

Photoperiodic and temperature control of nymphal development and induction of reproductive diapause in two predatory *Orius* bugs: interspecific and geographic differences

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Abstract. The effects of day-length and temperature on pre-adult growth and induction of reproductive diapause are studied in *Orius sauteri* and *Orius minutus* (Heteroptera: Anthocoridae) from northern (43.0°N, 141.4°E) and central (36.1°N, 140.1°E) Japan. In the north, at 20 °C, pre-adult growth is slower under an LD 14 : 10 h photoperiod than under shorter or longer photophases. At 24 and 28 °C, the longer photophases result in shorter pre-adult periods. Acceleration of nymphal growth by short days in autumn appears to be adaptive. In the central region, this response is less pronounced, suggesting that timing of adult emergence is less critical than in the north. Day length also influences the thermal requirements for pre-adult development. The slope of the regression line representing temperature dependence of pre-adult development is significantly smaller and the lower development threshold (LDT) is significantly lower under an LD 12 : 12 h photoperiod than under long-day conditions. The weaker dependence of nymphal growth on temperature and the lower LDT in autumn might be adaptive. In the north, increased temperature shifts the critical day length of diapause induction and suppresses the photoperiodic response in *O. sauteri* but not in *O. minutus*. Further south, the incidence of diapause in both species is low even under short-day conditions but the same interspecific difference is observed (i.e. increase of temperature affects the response in *O. sauteri* but not in *O. minutus*). This suggests seasonally earlier diapause induction with weaker temperature dependence in *O. minutus* than in *O. sauteri*.

Key words: Biological control, natural enemies, photoperiodic response, photoperiodism, temperature-regulated protandry, thermal requirements for development.

Introduction

Most insects in the Temperate Zone use day-length as a token stimulus to enter seasonal dormancy (usually diapause) and to stabilize their seasonal cycles. Whereas temperature is not always a reliable seasonal cue, its role is vital in insect seasonality, particularly because temperature can greatly

influence the processes of diapause induction. Insects demonstrate extraordinary diversity of seasonal cycles and even systematically close species might differ in their voltinism and seasonal adaptations (Danilevsky, 1965; Tauber *et al.*, 1986; Danks, 1987).

A large number of insect species are used worldwide in biological control programmes and more than 100 species are commercially produced and available to control agricultural pests (van Lenteren *et al.*, 1997). Among them, there are at least five species of the minute pirate *Orius* bugs (Heteroptera: Anthocoridae): *Orius albidipennis*, *Orius insidiosus*, *Orius laevigatus*, *Orius majusculus* and *Orius*

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strigicollis (van Lenteren *et al.*, 1997; Glenister, 1998; Yano, 2004). *Orius* spp. are effective generalist predators attacking many species of thrips, aphids, spider mites and other arthropods (Riudavets, 1995; Yasunaga, 1997). Commercial application of these predators, however, sometimes encounters serious problems. The major limitation is the insufficient predatory efficacy of *Orius* species in late autumn and winter in greenhouses lacking supplementary lighting because of diapause induction (Yano, 1999). In most species of *Orius* studied so far, only adult females overwinter in a state of adult (= reproductive) diapause and day-length appears to play a leading role in diapause induction in these insects. Males usually copulate in autumn and die before or during the winter (van den Meiracker, 1994; Ito & Nakata, 1998; Ruberson *et al.*, 1998; Shimizu & Kawasaki, 2001; Musolin *et al.*, 2004).

The objective of the present study was to determine the effects of day length and temperature, and their interaction, in the control of pre-adult (i.e. egg and nymphal) growth and adult diapause induction in two Japanese species, *Orius* (*Heterorius*) *sauteri* (Poppius) and *Orius* (*H.*) *minutus* (L.). Two populations of each species are tested, allowing clarification of not only interspecific, but also intraspecific differences in ecophysiological responses to day length and temperature. The findings provide insights into the control of nymphal growth and adult diapause induction and suggest how commercial rearing might be facilitated.

Materials and methods

Collection sites

Insects were collected in two distant locations: a northern site (Sapporo City; 43.0°N, 141.4°E; Hokkaido Prefecture, northern Japan) and a southern site (Tsukuba City; 36.1°N, 140.1°E; Ibaraki Prefecture, central Japan) (Fig. 1a). These two locations differ considerably in the seasonal pattern of day-length and temperature. Thus, the seasonal range of day length is wider in Sapporo (8 h 59 min in winter to 15 h 20 min in summer; not including civil twilight) than in Tsukuba (9 h 42 min in winter to 14 h 36 min in summer) (Japan Meteorological Agency, 2007; Fig. 1b). The northern site is also much colder than the central one: the long-term mean of the highest daily air temperature reaches only +27.2 °C in Sapporo, whereas it is +30.5 °C in Tsukuba, and the mean of the lowest daily air temperature drops to -8.4 °C in Sapporo, whereas it is only -3.1 °C in Tsukuba (means for 1971–2000 for Sapporo and 1990–2000 for Tsukuba; Japan Meteorological Agency, 2007; Fig. 1c).

Insects

Females of *Orius* spp. were collected on white clover *Trifolium repens*, annual fleabane *Erigeron annuus*, common

