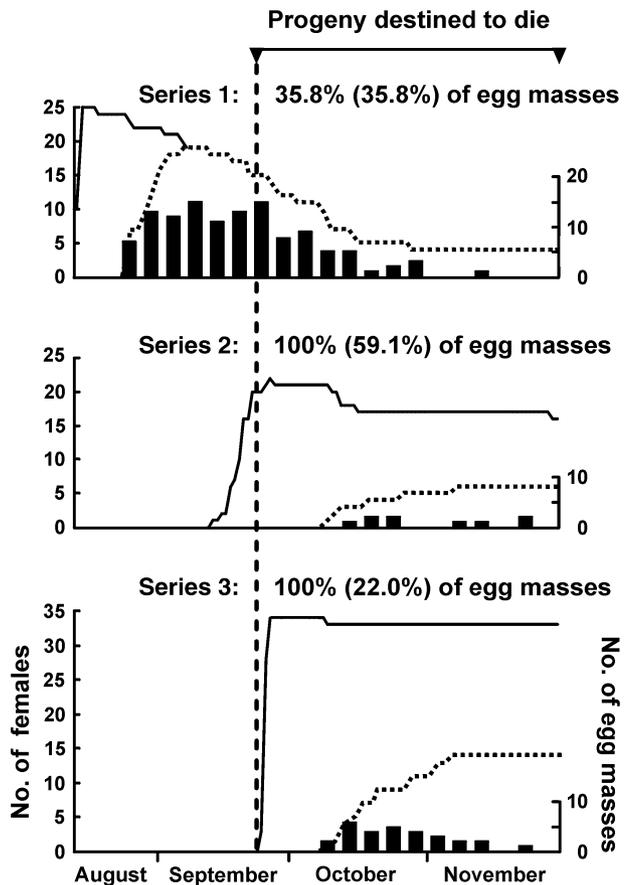


Fig. 2. Late-season reproduction and fate of the progeny in three early series of the experiment. Histograms show all egg masses produced during August–November; solid line, surviving females; broken line, ovipositing females. Percentages show the proportion of egg masses laid before winter, and from which all emerging nymphs died during the winter (in parentheses: the same egg masses as a percentage of the total number of egg masses produced before and after winter).

The relationship between adult body colour and reproductive state of adults

To assess the relationship between the body colour of adults and their reproductive state, all insects of both sexes were considered as three phenological groups: *summer* (adults from series 1 that all reproduced in summer 1999), *autumn* (those from series 2 and 3 that reproduced in autumn 1999), and *spring* (those from series 2, 3, and 4 that reproduced in spring 2000). All adults on the day of the first copulation and all females on the day of first oviposition were green in the *summer* group. However, a significant proportion of adults was intermediately coloured or russet on the day of the first copulation and/or oviposition in the *autumn* and *spring* groups (8.3–30.2%, $n = 12$ –53, Tukey-type multiple comparison test for proportions, $P < 0.05$).

Life-history traits in different series of the experiment

Egg production. Females in different series of the experiment produced eggs before winter, after winter, or in both seasons. Before winter, females of only the three early series reproduced and reproductive females from series 1 produced significantly more egg masses than reproductive females from the two later series (Fig. 3a). After winter, females of only series 2–4 reproduced. In contrast to the pre-winter reproduction, the number of egg masses deposited after winter was significantly larger in series 4 than in series 2 or 3 (Fig. 3b). Thus, the later the adults emerged in autumn, the greater the number of egg masses produced after overwintering. The data in Fig. 1 and records of individual rearing suggested that each female produced eggs only either before or after overwintering. If so, the emergence of adults in September resulted in a reduced reproductive output in females. Thus, females in series 2 and 3 that were reproductive before winter produced significantly fewer egg masses than females that emerged as adults and started reproduction earlier in series 1 (Fig. 3a), whereas females in these series that survived winter and started reproduction only in spring produced significantly fewer egg masses than females in series 4 that emerged later in the previous autumn and all reproduced only in spring after winter diapause (Fig. 3b).

Records of the size of egg masses deposited after overwintering showed that females of series 4 produced not only significantly more egg masses than did females from the earlier series, but also significantly more eggs (Fig. 3c), because the size of the egg masses was significantly larger in this series than in the two previous series (Fig. 3d).

Reproductive life span. Before winter, the period of oviposition was significantly longer in series 1 than in series 3 (Fig. 4a); however, after overwintering, females from series 4 produced eggs for twice as long as those in series 3 (Fig. 4b). In series 2, only three females survived until spring and only one of them produced more than one egg mass.

