Timing of diapause induction outside the natural distribution range of a species: an outdoor experiment with the bean bug *Riptortus clavatus*

Dmitry L. Musolin\(^1\), Hideharu Numata\(^2\) & Aida H. Saulich\(^1\)

\(^1\)Biological Research Institute of St. Petersburg State University, Oranienbaumskoye sh., 2, Stary Peterhof, St. Petersburg, 198904, Russia (Fax: +7-812-427-7310; E-mail: saulich@AS1061.spb.edu); \(^2\)Department of Bio- and Geosciences, Graduate School of Science, Osaka City University, Osaka 558-8585, Japan (Phone: +81-6-6605-2573; Fax: +81-6-6605-2574); \(*\)Present address: Entomology Laboratory, Department of Agro-Environmental Sciences, National Agricultural Research Center for Hokkaido Region, Hitsujigaoka, Sapporo, Hokkaido 062-8555, Japan (Fax: +81-011-859-2178; E-mail: musolin@DM1037.spb.edu)

Accepted: May 1, 2001

**Key words:** Heteroptera, Alydidae, photoperiod, diapause induction, life cycle, seasonal adaptations, voltinism, range expansion

**Abstract**

The phytophagous bug *Riptortus clavatus* (Thunberg) (Heteroptera: Alydidae) produces two or three generations per year in Central Japan and overwinters in the adult stage. In bugs from the Kyoto population (35°00′ N, 135°45′ E), we studied (1) the effects of day-length on the nymphal and preoviposition periods under constant photoperiod at 20.5 °C, and (2) photoperiodic induction of adult diapause at 20.5 °C and under a combination of constant photoperiod and natural daily rhythm of temperature in the forest-steppe zone of Russia (50°38′ N, 35°58′ E). Then, we examined (3) the timing of diapause induction under quasi-natural conditions in the same region, far outside the species’ natural geographical range. At 20.5 °C, the nymphal period in both males and females was significantly longer under regimes with shorter photophases than under those with longer photophases. The preoviposition period in females was significantly longer under regimes with shorter photophases than under those with longer photophases. 

The preoviposition period in females was significantly longer under the near-critical long-day regime L14:D10 than under typical long-day regimes (L15:D9, L16:D8, and L17:D7). The critical day-length for diapause induction was shorter under conditions of natural daily rhythm of temperature than those reported at constant 20, 25, and 30 °C. Under quasi-natural conditions in the forest-steppe zone, *R. clavatus* entered diapause in September, much later than the local populations of true bugs studied to date. This experiment showed that *R. clavatus* was maladapted to new environmental conditions: diapause was induced too late with the result that all or most nymphs hatched in late August or early September will die.

**Introduction**

Studies on seasonal development of insect species outside their natural geographical range are of considerable importance not only for fundamental knowledge of general biology but also for the theory and practice of biological control, pest management, and analysis of range expansion (Danilevsky, 1961; Tauber et al., 1986; Saulich, 1994, 1999). In this study, we used the bean bug *Riptortus clavatus* (Thunberg) (Heteroptera: Alydidae) as a model species and investigated the physiological mechanisms controlling seasonal development and timing of diapause induction far outside the species’ natural geographical range.

*Riptortus clavatus* inhabits south-eastern Asia, viz., the Japanese Archipelago, Korean Peninsula, and Taiwan (Tomokuni et al., 1993), and is a pest of cultivated soybean, but occurs naturally on different species of wild legumes. *Riptortus clavatus* produces two generations per year in Kyoto (Central Japan) and three generations further south (e.g., in Shikoku Island). This species overwinters in the adult stage (Kobayashi, 1972; Numata & Hidaka, 1982; Kono, 1989). Diapause in this species is facultative...
and controlled by a long-day type photoperiodic response (Kidokoro, 1978; Numata & Hidaka, 1982). Day-length experienced by nymphs beginning with the 4th stadium is critical for induction of diapause (Kobayashi & Numata, 1993).