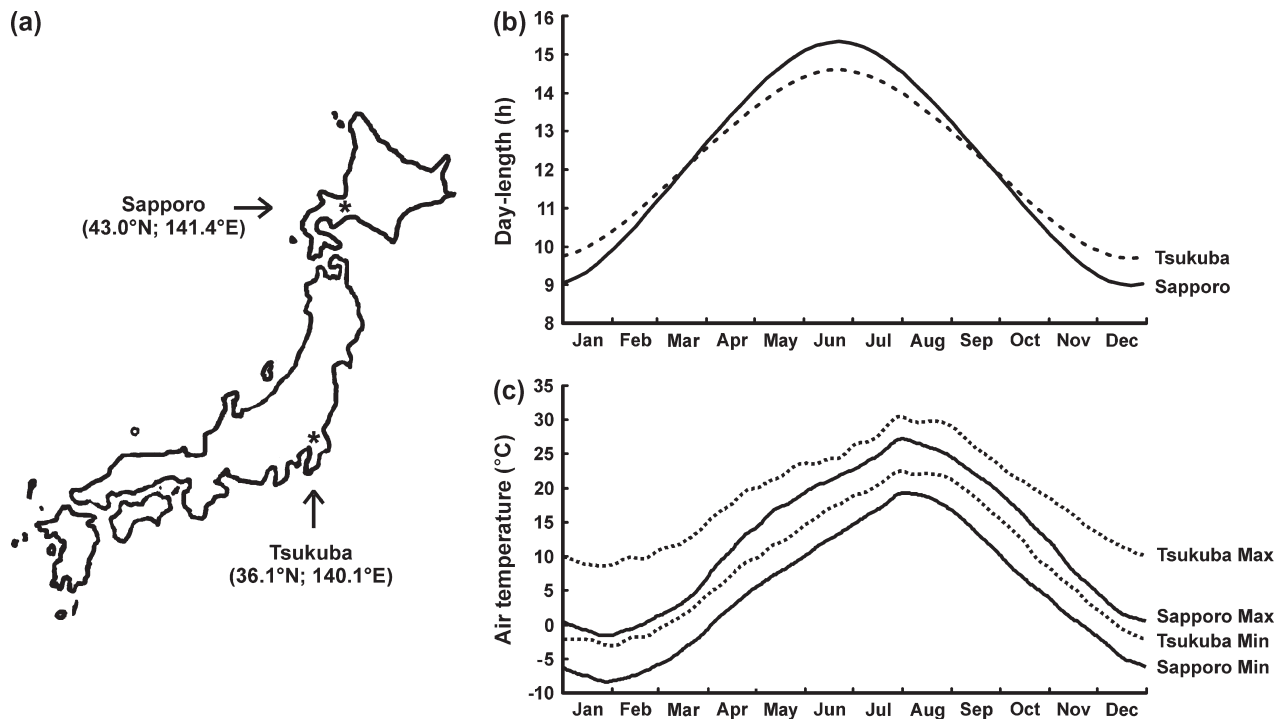


Fig. 1. (a) A map of Japan showing the origin of populations used in the experiment: the northern site (Sapporo) and the southern site (Tsukuba). (b) Seasonal changes of natural day-length in Sapporo and Tsukuba (without civil twilight; Japan Meteorological Agency, 2007). (c) Seasonal changes of natural temperature in Sapporo and Tsukuba (daily minimum and maximum air temperatures averaged for 1979–2000 for Sapporo and Tsukuba; Japan Meteorological Agency, 2007).

fleabane *Erigeron philadelphicus*, and other species of concurrent vegetation (mostly Asteraceae) in July to September 2001 and 2002 in the northern site and in July 2001 and September 2002 in the southern site. Females were then reared individually in plastic Petri dishes (20 × 90 mm) under an LD 16 : 8 h photoperiod at 24 ± 1 °C in an incubator. Frozen eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, were provided as food. Sprouts of fresh soybean, *Glycine max* (L.) Merrill (with a piece of moist cotton wool) were used as an oviposition substrate and a source of moisture. Food and water were replenished every other day. Upon emergence of the first adult males in the progeny of each female, the species was identified based on the shape of the paramere (Yasunaga, 1997). The number of identified wild-collected females exceeded 500 for *O. sauteri* and 100 for *O. minutus* from Sapporo and 120 for *O. sauteri* and 30 for *O. minutus* in Tsukuba. Their progeny were used to establish laboratory cultures. Adults were reared in transparent plastic containers (90 × 150 mm; with openings in the lids covered with mesh for aeration) at a density of 100–300 per container under an LD 16 : 8 h photoperiod at 24 ± 1 °C. Approximately 0.1 g of frozen eggs of *E. kuehniella* was provided to each container every other day; fresh soybean sprouts were provided every fourth day. The containers were filled to a depth of 1.0–1.5 cm with unhulled rice grains to provide shelter for bugs, and thus avoid cannibalism. Soybean sprouts with eggs of *O. sauteri* and *O. minutus* were transferred to Petri dishes, which were also half-filled with unhulled rice grains. Hatched nymphs (approximately 50–150 per Petri dish) were given the same food (0.03–0.05 g per dish) and water every other day. Upon moulting to adults, bugs from one to three Petri dishes were transferred to new transparent plastic containers as described above.

Experimental procedure

Soybean sprouts with eggs produced within 24 h were collected from the cultures and transferred to new Petri dishes half-filled with unhulled rice and kept under different photoperiodic conditions ranging from an LD 10 : 14 to 16 : 8 h photoperiod (at hourly increments) at 20, 24, and 28 ± 0.5 °C. Nymphs and then adults were fed as described above. The pre-adult period was assessed as the egg to adult period. The density of nymphs was kept at 50–150 per Petri dish. Sufficient food and unhulled rice prevented cannibalism. The emergence of adults was recorded daily. Upon emergence, adults of both sexes 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.

Determination of duration and thermal requirements of pre-adult development

The duration of the pre-adult period was statistically examined for each sex and photoperiod/temperature treatment

using a *t*-test (between sexes within each treatment) and a Tukey multiple comparison test (between different photoperiods; Zar, 1999).

To further examine the effect of day length on thermal requirements for development, the data on pre-adult period of females were analysed separately for each of four selected photoperiods (LD 10 : 14, 12 : 12, 14 : 10 and 16 : 8 h).

The rate of development (R_T) at a given temperature T was calculated for each individual as:

$$R_T = 1/D$$

where D is developmental time from egg to adult (= the pre-adult period, in days). As it is widely accepted that, within a range of moderate favourable temperatures, the relationship between rate of development (R_T) and temperature is close to linear (Campbell *et al.*, 1974; Gilbert & Raworth, 1996), a linear regression equation was applied:

$$R_T = a + bT$$

where a is an intercept, b is a coefficient of linear regression (and a slope of the regression line), and T is temperature. Based on this equation, a lower development threshold (LDT; or the lower thermal threshold of development) was calculated as:

$$\text{LDT} = -a/b$$

The sum of effective temperatures (SET), or the number of degree-days above the LDT, for pre-adult development was calculated as:

$$\text{SET} = 1/b$$

Standard errors (SE) of thermal parameters were calculated according to Campbell *et al.* (1974) and Lopatina *et al.* (2007). Regression lines (intercepts, slopes and LDTs) were compared within each population in accordance with the procedure of Sokal & Rohlf (1995) (unplanned comparison among a set of regression coefficients with the Tukey–Kramer test).

