Photoperiodic and temperature control of reproductive diapause induction in the predatory bug *Orius strigicollis* (Heteroptera: Anthocoridae) and its implications for biological control

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Abstract

The effect of day-length and temperature on the duration of the egg to adult period and reproductive diapause in *Orius strigicollis* (Poppius) (Heteroptera: Anthocoridae) from central Japan (36°N; 140°E) was studied in the laboratory. At 20°C, the egg to adult period tended to increase with lengthening of photoperiod from short day [10:14 (L:D) h] to long day [14:10 (L:D) h], but slightly decreased with further lengthening of photoperiod, whereas at 24°C, an opposite trend was recorded. At 28°C, the developmental period was much shorter and there were no obvious trends. It is assumed that photoperiod-mediated acceleration of nymphal growth takes place in autumn when ambient temperature and natural day-length are decreasing, thus promoting earlier emergence of adults prior to the deterioration of environmental conditions. The species showed a long-day photoperiodic response of reproductive diapause induction: at 20 and 24°C, 83–100% females entered diapause under short-day conditions [10:14 (L:D), 11:13 (L:D), and 12:12 (L:D) h], whereas 82–91% of females were reproductive under long-day conditions [14:10 (L:D), 15:9 (L:D), and 16:8 (L:D) h]. The critical day-length was close to 13 h at 20°C and to 12.5 h at 24°C. Diapause induction was strongly suppressed at 28°C: its incidence did not exceed 50% even at 10:14 (L:D) h. It is estimated that *O. strigicollis* produces four or five generations in central Japan. The seasonal cycle and tendency to enter diapause are compared with other *Orius* species and other geographical populations of *O. strigicollis*. Implications of diapause-related traits for biological control are discussed.

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1. Introduction

Diapause plays a pivotal role in survival during unfavorable seasons and life cycle synchronization in the overwhelming majority of insect species in the temperate zones. Among all environmental factors and cues, photoperiod has been proven to be most often used in seasonal positioning because of its astronomic preciseness (Danks, 1987). Whereas temperature itself is not a reliable seasonal indicator, its effect upon photoperiodic response of diapause induction is significant in many insect species. The mode of action of temperature may differ even among related species.

Biological control of pests has been practiced for over 100 years and both impressive results and commercial-scale applications have been reported over the last few decades (van Lenteren et al., 1997). Several predatory Heteroptera species are currently mass-produced and...
used as biological control agents against a wide range of agricultural arthropod pests (Glenister, 1998; van Lenteren et al., 1997). The rearing of biological agents on an industrial scale requires a profound knowledge of the species’ biology. The task is even more difficult when it is realized that not only species, but also populations differ in their eco-physiological features (e.g., diapause response) which might affect the success of mass rearing and pest control practices.

The minute pirate bug Orius (Heterorius) strigicollis (Poppius) (Heteroptera: Anthocoridae) is a small predatory flower bug distributed in the southwestern part of Japan, in Taiwan, and in the southern part of China (Yasunaga, 1997). The species is polyvoltine and supposed to produce up to eight generations in southern Japan (Kakimoto et al., 2003). Adults of the last summer generation copulate, but females enter diapause without starting reproduction; males, most probably, do not normally survive winter as known in some other anthocorid species (Ito and Nakata, 1998; Ruberson et al., 1998; Shimizu and Kawasaki, 2001). O. strigicollis is considered a promising biological control agent against Thrips palmi Karny, Frankliniella occidentalis (Pergande), and many other small pest arthropods (Song et al., 2001; Wang et al., 2001). The species has demonstrated good performance in the control of pests in both greenhouses and open fields (Song et al., 2001; Wang et al., 2001). It is registered as a biotic pesticide in Japan (Yano et al., 2002), where it is commercially available (e.g., Arysta LifeScience Corporation, 2003). However, the application of *Orius* species in greenhouses and open fields has encountered problems: the pest control effect was insufficient in late autumn and winter because adults entered reproductive diapause (Yano, 1999). Moreover, for mass production and long-term storage of dormant adults, it is important to know the environmental conditions necessary for diapause induction, maintenance, and termination. Finally, for inter-zone transfers and applications in the open field, it is critical to evaluate risks of unwanted establishment and negative effects on non-target species.