In the present study, we first examined photoperiodic induction of diapause and the effects of day-length on the duration of the nymphal period in males and females and on the preoviposition period in females under laboratory conditions of constant photoperiod and temperature of 20.5 °C as well as under a combination of constant photoperiod and natural daily rhythm of temperature in the forest-steppe zone of Russia. Then, we examined seasonal timing of diapause induction in this species under quasi-natural conditions in the same region, far outside the species' natural geographical range.

Materials and methods

Insects and rearing procedure. Riptortus clavatus used in these experiments were descendants of the bugs collected in Kyoto, Japan (35°00’ N, 135°45’ E) three months before the start of the experiments. The culture was maintained in 1 l glass containers covered with gauze netting with zigzag-folded strips of filter paper at constant temperature of 24.0 ± 1.0 °C and long-day photoperiod of L16:D8. Nymphs and adults were reared on dry soybean, Glycine max, grains and water supplemented with 0.05% sodium L-ascorbate (water-soaked balls of cotton wool). Then, adults were transferred to diapause-inducing conditions of short day L10:D14 and 15.0 ± 1.0 °C for three months and finally returned to diapause-averting conditions (L16:D8 and 24.0 ± 1.0 °C). The eggs laid after diapause termination were kept under the same conditions and newly hatched nymphs were used for experiments.

In all experiments nymphs were reared in 0.8 l glass containers covered with gauze netting with zigzag-folded filter paper strips inside. The density of the nymphs was kept at 20–30 per container for the 1st and 2nd instars and was then gradually reduced to 10–15 by the final (5th) instar. In addition to dry soybean grains and water fresh bunches of cow clover, Trifolium pratensis, alfalfa, Medicago sativa, as well as green pea, Pisum sativum, and haricot bean, Phaseolus vulgaris, seedlings were provided. The food and water were replenished every other day.

Adults were paired (male and female) in plastic 100 mm Petri dishes with openings for aeration covered by gauze and fed in the same manner as nymphs. The emergence of adults and appearance of first eggs was recorded daily. Females that had not commenced oviposition on day 30 after adult ecysis were dissected and their physiological status (diapause vs. reproductive state) was judged by the criteria previously established for this species (Numata & Hidaka, 1982; Numata & Kobayashi, 1989).

Laboratory experiment. The following set of constant photoperiodic regimes were used: L13:D11, L14:D10, L15:D9, L16:D8, and L17:D7. The light intensity in the incubators ranged between 180 and 250 lx and was supplied by 20 W fluorescent lamps. The temperature was maintained at the level of 20.5 ± 1.0 °C.

Experiment under conditions of constant photoperiod and natural daily rhythm of temperature. The experiment was carried out in the ‘Forest on the Vorskla River’ nature reserve (Belgorod province of Russia, forest-steppe zone, 50°38’ N, 35°58’ E). To determine how photoperiodic response of the species might be influenced by natural daily rhythm of temperature, we used a special semi-automatic device that allowed rearing of insects under conditions of constant photoperiod and natural daily temperature rhythm (Braun & Goryshin, 1984). Special two-layer frame cowls were used to cover glass containers with insects and thus provided darkness during the scotophase. Electric lamps were used to extend the natural day-length up to the pre-set duration in the long-day regimes. The insects experienced natural ambient temperature (the framed cowls did not increase temperature significantly during the scotophase). Totally, six photoperiodic regimes were used: L14:D10, L15:D9, L16:D8, and L17:D7 in 1995 and L12:D12, L13:D11, and L14:D10 in 1996 (a priori we expected that photoperiod L14:D10 would induce diapause in 100% of females and did not test regimes with shorter day-length in 1995; the next year, we tested two shorter photoperiods and repeated the L14:D10 regime to confirm the results of the previous year). Ambient temperature was recorded daily with a thermograph and checked using minimum and maximum thermometers. Insects were reared as in the laboratory experiment and females that did not lay eggs on day 30 after adult ecysis were dissected to determine their physiological status (the calendar dates of the experiment and the temperature conditions are shown in Figures 2 and 4).
Figure 1. Duration of nymphal period in *Riptortus clavatus* under different photoperiodic conditions at 20.5 °C. Means (± S.D.) followed by the same letters were not significantly different (P > 0.05 by the Tukey multiple comparison test; Zar, 1999); n = 28–54.