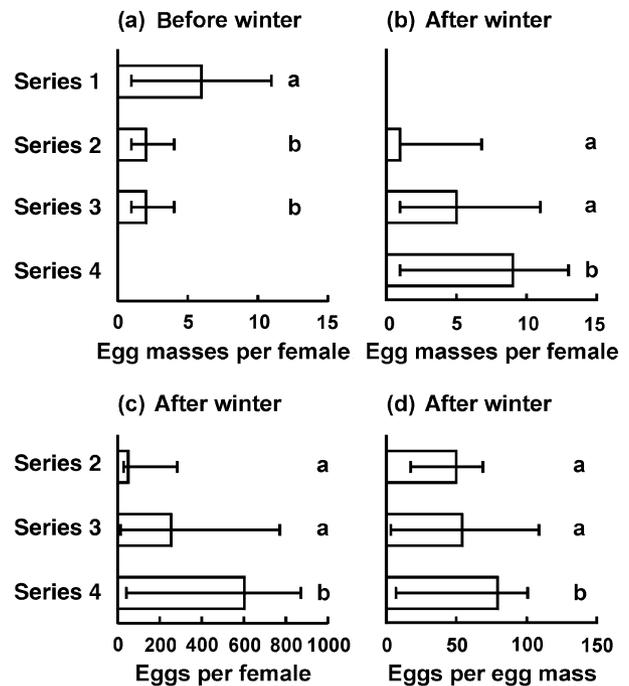


Fig. 3. Parameters of reproduction in different series of the experiment before and after winter (December–February): number of egg masses per female before winter (a) and after winter (b); number of eggs per female after winter (c); and egg mass size after winter (d). Medians (with range) followed by the same letters within each histogram are not significantly different ($P > 0.05$ by the Kruskal–Wallis test followed by the Steel–Dwass test). Females $n = 6$ –19 (a), 3–29 (b), 3–29 (c). Egg masses $n = 8$ –254 (d).

Start of copulation and oviposition after overwintering. In spring, adults started to copulate somewhat earlier in series 3 than in series 4: the median date of the first copulation was 25 May in series 3 and 28 May in series 4 (Mann–Whitney

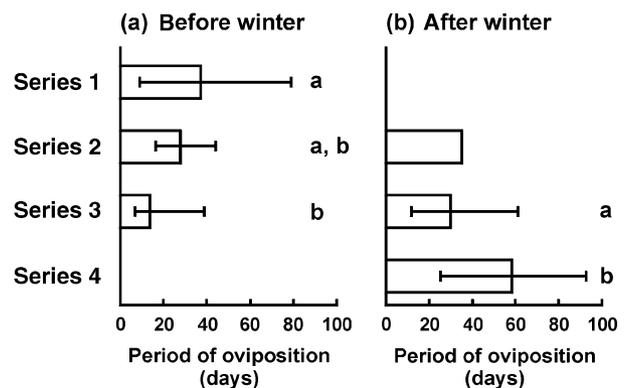


Fig. 4. Period of oviposition (from the first to the last oviposition) in different series of the experiment relative to winter (December–February). Medians (with range) followed by the same letters within each histogram are not significantly different [$P > 0.05$ by Kruskal–Wallis test followed by Steel–Dwass test (a), or by Mann–Whitney U -test, $n > 2$ (b)]. $n = 4$ –17 (a), 1–27 (b).

U-test, Julian days, $U=207$, $P=0.032$). Females also started oviposition significantly earlier in series 3 than in series 4: the median date of the first egg mass deposited was 3 June in series 3 and 8 June in series 4 (Mann–Whitney *U*-test, Julian days, $U=143$, $P=0.001$). In series 2 only two pairs copulated (29 May and 16 June) and only three females produced eggs (starting on 11 May, 15, and 18 June).

Longevity. The longevity of adults was measured only in series 3 and 4, in which more than 50% of adults survived until spring. Adults in these series were divided into two groups: (1) those that died before or during overwintering (i.e. before the spring reproduction), and (2) those that reproduced in spring and died later. In both groups and in both sexes, adults from series 4 lived longer than those from series 3, but the difference was significant only in females that reproduced in spring (Mann–Whitney *U*-test, $U=180$, $P=0.009$). These results suggest that adults (at least females) that had emerged and entered diapause later in autumn lived longer in spring.