Diapause status determination

As males of many, if not all, *Orius* spp. do not appear to enter reproductive diapause (Ito & Nakata, 1998; Ruberson *et al.*, 1998; Musolin *et al.*, 2004), only females were dissected under a dissecting microscope 15–16 (at 20 °C), 12–13 (at 24 °C), or 7 (at 28 °C) days after emergence as adults. The timing of dissection was based on preliminary experiments, in which most of the nondiapause females started oviposition 12–13, 8–10, and 5–6 days after adult emergence at the respective temperatures. Females with no visible eggs in their ovarioles were considered to be in reproductive diapause (Musolin *et al.*, 2004; Fig. 2b). To determine more precisely the critical day-length of diapause induction, additional photoperiods were used: LD 13.5 : 10.5 h at 20 °C for *O. sauteri* from Sapporo, LD 14.5 : 9.5 h at 20 and 24 °C for *O. minutus* from Sapporo, and LD 12.5 : 11.5 h at 24 °C for *O. minutus* from Tsukuba. Because of a large variation of

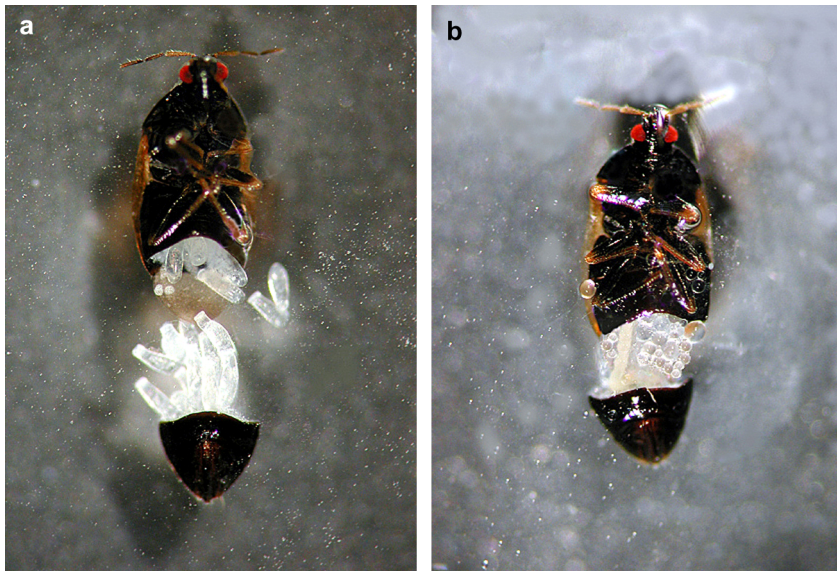


Fig. 2. Dissected females of *Orius* sp.: developed ova and reproductive structures in a nondiapause female (a) and well-developed fat body and undeveloped reproductive structures in a diapause female (b).

the photoperiodic response in *O. sauteri* from Tsukuba under short-day conditions (see below), the whole experiment under these conditions was replicated three times and the results were combined.

The critical day length of diapause induction was determined as the point on the photoperiodic response curve, at which diapause is induced in 50% of only those individuals inclined to diapause in the particular population. This method was applied because the incidence of diapause in both species in Tsukuba did not normally exceed even 50% at any tested combination of photoperiod and temperature (for details, see Results).

Results

Effect of day-length and temperature on duration of the pre-adult period

In both species and in all populations, males mostly completed pre-adult development on average 1–2 days earlier than females (Fig. 3). Thus, at 20 °C, the mean pre-adult period was significantly shorter in males than in females in 21 out of 22 trials. The trend was also pronounced at 24 °C (in 15 cases males developed significantly faster than females; the opposite was recorded only once) but it became less evident at 28 °C (12 versus three cases).

Both temperature and day length affected the total duration of the pre-adult period. At 20 °C, in the northern populations of both species, the pre-adult development was significantly slower under an LD 14:10h photoperiod and tended to be faster under conditions with shorter or longer photophases (Fig. 3a,b). The difference between the mean values under different photoperiodic conditions amounted to 3–5 days in each species and sex. In the southern populations at the same temperature, the tendency was similar (i.e. prolonged pre-adult

period under an LD 14:10 and/or 15:9h photoperiod), although the peak was less pronounced (Fig. 3c,d). By contrast, at 24 and 28 °C, the pre-adult period tended to slightly decrease with lengthening of photoperiod from short-day to long-day conditions, although the difference in the mean values under different photoperiodic conditions was less than that at 20 °C (1–4 days).

Effect of day-length on thermal requirements for development

Day length experienced by the pre-adult stages also influenced the thermal requirements of females (as the pre-adult development of males basically followed the same pattern as that of females, data for only females under photoperiods with even photophases were analysed). When rates of the pre-adult development were plotted against temperature (Fig. 4) and parameters of the linear regression equations were calculated (Table 1), a complicated pattern appeared. The slope of the regression line, which directly characterizes the dependence of developmental rates on temperature, was significantly smaller under short-day conditions of LD 12:12h (and sometimes of LD 10:14h) than under long-day conditions (i.e. LD 14:10 and 16:8h) ($P < 0.05$; Tukey–Kramer test).

The LDT showed an interesting but complicated pattern of variation. It varied from 8.4 °C (*O. minutus* from Sapporo at LD 12:12h) to 13.4 °C (*O. sauteri* from Sapporo at LD 14:10h) and in each population it ranged within 1.6–3.7 °C (Fig. 4, Table 1). The LDT was always significantly lower under an LD 12:12h photoperiod than under any other photoperiod, particularly, long-day conditions (LD 14:10 and 16:8h) ($P < 0.05$; Tukey–Kramer test).

By contrast, the SET was almost always higher at LD 12:12h than at any other photoperiod (Table 1).