**Experiment under quasi-natural conditions.** A special experimental procedure for clarification of voltinism patterns in insects was proposed by Danilevsky (1961) and has been successfully used in experiments with several insect species (e.g., Danilevsky & Kuznetsova, 1968; Goryshin et al., 1986; Teraoka & Numata, 1995). The techniques involve rearing several series of insects (usually from eggs to the stage that can enter diapause) under quasi-natural conditions and commencement of series throughout the season with a particular time interval (e.g., every ten days).

Our experiment using this procedure was performed in the same nature reserve in seven time series: 1st-instar nymphs were transferred from the laboratory conditions (described above) to the specially modified meteorological booth on the day of hatching every ten days from 29 May to 29 July, 1995. The shelf on which the Petri dishes with insects were kept was about 130 cm above the ground. Only the northern side of the booth was open and the insects inside the booth were sheltered from direct sunlight and rain. The temperature in the booth was recorded with a daily thermograph and checked using minimum and maximum thermometers. Insects were reared as in other experiments, and those females that did not lay eggs on day 30 after adult ecysis were dissected to determine their physiological status.

**Results**

**Nymphal development at a constant laboratory temperature of 20.5 °C.** If compared within each photoperiodic regime, the duration of the nymphal period did not differ significantly between males and females with the only exception of the L17:D7 regime, where development of female nymphs was longer (Figure 1). In males, the nymphal period was longest at L14:D10 and gradually decreased significantly between regimes, being shortest in that with the longest photophase tested at this temperature (L17:D7). In females, the duration of the nymphal stage was longer in the three regimes with shorter photophases and significantly shorter in the two regimes with longer photophases (Figure 1).

**Diapause induction in females at a constant laboratory temperature of 20.5 °C.** All females entered diapause in the short-day regime of L13:D11 but at daylengths 14 h or longer more than 90% females were non-diapause (Figure 2). However, the preoviposition period was significantly longer at L14:D10 than at longer photoperiods (Figure 3). Visual observations revealed that diapause adults in the L13:D11 regime spent more time sitting under zigzag-folded filter paper strips, while non-diapause individuals in other regimes were more active.

**Diapause induction in females under conditions of constant photoperiod and natural daily rhythm of temperature.** In 1995, when regimes with photophases of 14 h and longer were tested, 98 to 100% females were non-diapause (Figure 2). In 1996, in the L14:D10 regime almost 100% were non-diapause, but 39% females entered diapause in the L13:D11 regime and 100% did so in the L12:D12 regime. Thus, critical day-length (a day-length at which 50% of the individ-
Figure 2. Photoperiodic response of diapause induction in females of Riptortus clavatus at 20.5 °C and under conditions of natural daily rhythm of temperature. In 1995, nymphs hatched on 12–15 June, adults emerged on 10–27 July, and females that did not lay eggs were dissected on 10–27 August; in 1996, nymphs hatched on 30 May–5 June, adults emerged on 5–17 July, and females were dissected on 5–15 August. In parentheses: mean air temperature; means of daily lowest:highest air temperatures during the first 20 days after adult ecdysis; \( n = 27–44 \).

Figure 3. Duration of the preoviposition period in females of Riptortus clavatus under different photoperiodic conditions at 20.5 °C. Means (± S.D.) followed by the same letters were not significantly different (\( P > 0.05 \) by the Tukey multiple comparison test: Zar, 1999); \( n = 26–43 \).