Diapause syndrome has been studied in several predatory *Orius* species from different parts of the world (e.g., Ito and Nakata, 1998; Ruberson et al., 1998; Shimizu and Kawasaki, 2001; van den Meiracker, 1994). Most attention was paid to the role of photoperiod in diapause induction, whereas the effect of temperature conditions and interaction between photoperiod and temperature in the induction of diapause remain poorly understood. The objectives of this study were to examine the photoperiodic response of diapause induction in *O. strigicollis* from central Japan, the effect of temperature on this response, and the effects of both day-length and temperature on nymphal development in this species. Based on these data, the seasonal cycle of *O. strigicollis* and implications for biological control are discussed.

2. Materials and methods

2.1. Insects and the laboratory culture

Females of *Orius* spp. were collected in late July 2001 and early September 2002 on Glossy abelia Abelia × grandiflora and concurrent vegetation in Tsuchiura City (36.1°N; 140.2°E; Ibaraki Prefecture, central Japan) and then reared individually in plastic petri dishes (20 mm × 90 mm) under a photoperiod of 16:8 (L:D) h at 24 ± 1°C in an incubator. Frozen eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, were provided as food and fresh soybean, Glycine max (L.) Merrill, sprouts (with a piece of moist cotton wool) as an oviposition substrate and source of moisture. Food and water were replenished every other day. Upon emergence of the first male adults in the progeny of each female, the species was determined based on the shape of the paramere (Yasunaga, 1997). More than 120 wild-collected females of *O. strigicollis* and their progeny were then used to establish a laboratory culture. Adults were reared in transparent plastic containers (90 mm × 150 mm; with openings in the lids covered with mesh for aeration) with a density of 100–300 per container under a 16:8 (L:D) h photoperiod at 24 ± 1°C. About 0.1 g of frozen eggs of *E. kuehniella* and fresh soybean sprouts were provided to each container every other day. The containers were filled to a depth of 1–1.5 cm with unhulled rice grains to avoid cannibalism. Soybean sprouts with eggs of *O. strigicollis* were transferred to petri dishes, which were also half-filled with unhulled rice grains. Hatched nymphs (ca. 50–150 per petri dish) were given the same food (0.03–0.05 g per dish) and water every other day. Upon molting to adults, bugs from 1 to 3 petri dishes were transferred to new transparent plastic containers as described above.

2.2. Experimental procedure

Soybean sprouts with eggs produced within 24 h were collected from the culture and transferred to new petri dishes half-filled with unhulled rice and kept under different photoperiodic conditions ranging from 10:14 (L:D) to 16:8 (L:D) h (at hourly increment) at 20, 24, and 28 ± 0.5°C. Nymphs and then adults were fed as described above. Hatching of nymphs was not recorded because of the very small size of the first instar nymphs, and thus the pre-adult period was assessed as the egg to adult period. The density of nymphs was kept at 50–150 per petri dish. The emergence of adults was recorded daily. Upon emergence, adults were transferred to new petri dishes with rice, food, and soybean sprouts under the same photoperiod and temperature conditions. The density of adults was less than 150 individuals per petri dish with the sex ratio close to 1:1.
Females were dissected under a stereomicroscope 15–16 (20°C), 12–13 (24°C), or 7 (28°C) days after adult emergence. The timing of dissection was determined based on preliminary experiments, in which most of non-diapause females started oviposition 12–13, 8–10, and 5–6 days after adult emergence at the same temperatures. Diapause status of females was judged based on the state of development of gonads: females with no visible eggs in their ovarioles were considered to be in reproductive diapause. To determine the critical photoperiod of diapause induction more precisely, an additional photoperiod of 12.5:11.5 (L:D) h at 24 ± 0.5°C was used.

2.3. Statistical analysis

The duration of the egg to adult period was statistically examined using the \( t \) test (between sexes within each treatment) and the Tukey multiple comparison test (among different photoperiods; Zar, 1999).