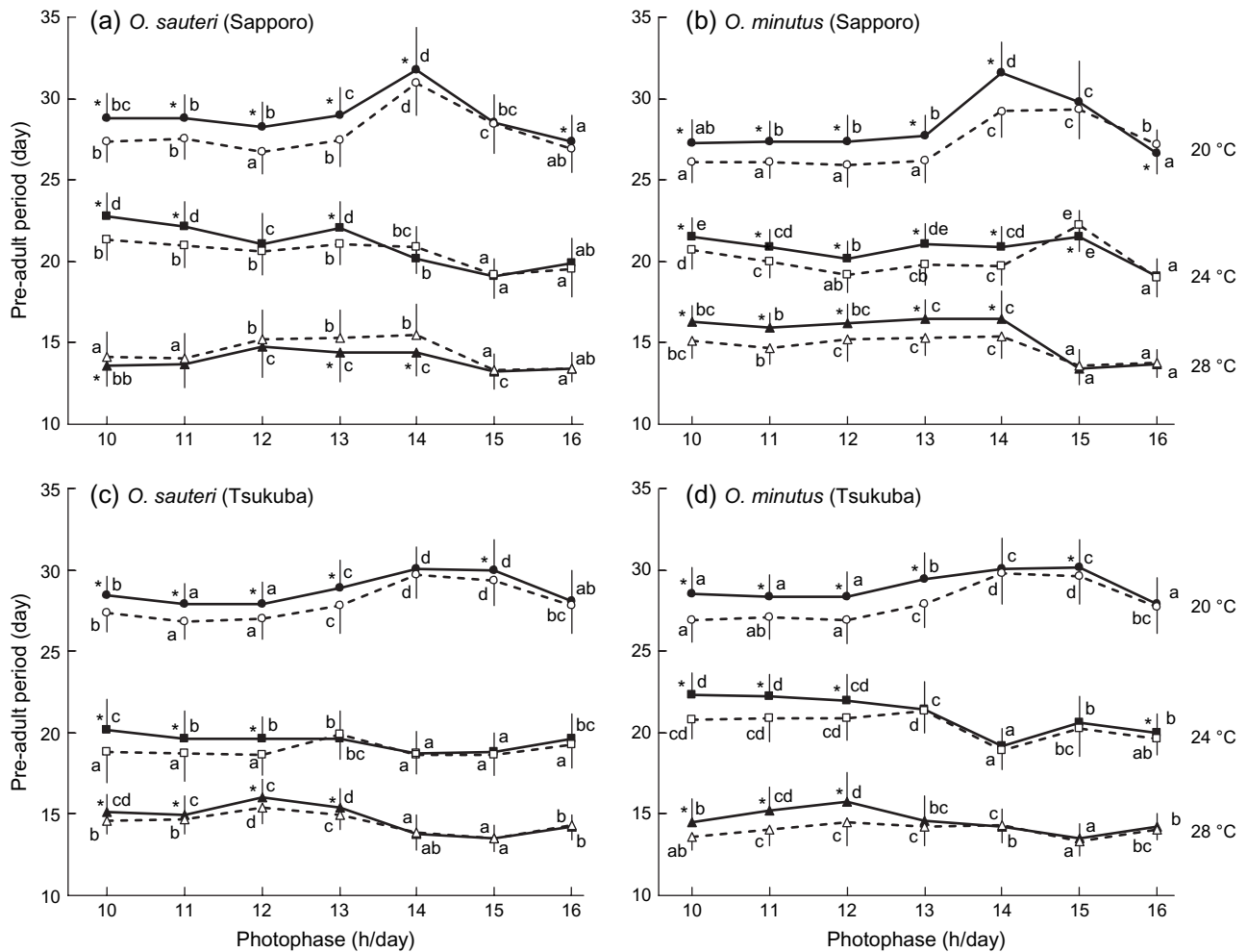


Fig. 3. The effect of day-length and temperature on duration of the pre-adult period in the northern and southern populations of *Orius sauteri* (a and c) and *Orius minutus* (b and d). Females, solid lines and closed symbols; males, broken lines and open symbols. Asterisks indicate that means \pm SD are significantly different between females and males under the same conditions ($P < 0.01$, *t*-test). Means with the same letters within each temperature/sex data set (each response curve) are not significantly different ($P > 0.01$, Tukey multiple comparison test). Sample size (in each sex): *O. sauteri*: $n = 100$ –577 (20 °C), 54–704 (24 °C) and 89–260 (28 °C); *O. minutus*: $n = 115$ –246 (20 °C), 78–152 (24 °C) and 80–216 (28 °C).

Photoperiodic response of diapause induction

The effect of photoperiod and temperature on induction of reproductive diapause differed markedly between the two species and two locations. At the moderate temperatures (20 and 24 °C), the majority of females from the northern populations of both species entered diapause under short days, whereas most females under long-day conditions did not enter diapause, thus showing a typical long-day type photoperiodic response of diapause induction (Fig. 5a,b). At 20 °C, the critical day-length of diapause induction was a little shorter than 14 h in *O. sauteri* and 14.5 h in *O. minutus*. The higher temperature (24 °C) markedly decreased the incidence of diapause in *O. sauteri* and shifted the critical day-length by almost 1 h, whereas a further increase of temperature (up to 28 °C) almost completely suppressed

induction of diapause even under the short days. In *O. minutus* from the same location, however, increase of temperature neither influenced the incidence of diapause, nor shifted markedly the critical day length.

The photoperiodic responses of the southern populations differed from those of the northern populations. In both species and in all the treatments, the incidence of diapause hardly exceeded 50%, showing that the majority of the southern population does not enter diapause even in response to the very short day length (Fig. 5c,d). A large variation in the photoperiodic response of *O. sauteri* was recorded under the short-day conditions at 20 and 24 °C (data not shown) and even when three replicates of the experiment were pooled the resulting incidence of diapause was somewhat lower at 20 °C than 24 °C (Fig. 5c). The further increase of temperature (to 28 °C) almost completely suppressed induction of diapause