The relationship between reproductive life span and egg production after overwintering. When the total number of eggs produced after overwintering was plotted against the duration of the period of oviposition in each female together in series 3 and 4, a significant positive correlation was found: the longer the period of oviposition in spring–summer, the larger the number of eggs produced (Spearman's rank correlation $r_s=0.81$, $n=46$, $P=4 \times 10^{-8}$); however, when the data on females from these two series were tested separately, it became obvious that the relationships between the period of oviposition and egg production after overwintering are different in the two series: the correlation was still significant in series 3 ($r_s=0.86$, $n=19$, $P=2.6 \times 10^{-4}$; Fig. 5), but not in series 4 ($r_s=0.36$, $n=27$, $P=0.064$). It seems that females from series 4 realised their reproductive potential completely, whereas females from series 3 did not live long enough to do so.

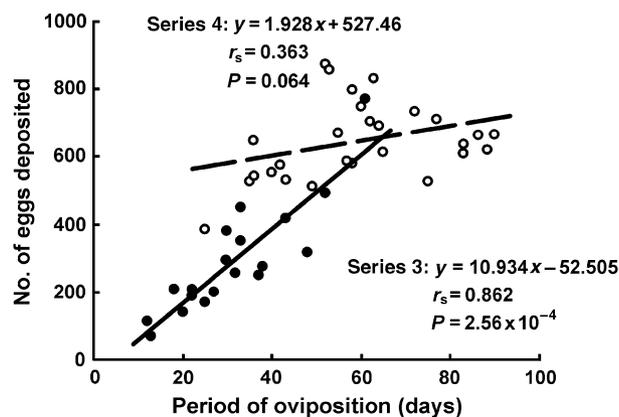


Fig. 5. The relationship between duration of the period of oviposition and total number of eggs produced after overwintering for series 3 and 4. Spearman's rank correlation coefficients r_s , P , corresponding regression equations, and trend lines are shown separately for series 3 (●, solid line) and series 4 (○, broken line). x in the regression equations is the period of oviposition (in days).

Egg mass size as a function of age. When all egg masses produced in series 3 and 4 after overwintering were plotted against a calendar scale (Fig. 6), the difference between these series in the patterns of the post-overwintering reproduction became even more evident. In series 3, the size of egg masses varied markedly throughout the period of oviposition without any directional trend (Fig. 6a). In contrast, in series 4, the size of egg masses tended to increase for a short period (the third and fourth egg masses were usually the largest) and then slowly decreased until death (Fig. 6b).

Discussion

Range expansion

Nezara viridula has a worldwide distribution, occurring throughout the tropics and subtropics between latitudes 45°N and 45°S, and this wide range is still spreading (Todd, 1989; Panizzi *et al.*, 2000). In Japan, the species has long been distributed in the southern part of the archipelago (Kiritani, 1971). Based on a wide-scale field survey, Kiritani *et al.* (1963) showed that in the early 1960s the northern limit of distribution of *N. viridula* in central Japan was in Wakayama Prefecture, about 70 km south from Osaka.

Results of the field collection in the present study and those performed in 1998–2000 in Osaka (Musolin & Numata, 2003) demonstrate that *N. viridula* has expanded its distribution range in central Japan during the last 40 years as predicted by Mochida (1991) and reached Osaka. Kiritani *et al.* (1963) showed that the northern limit of the species' range coincided with the +5°C isotherm of mean January temperature and suggested that climatic conditions primarily determined the northern limit of its distribution in central Japan. Since the early 1960s, mean ambient air temperature for the three coldest months (December–February) and annual mean air temperature have been tending to increase in Osaka (Fig. 7; National Astronomical Observatory, 2001). Mean temperature for each of these three months and annual mean in the 1990s were higher by approximately 1–2°C than those in 1960s. Furthermore, from 1987 to 2000, the mean temperature of January, the critical month according to Kiritani *et al.* (1963), always exceeded +5°C. Besides, temperature was compared between Osaka and Tadono (at a meteorological stations located within the distribution range of *N. viridula* in Wakayama Prefecture close to its northern limit as recorded in the early 1960s). The mean decade temperatures for each of the three winter months were lower in Osaka in the 1960s than in Tadono during a decade prior to the survey of Kiritani *et al.* (1963), but have increased and become comparable or higher in Osaka by the 1990s (The Central Meteorological Observatory, 1954–1963; Japan Meteorological Agency, 2003).