Seasonal development under quasi-natural conditions in the forest-steppe zone. In five early series of the experiment, nymphs hatched between 29 May and 10 July and adults emerged between 24 June and 24 August (Figure 4). All females in these series were non-diapause and preoviposition period ranged from 9.0 ± 1.81 to 16.5 ± 3.61 days (mean ± S.D.) without a clear trend, mostly dependent on ambient temperature and weather. The appearance of diapause females was recorded for the first time in the sixth series, in which adults emerged on 25–29 August: only 55% females laid eggs and after dissection 35% females were judged to be in diapause. In the next series, in which adults emerged on 3–12 September, no females laid eggs, and after dissection 93% were judged to be in diapause.

Discussion

Effects of day-length on duration of nymphal period. The effect of photoperiod on duration of the nymphal period has been reported in a number of terrestrial heteropteran species and seems to be a widely used seasonal adaptation (Musolin & Saulich, 1997, 1999). In Dolycoris baccarum (Conradi-Larsen & Sømme, 1973), Nezara viridula (Ali & Ewiess, 1977), and Carbula humerigera (Kiritani, 1985) nymphal growth was shown to be accelerated by long- and retarded by short-day conditions. These findings led Hori (1986) to the conclusion that the majority of heteropterans studied may be classified as species with long-day type acceleration of nymphal growth. However, our recent review (Musolin & Saulich, 1997) revealed that nymphal development was more often retarded by long- and accelerated by short-day photoperiods.

In species with facultative diapause and bi- or multivoltine seasonal development, such responses allow acceleration of development both at the beginning and the end of the summer season when days are shorter, and thus promote bi- or multivoltinism and more complete use of seasonal resources. In the case of univoltine seasonal development with either obligatory or facultative diapause, the value of this photoperiodic adaptation is likely to be limited by synchronization of adult emergence (Musolin & Saulich, 1997, 1999).

Our experiments with R. clavatus at a constant temperature of 20.5 °C showed that in both sexes long day-length accelerated nymphal growth, while short (near-critical) day-length retarded nymphal growth (Figure 1). Unfortunately, photoperiods with even shorter (typical short-day, autumn) photophases were not tested in this experiment and the ecological significance of this response in R. clavatus remains unclear.

Effects of constant and fluctuating temperature on photoperiodic induction of diapause. The results obtained at a constant temperature of 20.5 °C were in close agreement with those of Kobayashi & Numata.
Figure 4. Induction of diapause in females of *Riptortus clavatus* under quasi-natural conditions in 1995. Each horizontal line represents an experimental series. Arrows indicate the dates of hatching of the nymphs. Histograms show emergence and physiological state of females: non-diapause (white) or diapause (black); the incidences of diapause in each series are shown as percentages. Asterisks indicate the period in which females start to oviposit. Solid line, natural day-length including a half of civil twilight (after Sharonov, 1945). Temperature was calculated as means for five- or six-day periods in the experimental meteorological booth; \( n = 24–45 \).
(1995) and confirmed that within a range of ecologically relevant constant temperatures, a long-day type photoperiodic response of *R. clavatus* is remarkably stable, especially around its critical value (13 h 30 min).

Natural daily fluctuation of temperature experienced by *R. clavatus* in our outdoor experiments considerably suppressed a tendency towards diapause: 61% females were non-diapause under a photoperiod of L13:D11 and natural temperature, while all females entered diapause under the same photoperiod at a constant temperature of 20.5 °C in the present study (Figure 2); almost all females were reported to enter diapause in the experiments conducted by Kobayashi & Numata (1995) at 20, 25, and 30 °C. A similar effect of temperature fluctuation was revealed recently in several species of green lacewings (Volkovich & Blumental, 1997) and in the linden bug, *Pyrrhocoris apterus* (Nasir et al., 1999). In our experiment under conditions of natural temperature fluctuation, the critical photoperiod was shifted to some value slightly shorter than 13 h. Outdoor temperature during the experiment roughly corresponded to September–October ambient temperature in Kyoto, the place of origin of the bugs used in this study (Figures 2, 4, and 5). This finding, therefore, supported the assumption suggested by Numata (1985 and unpubl.) based on the laboratory experiment at constant temperature and captures of insects in the wild: diapause in Kyoto population of *R. clavatus* should be naturally induced in September.