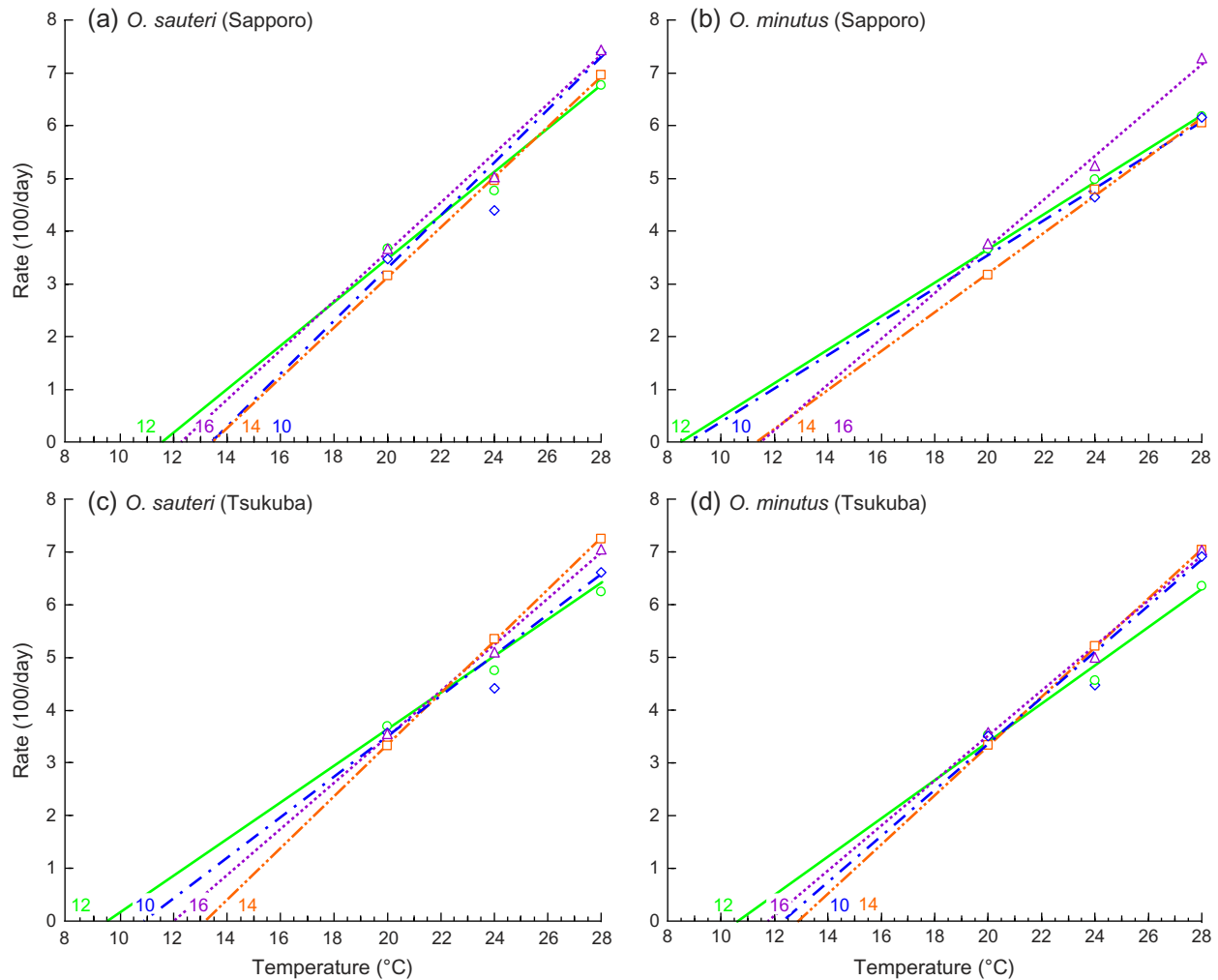


Fig. 4. Regression lines of rate of pre-adult development of females under four photoperiods in the northern and southern populations of *Orius sauteri* (a, c) and *Orius minutus* (b, d). Diamonds and dash-dot-dash lines are for an LD 10 : 14 h photoperiod; circles and solid lines are for an LD 12 : 12 h photoperiod; squares and dash-dot-dot-dash lines are for an LD 14 : 10 h photoperiod; triangles and dotted lines are for an LD 16 : 8 h photoperiod [numbers showing photophases (h) are provided next to the corresponding lines].

even under the short-day conditions. In the central population of *O. minutus*, however, all the photoperiodic response curves obtained at three temperatures had similar shapes with clearly higher incidence of diapause under short days than under long days. Thus, increase of temperature did not have marked influence on either the incidence of diapause or the critical day length in this population (Fig. 5d).

Discussion

Male development at a low temperature

Males of both *Orius* species and in both populations emerge as adults earlier than females and this trend is most pronounced at the lower temperatures (Fig. 3).

Similar situations are recorded, for example, in the mirid *Deraeocoris nebulosus* (Boyd & Alverson, 2004) and in the anthocorid *O. strigicollis* (Musolin *et al.*, 2004) and termed 'temperature-regulated protandry' (Boyd & Alverson, 2004). Faster development of male nymphs might be related to the earlier observation that males are always smaller than females in *O. sauteri* (Nakata, 1995) and probably in most of other *Orius* species. Whether or not protandry disappears at higher temperatures, the faster pre-adult development and earlier emergence of males might be of ecological significance: by the time when females emerge as adults, males might be ready to mate. This adaptation appears to be particularly important for the first generation in the early summer, when the temperature is still low and no males survive from the previous (i.e. overwintering) generation.

Table 1. Parameters of linear regression of the rates of pre-adult development, lower developmental thresholds (LDT) and sums of effective temperatures (SET) of females under four photoperiods in northern and southern populations of *Orius sauteri* and *Orius minutus*.