The trends of the monthly lowest air temperatures during the winter in Osaka were similar to those of the mean temperatures: in the 1990s the monthly lowest temperatures

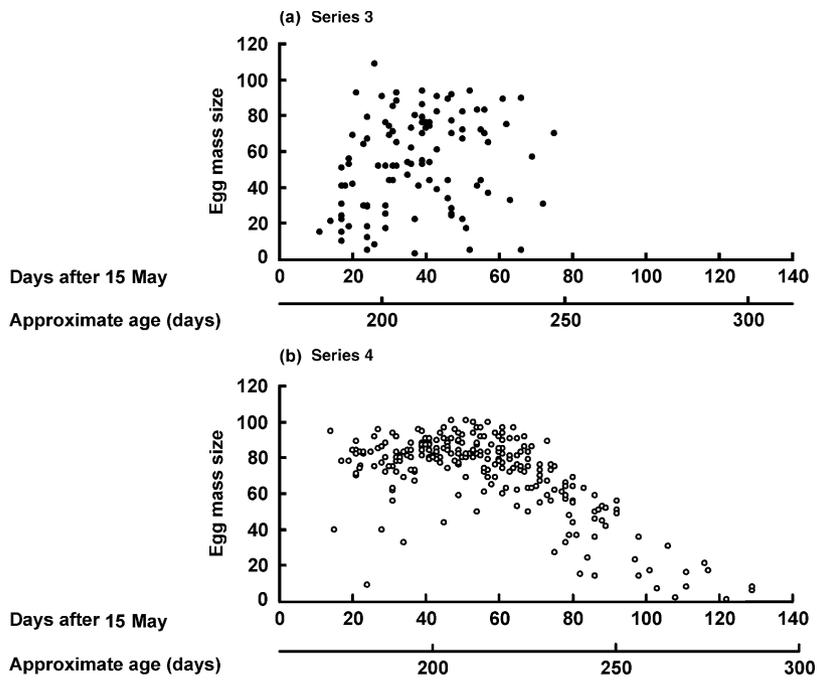


Fig. 6. Size of egg masses produced after overwintering as a function of age. Female age is approximate because females moulted to adults within 3 days in 1999 and were reared in groups during winter. Females $n = 19$ for series 3 (a) and 27 for series 4 (b).

of the three winter months were approximately 2°C higher than those in the 1960s. Furthermore, decade means of the lowest temperatures were always higher in Osaka in the

1960s–1990s than in Tadono in the 1950s–1960s (The Central Meteorological Observatory, 1954–1963; Japan Meteorological Agency, 2003).

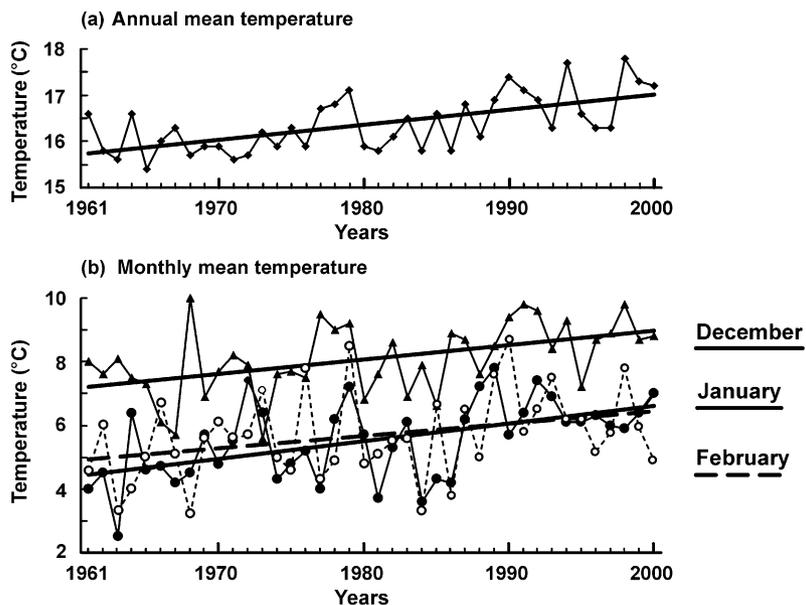


Fig. 7. Changes in air temperature in Osaka between 1961 and 2000 (data from National Astronomical Observatory, 2001). Annual mean (a) and monthly means for the three coldest months (b) are shown along with linear regression trend lines. For annual mean, $F_{1,39} = 24.18$, $P < 0.01$; for monthly means: January, solid lines and solid circles, $F_{1,39} = 14.31$, $P < 0.01$; February, broken lines and open circles, $F_{1,39} = 4.85$, $0.01 < P < 0.05$; and December, solid lines and triangles, $F_{1,39} = 10.66$, $P < 0.01$ (ANOVA).