**Effects of day-length on duration of the preoviposition period in females.** Duration of the preoviposition period in non-diapause females differed significantly between the near-critical long-day regime L14:D10 and the typical long-day regimes L15:D9, L16:D8, and L17:D7 (Figure 3). Many examples of photoperiodic conditions controlling different aspects of development and reproduction (Saunders, 1982; Philogène & McNeil, 1984) as well as progression or termination of diapause and post-diapause reproduction (Danks, 1987, pp. 133–159; Zaslavski, 1988; Hodek, 1996) have been reported in insects. Among heteropteran species, differences in the duration of preoviposition period were reported to be associated with the production of different wing morphs in species that show alary polymorphism (e.g., Harada, 1992, 1998) or with production of diapause/non-diapause eggs (e.g., Musolin et al., 1999). Ruberson et al. (1991) showed that there was a difference in the duration of the preoviposition period in the anthocorid bug, *Orius insidiosus*, raised under different photoperiodic conditions and suggested the existence of a low-intensity reproductive diapause that might be terminated within a relatively short period. A similar hypothesis was suggested by Kohno in respect to the results of experiments with the anthocorids *Orius sauteri* and *Orius minutus* (Kohno, 1997, 1998). Moreover, Nakamura & Numata (2000) discussed the relationships between initial intensity of diapause, rates of diapause development, and preoviposition period in *R. clavatus*. However, all these considerations cannot be applied directly to our results, because under all photoperiodic conditions ranging from L14:D10 to L17:D7 almost all females were reproductive (Figure 2) and it seems unlikely that diapause was induced and terminated in females under these conditions.

**Timing of diapause induction inside and outside the natural geographical range.** The environmental conditions of the forest-steppe zone differ significantly from those at the origin of the *R. clavatus* population used in this experiment (Figure 5). Thus, in Belgorod, summer temperature is much lower, days in summer are longer but during the second part of summer days shorten more quickly than in Kyoto. Nevertheless, the experimental insects successfully completed their development with minimal mortality and all females were reproductive in the five early series (Figure 4). The same procedure was followed throughout the

![Figure 5](image-url)
whole experiment and eggs obtained only from the laboratory culture were used in all series. However, the results of the experiment suggested that *R. clavatus* could produce two complete generations: females from the first series laid eggs from the beginning of July (Figure 4) and nymphs from these eggs hatched within several days, between the dates when nymphs in the experimental series five and six hatched (10 July and 20 July, respectively).

In the present study, females entered diapause in late August–September. These results were in good agreement with the main features of the photoperiodic response in this species: i.e., photoperiodic sensitivity of adults, thermostability of the response and critical day-length a little shorter than 13 h. Natural day-length in the region becomes shorter than 13 h on 15 September [if half of civil twilight (sensu Danks, 1987, p. 88) is included; Figure 4; Sharonov, 1945] or in the first half of September (without twilight).

*Riptortus clavatus* produces two generations per year in Kyoto and three generations further south (Kobayashi, 1972; Numata & Hidaka, 1982; Kono, 1989). Timing of diapause induction in the Kyoto population of this species has not been investigated previously using similar experimental techniques. However, based on captures of insects in the wild and dissection, it was suggested that this population naturally enters diapause in September (Numata, 1985 and unpubl.). Day-lengths in Kyoto and Belgorod are similar in September: 12 h 27 min in Kyoto and 12 h 42 min in Belgorod on 15 September, but by 1 October, the day is getting shorter in Belgorod than in Kyoto (11 h 38 min vs. 11 h 48 min without twilight; Beck, 1980). In good accordance with this similarity of natural day-length conditions, the results of the present study showed that timing of diapause induction was similar in Belgorod and Kyoto.