Species (population)	Photophase (h)	n	Parameters of the linear regression equation ($\times 10^{-2}$)		R^2 of regression	LDT \pm SE ($^{\circ}$ C)	SET \pm SE (degree-days)
			Intercept (a) \pm SE	Slope (b) \pm SE			
<i>Orius sauteri</i> (Sapporo)	10	453	-6.714 ± 0.17^a	0.5 ± 0.007^a	0.906	13.41 ± 0.16^a	199.74 ± 3.03
	12	379	-4.8 ± 0.178^b	0.414 ± 0.007^b	0.864	11.59 ± 0.22^b	241.53 ± 4.93
	14	402	-6.452 ± 0.177^a	$0.48 \pm 0.007^{a,c}$	0.928	13.44 ± 0.16^a	208.24 ± 2.9
	16	402	-5.75 ± 0.171^c	0.468 ± 0.007^c	0.949	12.29 ± 0.17^b	213.78 ± 2.47
<i>Orius sauteri</i> (Tsukuba)	10	1175	-4.218 ± 0.08^a	0.386 ± 0.003^a	0.914	10.92 ± 0.11^a	258.92 ± 2.32
	12	1406	-3.312 ± 0.08^b	0.348 ± 0.003^b	0.894	9.51 ± 0.14^b	287.21 ± 2.64
	14	452	-6.53 ± 0.127^c	0.494 ± 0.005^c	0.933	13.21 ± 0.12^c	202.35 ± 2.55
	16	414	-5.234 ± 0.126^d	0.437 ± 0.005^d	0.945	11.97 ± 0.15^d	228.67 ± 2.71
<i>Orius minutus</i> (Sapporo)	10	438	-2.762 ± 0.13^a	0.316 ± 0.005^a	0.907	8.73 ± 0.26^a	316.21 ± 4.86
	12	414	-2.68 ± 0.12^a	0.318 ± 0.005^a	0.92	8.43 ± 0.25^a	314.52 ± 4.58
	14	437	-4.2 ± 0.118^b	0.371 ± 0.05^b	0.901	11.33 ± 0.17^b	269.76 ± 4.28
	16	402	-4.96 ± 0.124^c	0.434 ± 0.005^c	0.952	11.43 ± 0.15^b	230.53 ± 2.58
<i>Orius minutus</i> (Tsukuba)	10	443	-5.372 ± 0.153^a	$0.437 \pm 0.006^{a,c}$	0.884	$12.29 \pm 0.18^{a,c}$	228.82 ± 3.95
	12	445	-3.843 ± 0.162^b	0.363 ± 0.007^b	0.838	10.58 ± 0.26^b	275.34 ± 5.76
	14	440	-6.005 ± 0.154^c	0.467 ± 0.006^c	0.951	12.85 ± 0.16^a	213.92 ± 2.32
	16	325	-5.009 ± 0.183^a	0.427 ± 0.008^a	0.956	11.73 ± 0.22^c	234.09 ± 2.8

Data are the mean \pm SE. Values followed by the same superscript letters within each column and population are not significantly different ($P > 0.05$; Tukey–Kramer test).

Effect of day length and temperature on duration of the pre-adult period

Rates of nymphal growth and development in insects can be controlled not only by obvious environmental factors and cues such as temperature or food availability, but also by day length (Danks, 1987; Saunders *et al.*, 2002). Photoperiodic responses reported in heteropteran species range from a slight retardation or acceleration of nymphal growth to prolonged nymphal diapause (Saunders, 1983; Kiritani, 1985; Musolin & Saulich, 1997; Tanaka *et al.*, 2002; Lopatina *et al.*, 2007; Pfenning *et al.*, 2007; Saulich & Musolin, 2007). In insects that overwinter in the adult stage in the Temperate Zone, acceleration of nymphal growth under the late-season short-day conditions may ensure completion of nymphal development and synchronization of adult emergence before the autumnal deterioration of environmental conditions (Musolin & Saulich, 1997, 1999).

Among anthocorids, the effect of day length on the duration of the nymphal period is reported for only three species. In *O. insidiosus* from Arkansas, U.S.A., short-day conditions accelerate growth of nymphs at 20 $^{\circ}$ C (Ruberson *et al.*, 1991), but the trend is not consistent in the same species from Georgia, U.S.A., at 18 $^{\circ}$ C (van den Meiracker, 1994). In *O. majusculus* from the Netherlands, short-day conditions significantly accelerate the growth of nymphs at 18 $^{\circ}$ C, although the trend is also inconsistent (van den Meiracker, 1994). In *O. strigicollis* from central Japan, at 20 $^{\circ}$ C, the pre-adult period tends to increase with lengthening of the photoperiod from LD 10:14 to 14:10 h, although there is a slight decrease with further lengthening of the photoperiod. At higher temperatures (24 and 28 $^{\circ}$ C),

the trend is opposite or not pronounced (Musolin *et al.*, 2004).

In the present study, the responses of *O. sauteri* and *O. minutus* are somewhat similar, although they differ between the geographic locations and temperature regimes. In the northern populations at 20 $^{\circ}$ C, pre-adult growth is slow under long-day conditions of LD 14:10 h and tends to be significantly faster under conditions with shorter or longer photophases (Fig. 3a,b). At higher temperatures, the longer photophases result in shortening of the pre-adult period or the influence of the day length is not pronounced. Although in early- and mid-summer, when the day length is long and time is not a limiting seasonal factor, the nymphal growth is apparently mostly controlled by temperature, and the situation might be different in early autumn, when acceleration of nymphal growth by short (or shortening) day length at low (or decreasing) ambient temperature might well be of adaptive significance. The late-season nymphs might use short day as a signal to hasten growth. Earlier and better synchronized emergence of adults will increase chances to encounter mates in autumn and gain reserves for overwintering in females.

In the central populations of both species, this photoperiodic response is less pronounced than in the north (Fig. 3c,d), possibly because temperature and proper timing of adult emergence are less critical in the south than in the north: ambient temperature exceeds LDT much longer in Tsukuba than in Sapporo (Fig. 1c, Table 1).

The same results might also be interpreted as follows: the near-critical photoperiods (LD 14:10 and/or 15:9 h) retard nymphal development in the northern populations at 20 $^{\circ}$ C. A similar, though more pronounced, situation is reported in the linden bug, *Pyrrhocoris apterus* from Prague (Saunders,