These meteorological data indicate that the winter temperatures were higher, and thus overwintering conditions for *N. viridula* were less severe, in Osaka in the 1990s than they were within the northern part of the species' range in Wakayama Prefecture in the early 1960s. Thus, it is suggested that climate warming is an important factor that has favoured the northward range expansion of *N. viridula* in central Japan, similarly to some other insect species in Japan and worldwide (Yoshio & Ishii, 1998; Hughes, 2000; Bale *et al.*, 2002; Parmesan & Yohe, 2003). Such biological traits of *N. viridula* as its wide distribution range, strong ability to migrate over long distances, and wide polyphagy (Todd, 1989; Panizzi *et al.*, 2000) also may have contributed to the range expansion as general predictions suggested (Butterfield & Coulson, 1997; Cannon, 1998).

Overwintering and seasonal development at the edge of the distribution range

Nezara viridula can survive winter in Osaka in a state of adult diapause. If adults emerge and enter diapause in late September–October, winter mortality caused by temperature conditions may be very low. However, nymphs are unable to survive winter even in later instars when food is available throughout the winter (Fig. 1, series 5 and 6), contrary to subtropical regions (e.g. Brazil) where adults can breed on different host plants all the year round and nymphs can survive a cold season (Panizzi, 2000).

There is no direct evidence that adults of *N. viridula* overwinter in Osaka in the wild, although the present experiment shows that it is possible. If adults normally do not overwinter in Osaka, it may be supposed that they migrate annually from some regions further south, for example, Wakayama Prefecture. In both cases (i.e. local overwintering and annual migrations) the species can produce three generations during the summer season in Osaka. Adults of the overwintered generation change body colour (and probably migrate, at least locally) in April–May, copulate starting from late May, lay eggs from early June, produce two non-diapause generations and a third one that mostly does not start reproduction, probably migrates (at least locally), changes body colour, and enters reproductive winter diapause.

Records of adult body colour on the day of the first copulation/oviposition further suggest that the coloration of adults serves as a good but not absolute indicator of reproductive status, especially during the periods of diapause induction and termination as supposed earlier (Seymour & Bowman, 1994; Jones & Westcott, 2002) and shown in laboratory experiments (Musolin & Numata, 2003).

Timing of diapause induction and its life-history consequences

The timing of diapause induction in series 2–4 was in good agreement with the results of the previous laboratory

experiments. In Osaka, adult diapause induction in this species is controlled by a long-day photoperiodic response with a critical day-length close to 12.5 h at 20 °C and 25 °C (Musolin & Numata, 2003). Natural day-length (without twilight) in Osaka is 12 h 53 min on 1 September, 12 h 27 min on 15 September, and 11 h 48 min on 1 October. Day-length determined reproduction in all females that emerged before 1 September (Fig. 1; series 1), but induced diapause in approximately 60% of females that attained adulthood in the second half of September (series 2 and 3) and in 100% of those that attained adulthood during the first half of October (series 4). The critical day-length for induction of reproductive diapause is shorter in *N. viridula* than in several other seed-sucking heteropterans that have long lived in the Osaka area (Numata & Nakamura, 2002). Consequently, *N. viridula* enters diapause later in the season than those species do.