**Timing of diapause induction: Ecological perspectives.** It was also recorded that when nymphs of *R. clavatus* of the last (i.e., 7th) series failed to complete their development by the middle of September, their growth was markedly retarded, they often could not moult into the next stage and showed high mortality, probably because of low night temperatures. Thus, it was supposed that the progeny of females beginning with the fourth series (i.e., nymphs hatched in the second half of August or later) would also fail to reach adulthood and they would die when the temperature drops in September. Moreover, in contrast with Kyoto where temperature remains favorable for pre-diapause activity for several weeks, in Belgorod the mean temperature quickly drops below +10 °C at the beginning of October, making successful pre-diapause feeding difficult.

An investigation of winter survival was not performed in this study. Thus, we can only speculate whether females emerging at the end of August or in September and considered to be in diapause were physiologically ready for overwintering. However, it does not seem likely that the bugs of such a southern population of this species can survive a long and cold winter in the region of the outdoor experiment where the mean air temperature is normally below −5 °C for more than three winter months (Karausheva, 1980).

**Comparison with local heteropteran species.** The early and marked deterioration of environmental conditions in the forest-steppe zone at the beginning of autumn determines an early induction of winter diapause in the local populations of true bugs with facultative winter diapause. Thus, for example, *Pyrrhocoris apterus* can produce one or two generations per year in this region and adults enter diapause without starting reproduction if they emerge in the second half of July or later (Saulich et al., 1994). *Graphosoma lineatum* was also shown to be able to produce two generations per year, but this may occur only if adults emerge not later than in June in the warmer microhabitats or in unusually warm years (Musolin & Saulich, unpubl.). In both species, the photoperiodic response of diapause induction is temperature-dependent: lower temperatures strongly promote induction of diapause (Numata et al., 1993; Saulich et al., 1994; Musolin & Saulich, 1995). Another heteropteran species, *Arma custos*, was shown to have a facultative diapause but in the forest-steppe zone always produces one generation and adults enter diapause even if they emerge as early as mid-July. This happens because non-diapause development is possible only under a combination of long photophase (more than 15–16 h) and high temperature (29–30 °C) (Volkovich & Saulich, 1994). Thus, differences in the parameters of photoperiodic response of *R. clavatus* and other species studied cause the observed differences in timing of diapause induction under the same conditions: relatively short critical day-length and thermostability of photoperiodic response in *R. clavatus* resulted in physiological activity of females emerging as adults from June to as late as the end of August.

The results obtained in the present study show that *R. clavatus* is maladapted to new environmental
conditions: under quasi-natural conditions diapause was induced too late (much later than in local heteropteran species) with the result that all or most nymphs hatched in late August or early September died. Some studies have been performed with the insect populations transferred from one part of the natural distribution range to another and with species that had been intentionally or accidentally introduced into new areas or that have expanded their distribution range (e.g., Danilevsky et al., 1970; Takeda & Chippendale, 1982; Goryshin et al., 1986; Gomi & Takeda, 1996; Saulich, 1999; for review see Tauber et al., 1986, pp. 238–245). Results of our experiment may contribute to understanding of seasonal development of insects as well as to the theory and practice of biological control and analysis of distribution range expansion. The experiment under quasi-natural conditions clearly illustrated the proposition that when a species with a photoperiodically controlled diapause is introduced into new environmental conditions far outside its natural distribution range, day-length and temperature remain the leading factors in the control of seasonal development and diapause induction.

Acknowledgements

We would like to thank Ms E. Davidian (the ‘Forest on the Vorska River’ nature reserve, Russia) for assistance and Dr. K. Nakamura (Okayama University of Science, Japan) for reading an early version of the MS. This research was partly supported by the Russian Foundation of Basic Research (Leading Scientific Schools project # 00-15-97934), Federal Programs Biodiversity and Integration for A.S., and the Ministry of Education, Science, Sports and Culture of Japan (Grant-in-Aid for JSPS Fellows # 98116) for D.M.

References