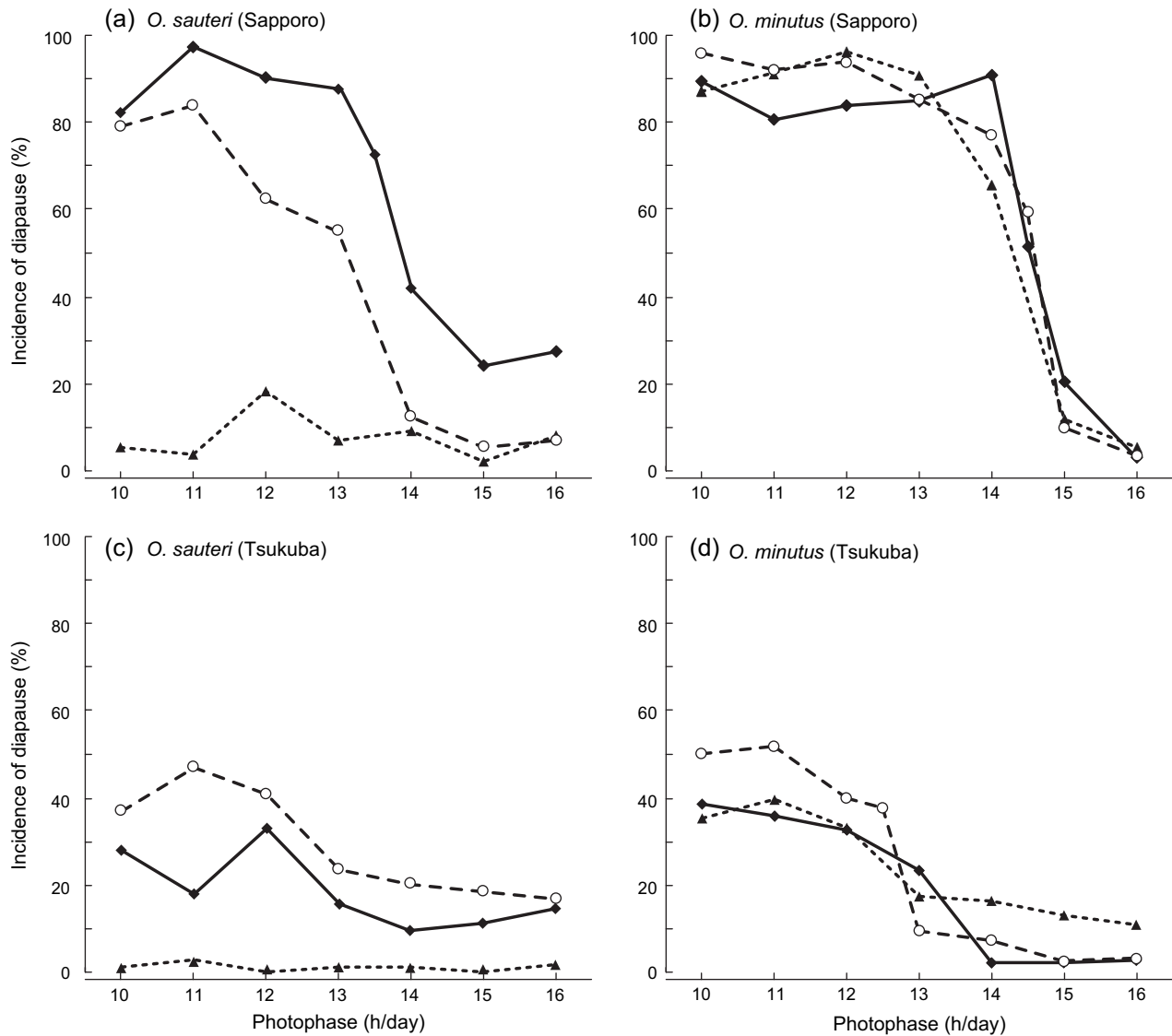


Fig. 5. Photoperiodic response curves for diapause induction in females of the northern and southern populations of *Orius sauteri* (a, c) and *Orius minutus* (b, d). 20 °C, solid lines and closed diamonds; 24 °C, broken lines and open circles; 28 °C, dotted lines and closed triangles. Sample size: *O. sauteri*: $n = 69\text{--}434$ (20 °C), $28\text{--}413$ (24 °C) and $38\text{--}162$ (28 °C); *O. minutus*: $n = 86\text{--}192$ (20 °C), $59\text{--}220$ (24 °C) and $46\text{--}178$ (28 °C).

1983). Two possible explanations are suggested to interpret the findings: a stationary (rather than changing), neither 'long' nor 'short', circadian system might be deleterious if maintained for long periods and/or the phenomenon serves to 'tighten up' the scatter for physiological ages among the nymphs hatched during the long summer season, thus encouraging univoltinism in the population (Saunders, 1983: 404). Neither explanation, however, can be applied to the present results for the three *Orius* species reported in the present study and elsewhere (Musolin *et al.*, 2004) because no obvious symptoms of deleterious effects are observed under near-critical conditions (such as higher mortality or morphological deformation; data not shown) and all species are multivoltine at least in the southern location. It might be, however, that

the photoperiodic systems of species with short life cycles (such as those of the *Orius* bugs) need more time (i.e. more days counted) for decision-making regarding whether to start reproduction or enter diapause under conditions that are neither long- nor short-day.

Effect of day length on thermal requirements for development

Relationships between photoperiodic conditions and thermal requirements, or thermal reaction norms, in insects have attracted substantial attention only recently (Lopatina *et al.*, 2007). Both LDT for nymphal development and a coefficient

of linear regression b between developmental rates R_T and temperature (i.e. a slope of the regression line) decrease significantly under short-day conditions in four tested populations of the linden bug, *P. apterus*. This finding indicates that the nymphal development in this species is less temperature dependent under short-day than under long-day conditions. It is argued that short-day conditions make nymphal growth of this species less dependent on temperature by decreasing LDT and a coefficient of linear regression b . Such a response is suggested to be adaptive for nymphs at the end of the summer season when both day-length and ambient temperature decrease and time becomes a limiting factor and when nymphs should reach the diapausing stage (i.e. adult in this species) before the autumnal deterioration of environmental conditions. Comparative reanalysis of previously published data on nymphal development of heteropterans and other insect species under different combinations of day length and temperature suggests that the phenomenon might not be restricted to *P. apterus*, although it is not universal (Lopatina *et al.*, 2007).

The data obtained in the present study for the two *Orius* species do not fit perfectly the pattern found in *P. apterus* (Lopatina *et al.*, 2007). Thus, for example, the changes in the slope of the regression line b and LDT do not appear to be gradual (Fig. 4, Table 1). Both these characteristics, however, have the lowest or very close to the lowest values under an LD 12 : 12 h photoperiod, the day length of the autumnal equinox (around September 22). The weaker dependence of the nymphal growth on temperature and the lower LDT in the autumn would be apparently adaptive. For example, it allows faster development under comparatively low temperatures and slower development under comparatively high temperatures. The shortest day length tested in the experiment (LD 10 : 14 h) takes place in both locations much later (i.e. in November; Fig. 1b) and is ecologically irrelevant to nymphal growth, at least at the northern site.