The timing of adult emergence and diapause induction in autumn is crucial in terms of fitness, winter survival, and post-diapause reproduction of *N. viridula* in Osaka. The life history theory suggests that life-history traits such as lifetime reproductive success (measured as number of offspring), reproductive lifespan, and survivorship to reproduction are linked and variation in these traits is constrained by trade-offs (Roff, 1992; Zera & Harshman, 2001). The incorporation of diapause with its specific physiological requirements, costs, and benefits makes these relationships and trade-offs even more complicated (Leather *et al.*, 1993; Nylin, 2001; Ellers & van Alphen, 2002). Winter diapause and its duration have been shown to considerably affect not only winter survival, but also post-diapause reproduction and patterns of the relationships may vary in different species. Thus, in the leaf miner *Bucculatrix pyriorella*, an overwintered generation had lower fecundity than non-diapause generations (Fujie, 1980), whereas in the water strider *Aquarius remigis*, post-diapause spring breeders had a higher lifetime reproductive potential and longer reproductive life span than non-diapause summer breeders (Blanckenhorn, 1994). Contrary to both these examples, in the chestnut weevil *Curculio elephas*, adult performance as measured by fecundity and longevity was similar between adults that emerged after 1- and 2-year (i.e. prolonged) diapause (Menu & Debouzie, 1993). Moreover, the duration of diapause was negatively correlated with egg production in the bruchid *Kytorhinus sharpianus* and in the spider mite *Tetranychus urticae*, but the relationships between diapause duration and longevity were opposite in these species: it was positive in *K. sharpianus* and negative in *T. urticae* (Ishihara & Shimada, 1995; Kroon & Veenendaal, 1998). In the present study, reproductive traits varied between non-diapause and post-diapause females and between those that emerged and entered diapause in late September and early October (Figs 3–6). Thus, females from the non-diapause series 1 produced significantly fewer egg masses than those from series 4 in which all females started reproduction only after winter diapause (Mann–Whitney *U*-test, $U = 146$, $P = 0.003$). Besides, females in series 4 that emerged and entered diapause later lived longer and performed better

than those in series 3 that emerged and entered diapause earlier (Figs 3b,d,e, and 4). The differences in the post-diapause reproductive performance may affect fitness of the progeny, as it is known that, for instance, egg mass size has an influence upon survival rates of nymphs in *N. viridula* (Kiritani *et al.*, 1966b).

The different patterns of post-diapause performance in series 3 and 4 (Figs 5 and 6) suggest that the observed differences were caused not by a possible difference in duration of diapause *per se*, but by the timing of diapause induction or, more precisely, by the environmental conditions during the period when diapause was induced. Day-length in late September (when adults of series 3 emerged) is shorter than the critical photoperiod but, perhaps, is still too close to it and temperature is, probably, too high for induction of deep diapause. It was shown in the bean bug *Riptortus clavatus* that different diapause-inducing photoperiods had quantitatively different effects on the initial intensity of diapause and rates of diapause development (Nakamura & Numata, 2000). In the present study, it is likely that the diapause induced later in the season under more typical short-day conditions was deeper. It reduced the physiological cost of diapause and, thus, provided a higher overwintering success and better post-diapause performance.

The present study suggests that *N. viridula* is still to a certain extent maladapted to the environmental conditions in Osaka. Thus, females from series 1–3 produced eggs until 13–25 November (Fig. 2), but the death of all nymphs in series 5 and 6 (Fig. 1) showed that nymphs have no chance of surviving winter or attaining adulthood if eggs are deposited after mid-September. Therefore, 35.8–100% of egg masses produced by females in series 1–3 in autumn (or 22–59.1% of the total number of egg masses produced in these series before and after winter) were destined to die and appeared to be an ineffective allocation of metabolic resources. Moreover, both females and males in these series suffered a significantly higher winter mortality than those that emerged as adults later in series 4 (Fig. 1). Furthermore, if oviposition is considered together in both seasons and it is assumed that females oviposit only before or after overwintering, the emergence of adults in September resulted in a reduced reproductive output: females in series 2 and 3 produced significantly fewer egg masses than those that emerged earlier and reproduced only before winter (series 1) and those that emerged later and reproduced only after winter (series 4) (Fig. 3a,b). This maladaptive late-season reproduction is a result of imperfect timing of diapause induction and may be considered the ecological cost of range expansion. As pointed out by Bradshaw *et al.* (2000), the timing of seasonal development presents the most immediate impediment to range expansion into the temperate zone.

To ensure a better adaptation to the new environmental conditions and complete establishment in the region, the local population of *N. viridula* will probably evolve a lengthening of the critical photoperiod of the response of diapause induction and, consequently, advance the timing of diapause induction. This adjustment will allow this species to

enter diapause not later than mid-September and, thus, avoid maladaptive reproduction in October–November but, perhaps, will entail additional costs of winter diapause as a result of an adverse impact of the pre-winter high temperature conditions on overwintering success (Han & Bauce, 1998; Gomi, 2000). Directional shifts in the timing of diapause induction are known to occur when selective pressures change (Hairston & Walton, 1986). A marked variation in the photoperiodic response recorded in *N. viridula* in Osaka (Musolin & Numata, 2003) can make such a shift feasible and quick, as this type of physiological variation is known to provide considerable scope for natural selection and rapid adaptation to climate change (Butterfield & Coulson, 1997).

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