3. Results

3.1. Effect of day-length and temperature on duration of the egg to adult period

When compared within each treatment, the mean duration of the egg to adult period was shorter in males than in females under 18 of 21 conditions, though the difference was significant only under 10 conditions (Fig. 1). The difference in means between sexes was less than 2 days at 20°C and even smaller at the higher temperatures tested. Both temperature and day-length affected the duration of the egg to adult period. At 20°C, in both sexes, this period tended to increase with lengthening of photoperiod from short day [10:14 (L:D) h] to long day [14:10 (L:D) h], but slightly decreased with further lengthening of photoperiod. The difference among the mean values under different photoperiods amounted to 3–4 days in each sex. In contrast, at 24°C, the egg to adult period tended to decrease with lengthening of photoperiod from short-day conditions [from 10:14 (L:D) to 13:11 (L:D) h] to long-day ones [from 14:10 (L:D) to 16:8 (L:D) h]. At the highest of the temperatures tested (28°C), the difference among treatments was small and no directional trend was observed.

3.2. Photoperiodic response of diapause induction

Induction of diapause in females of *O. strigicollis* is controlled by a long-day photoperiodic response and affected by temperature (Fig. 2). At 20 and 24°C, 83–100% of females entered diapause under short-day conditions [10:14 (L:D) to 12:12 (L:D) h], whereas 82–91% of females were reproductive under long-day conditions [14:10 (L:D) to 16:8 (L:D) h]. The shapes of the diapause induction curves were similar at these two temperatures and the critical day-length that induced diapause in 50% of females was close to 13 h at 20°C and to 12.5 h at 24°C. However, the higher temperature (28°C) strongly suppressed induction of diapause under short-day conditions: the incidence of diapause did not exceed 50% even at 10:14 (L:D) h. Furthermore, the
incidence of diapause at 28 °C may be slightly overestimated (especially, under long-day conditions) because of high adult mortality at this temperature: mortality was as high as 44.3% on average at 28 °C, whereas it was only 20.7% at 20 °C, even though females were dissected and the survival rate was estimated much earlier at 28 °C than at the lower temperatures (see Section 2). Non-diapause females may suffer a higher mortality rate than diapause females.

4. Discussion

4.1. Effect of day-length and temperature on nymphal growth

Day-length has been shown to affect nymphal growth and development in many species of true bugs and in different ways, ranging from acceleration or retardation of nymphal growth to prolonged nymphal diapause (Musolin and Saulich, 1997, 1999; Tanaka et al., 2002). In temperate populations of species with adult winter diapause, the late season short-day conditions may accelerate growth of nymphs at 20 °C (Ruberson et al., 1991), but the trend was somewhat reversed, though not consistent, in nymphs of the same species from Georgia, USA, at 18 °C (van den Meiracker, 1994). In O. majusculus (Reuter) from the Netherlands, short-day significantly accelerated the growth of nymphs at 18 °C, although the trend was also inconsistent (van den Meiracker, 1994).

In the present study, the effect of day-length on the egg to adult period showed different trends at different temperatures. At 20 °C, this period tended to increase with lengthening of the photoperiod from short day [10:14 (L:D) h] to long day [14:10 (L:D) h], but slightly decreased with further lengthening of the photoperiod. At 24 °C, the response showed a weak opposite trend (Fig. 1). At 28 °C, the developmental period was much shorter and there was no pronounced trend.

In Tsuchiura, the longest day is 14 h 36 min and day-length decreases to 13 h by the end of August, 12 h by late September, and 11 h by late October (Fig. 3; twilight not included). Minimum daily temperature exceeds 20 °C until mid-September (Fig. 3), whereas mean temperature does so until the end of September (Japan Meteorological Agency, 2003). It is probable that until late August, the duration of the nymphal period in O. strigicollis is mostly affected by temperature, whereas in September, when ambient temperature and natural day-length are decreasing, some photoperiod-mediated acceleration of the pre-adult development takes place. Such a response would promote an earlier and better-synchronized emergence of adults at the end of the warm season just before the deterioration of environmental conditions in autumn as is seen in other insect species (Danks, 1987; Musolin and Saulich, 1997, 1999; Nylin and Gotthard, 1998).