Apparently, photoperiodically induced modifications of nymphal growth rates and changing thermal requirements are inter-related, although the exact underlying mechanism remains unknown. Testing a wider range of temperatures and a direct measurement of LDT in nymphs reared under different photoperiodic conditions by exposing them to low temperatures close to the estimated LDT might be helpful to clarify this relationship. Overall, what have previously been considered thermal constants of development (such as LDT and SET) are, in reality, not constant but can be influenced by photoperiodic conditions, and thus vary during the season. This situation can make the precise insect phenology modeling and forecast more complicated.

Photoperiodic response of diapause induction

Temperature–photoperiod interactions have different effects on diapause induction responses in different true bug species. In some of them, rearing at higher constant temperatures can result in a markedly shifted threshold of the photoperiodic response curve. Thus, in *P. apterus* and in the pentatomid *Graphosoma lineatum*, increases in temperature

from 20 to 27 °C and from 24 to 28 °C, respectively, cause a shortening of the threshold value by approximately 90 min (Numata *et al.*, 1993; Musolin & Saulich, 1995; Saulich & Musolin, 2007). In other species, the response is weaker (e.g. in the pentatomid *Nezara viridula*; Musolin & Numata, 2003). In yet other species, a change in temperature does not shift the threshold as, for example, in the alydid *Riptortus pedestris* (formerly *Riptortus clavatus*) within the range 20–30 °C (Kobayashi & Numata, 1995; Musolin & Saulich, 1996) and in the pentatomid *Arma custos* within the range 27–30 °C (Volkovich & Saulich, 1994). As the present data demonstrate, in the northern site, two closely-related and sympatrically distributed species (*O. sauteri* and *O. minutus*) show quite different responses to the temperature–photoperiod interactions: in *O. sauteri*, an increase of temperature clearly shifts the photoperiodic threshold for diapause induction and a high temperature (28 °C) almost completely suppresses the photoperiodic response, whereas the same increase of temperature has no noticeable effect in *O. minutus* (Fig. 5a,b). Like many other insects, most probably including Heteroptera species (e.g. Nakashima & Hirose, 1997; Shimizu & Kawasaki, 2001), these two *Orius* species show a trend of suppression of diapause induction response towards the tropics and subtropics: in the southern populations of both species, the incidence of diapause scarcely reaches 50% even under very short-day conditions (Fig. 5c,d). Even then, however, the response to the temperature–photoperiod interactions follows the same pattern as in the north: increased temperature affects the photoperiodic response in *O. sauteri* but not in *O. minutus*. The interspecific differences in critical day-length of diapause induction and the temperature–photoperiod interactions might suggest that, at least in the north, *O. minutus* enters diapause somewhat earlier in late summer but its diapause induction response is less dependent on ambient temperature than that in *O. sauteri*.

Seasonal development and voltinism of *O. sauteri* and *O. minutus* in northern and central Japan

In Sapporo (the northern site), daily mean air temperature exceeds 12.7 °C (the mean LDT calculated for *O. sauteri*; Table 1) starting from the fourth week of May (Fig. 1c). The photoperiodic response curves (Fig. 5a) suggest that diapause in this species is induced in 50% of females when the natural day length decreases to less than 14 h in mid-August and when the mean ambient temperature is in the range 20–24 °C. The seasonal accumulation of heat for this period is approximately 480 degree-days above the threshold of 12.7 °C (Japan Meteorological Agency, 2007), although it should be noted that the real thresholds are always lower than those derived by regression equations because of the linearity of the regression model. The data from Figures 3(a) and 4(a) and Table 1, as well as those of Nakata (1995), suggest that a full generation of *O. sauteri* (including a preoviposition period) might require approximately 300 degree-days for development (pre-adult growth and maturation of females). Thus, thermal conditions in the northern site allow *O. sauteri* to produce only one or

two generations per year (the second generation might be incomplete i.e. only in warmer years and/or only in a part of the population; it can also overlap with the first generation).

A similar analysis for *O. minutus* suggests that this species enters diapause starting from early July and, thus, can accumulate effective heat for production of only one complete generation in Sapporo.

On the central site, the season suitable for growth and reproduction is much longer (Fig. 1c). The low incidence of diapause induction in both species in the laboratory experiment (Fig. 5c,d) makes it difficult to predict precisely when females enter diapause in the field in Tsukuba. Data of Shimizu & Kawasaki (2001) suggest that, if females of these species emerge as adults in late September in Tsukuba, some of them might become reproductive before overwintering. The present estimation based on the data from Figures 1, 3 and 4 and Table 1 suggests that, from April to September, both *O. sauteri* and *O. minutus* can produce approximately five (probably overlapping) generations in Tsukuba. This is similar to that suggested for *O. strigicollis* in the same region (Musolin *et al.*, 2004).

Implications for biological control

At least five predatory *Orius* species are used as biological control agents and have been commercialized in several countries (van Lenteren *et al.*, 1997; Glenister, 1998; Ohta, 2001; Yano, 2004). The large-scale application of these predatory bugs has, however, encountered serious problems. The major factor limiting the predatory efficacy of *Orius* is diapause induction in late autumn and winter in greenhouses lacking supplementary lighting (Yano, 1999). To extend the seasonal limits of biological control, four options are suggested: application of low intensity light in greenhouses to extend the photoperiod and prevent diapause induction (Gilkeson & Hill, 1986; Chambers *et al.*, 1993; Jacobson, 1993); selection for nondiapause strains (Gilkeson & Hill, 1986; Morewood & Gilkeson, 1991); maintenance of high temperature in greenhouses that prevents diapause induction (Morewood & Gilkeson, 1991; Kohno, 1998); and application of nondiapause species or strains from southern temperate or sub-tropical zones with a low diapause incidence (Gillespie & Quiring, 1993; Hirose *et al.*, 1999). The present results suggest that, when *Orius* spp. are used in greenhouses, high temperature can markedly reduce incidence of diapause in *O. sauteri* but not in *O. minutus*. In both species, however, artificial extension of photophase should result in decreased incidence of reproductive diapause because day-length is the leading cue for diapause induction. The results also confirm that southern populations have a lower incidence of diapause even under short-day conditions. Thus, if temperature permits, most females will remain reproductive even in late autumn and winter.

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