4.2. Effect of day-length and temperature on diapause induction

Temperature can affect the photoperiodic response of diapause induction in insects in different manners (Danilevsky, 1961; Denlinger, 2001; Saulich, 1999). In Heteroptera, some species demonstrate thermostability of the photoperiodic response, and changes of temperature neither affected the shape of the response curve nor shifted the critical day-length (Kobayashi and Numata, 1995; Musolin and Numata, 2003; Volkovich and Saulich, 1994), whereas, similar temperature changes affected the shape of the response curve or shifted it in other species (Musolin and Saulich, 1995; Numata et al., 1993). The temperature optimum of the photoperiodic response of diapause induction (i.e., a range of temperatures within which the photoperiodic response manifests itself clearly) may be broad (15–17 °C at constant temperatures), or narrow (3–5 °C; Saulich, 1999).
Among *Orius* species, the effect of temperature on the photoperiodic response of diapause induction has been studied, though partly, only in two species. In *O. insidiosus*, it was shown that a photoperiod of 10:14 (L:D) h induced diapause in ca. 75% of females at 18–25°C, but in less than 25% at 30°C (van den Meiracker, 1994). In *O. sauteri* (Poppius), two photoperiods were tested at two temperatures, and it was shown that both photoperiod and temperature affect the incidence of diapause (Kohno, 1998). The present results show that in *O. strigicollis* day-length plays a dominant role in diapause induction. The photoperiodic response curves have similar shapes at 20 and 24°C and the critical day-lengths differ only by approximately half an hour (Fig. 2). However, a further increase of temperature up to 28°C strongly suppressed induction of diapause even under short-day photoperiods. Thus, the photoperiodic response in this species is comparatively thermostable at 20–24°C, but its temperature optimum is comparatively narrow.

The diapause induction response is much more markedly pronounced in the Tsuchiura population (Fig. 2) than in those from Kochi, 33.5°N (incidence of diapause is less than 40%; Shimizu and Kawasaki, 2001), Kagoshima, 31.7°N (less than 30%; Kakimoto et al., 2003), and Okinawa, 26°N (less than 20%; Shimizu and Kawasaki, 2001) (all tested at 20°C). However, a further increase of temperature up to 28°C strongly suppressed induction of diapause even under short-day photoperiods. Thus, the photoperiodic response in this species is comparatively thermostable at 20–24°C, but its temperature optimum is comparatively narrow.

Photoperiodic induction of diapause was studied in *O. sauteri*, *O. nagaii* Yasunaga, and *O. minutus* (L.) in Tsukuba, 36.0°N, only 15 km West from Tsuchiura (Shimizu and Kawasaki, 2001). It was reported that the incidence of diapause in all these species did not exceed 50–70% even under such short-day conditions as 9:15 (L:D) and 10:14 (L:D) h at 20°C (females dissected 18–20 days after ecdysis). However, when these species were reared outdoors from early October, all females were non-reproductive when dissected in mid-January, suggesting that low outdoor temperatures or a decreasing natural day-length promote diapause induction in autumn (Shimizu and Kawasaki, 2001). In our experiments, the incidence of diapause in *O. strigicollis* was close to 100% under short-day conditions at 20–24°C (Fig. 2) showing that day-length might play a more important role in diapause induction in this species than in other *Orius* species in the same area.

### 4.3. Seasonal development and voltinism of *O. strigicollis* in central Japan

In Tsuchiura, the daily maximum temperature exceeds 12.3°C (= the developmental zero calculated based on the data in Fig. 1) starting from late March (Fig. 3) and the mean temperature does so starting from mid-April (Japan Meteorological Agency, 2003). Thus, the start of oviposition in spring may be expected in April.

The results suggest that under natural conditions, reproductive diapause in females is induced starting from the first half of September, when the natural day-length corresponds to the critical photoperiod for diapause induction (12.5–13 h), and ambient temperatures are close to 20–24°C used in the tests (Fig. 3). This is in agreement with the records of Shimizu et al. (2001) stating that by late October all females of *O. strigicollis* collected in Tsuchiura were in diapause. Then, from April to September, *O. strigicollis* can produce up to four or five (probably, overlapping) generations in Tsuchiura.

Many insect species are bi- or polyvoltine in the southern part of their distribution range and uni- or semivoltine closer to the northern limits of the range if their seasonal development is constrained by the amount of available heat and/or the period of their food sources.

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**Fig. 3.** Seasonal changes of natural day-length and temperature in Tsuchiura, central Japan (36.1°N; 140.2°E). Broken line, natural day-length; solid lines, daily minimum and maximum air temperatures averaged for 1979–2000 (Japan Meteorological Agency, 2003); shaded area, the period when natural day-length corresponds to the critical day-length for diapause induction (12.5–13 h at 20–24°C).
availability (Danks, 1987; Saulich and Musolin, 1996). However, this does not seem to be the case for *O. strigicollis*. The data presented here and those of Shimizu and Kawasaki (2001) suggest that in Tsuchiura, which is close to the northern limit of the species’ range, *O. strigicollis* can produce fewer generations than in the southern part of its range (e.g., eight generations estimated to be produced in Kagoshima, 31.7°N; Kakimoto et al., 2003), but the species is still polyvoltine in Tsuchiura. Shimizu et al. (2001) analyzed cold-hardiness in *O. strigicollis* as well as climatic conditions in the areas where this species is or is not distributed in Japan and suggested that the species’ range is limited by daily minimum air temperature in January (~1°C on average) and the number of cold days with a minimum air temperature below 0°C (50 days each winter). In Tsuchiura, parameters are very close to the critical limit: daily minimum air temperature is ~1.0°C on average in January and the number of days with a minimum air temperature below 0°C is 55 (data set for 1979–2000; Japan Meteorological Agency, 2003).

4.4. Implications for biological control

*Orius strigicollis* seems to be very attractive for application in Japan and neighboring countries even in cases of inter-zonal transfer, because the species is native to South-East Asia and the risks of unexpected negative effects on the environment (van Lenteren, 1997; van Lenteren et al., 2003; Yano, 1999) would be low. Thus, if *O. strigicollis* originated from the South is transferred northward into a moderate climate for use in biological control programs and escapes, the risk of establishment will be low, because, due to the low incidence of diapause and very short critical day-length for diapause induction within the responsive part of the population, the predator will fail to enter diapause before the arrival of cold conditions in autumn and die before or during winter. The opposite transfer of a northern population southward does not seem to be effective, because these predators will enter diapause too early in the season and may suffer high mortality before winter (Shimizu and Kawasaki, 2001). Additional field tests of winter survival and effects on non-target species are needed if the species is to be applied outside its natural range.

The large-scale applications of *Orius* spp. have encountered problems related to the reproductive physiology of these insects. The major limitation is the insufficient efficacy of these predators as biocontrol agents in late autumn and winter because of diapause induction (Yano, 1999). To extend seasonal limitations of biological control, four options have been suggested: (1) application of low intensity light in greenhouses to extend the photoperiod and prevent diapause induction (Chambers et al., 1993; Gilkeson and Hill, 1986; Jacobson, 1993); (2) selection for non-diapause strains (Gilkeson and Hill, 1986; Morewood and Gilkeson, 1991); (3) maintenance of high temperature in greenhouses that prevents diapause induction (Kohno, 1998; Morewood and Gilkeson, 1991); and (4) application of non-diapause species or strains from southern temperate or sub-tropical zones with a low diapause incidence (Gillespie and Quiring, 1993; Hirose et al., 1999). All these options seem feasible for the improvement of biocontrol programs with *O. strigicollis*. Our results show that high temperature markedly reduces incidence of diapause in a southern population of *O. strigicollis* (a combination of options 3 and 4 listed above).

It is believed that *O. strigicollis* has a lower diapause incidence and, thus, may be preferable to, for instance, *O. sauteri* (Yano et al., 2002). However, whereas qualitative characteristics of diapause (e.g., type of diapause response or diapause stage) are typically traits of a species level, quantitative characteristics (e.g., the critical day-length for diapause induction or incidence of diapause under particular photoperiodic conditions) are population-specific traits subject to pronounced geographic clines and variability (Danilevsky, 1965; Danks, 1987). *O. strigicollis* indeed showed a lower diapause incidence than *O. sauteri* and *O. minutus* in Kagoshima, 31.7°N (Kakimoto et al., 2003), but the results were apparently different in the Tsukuba–Tsuchiura area (Fig. 2 at 20°C and data from Shimizu and Kawasaki, 2001; also supported by our unpublished data). When the application of a biocontrol agent is planned, not only species-specific, but also population-specific diapause-related traits should be taken into consideration.

As our experiments showed (Fig. 2), lower temperatures promoted induction of diapause in *O. strigicollis*. A similar effect seems probable in open fields or greenhouses in late autumn or winter. Further studies on the effects of changing temperature (including daily cycles) and changing photoperiods using different populations of the species are needed to ensure successful application of this species as a biological control agent.

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