among different ages (KRUSKAL-WALLIS's test, $p > 0.05$).

The present findings concerning the relationships between age and time of day to the mating activity will be useful to establish techniques for isolating and monitoring the sex pheromone of *D. indica*.

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Photoperiodic Effect on Incidence of Reproductive Diapause in *Orius sauteri* and *O. minutus* (Heteroptera: Anthocoridae)¹

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Minute pirate bugs *Orius* spp. are known as predacious natural enemies on various agricultural pests like thrips, aphids, spider mites and whiteflies. Among these, *O. sauteri* (POPPIUS) and *O. minutus* (L.) are widely distributed throughout the main islands of Japan and are commonly observed in cultivated fields (YASUNAGA and KASHIO, 1993). Naturally occurring

Orius spp. effectively controlled *Thrips palmi* KARNY populations on eggplant in the field (NAGAI, 1993). On the other hand, no remarkable efficiency of *Orius* bugs for controlling thrips populations were confirmed in the greenhouse during winter in Japan. This may arise from the diminution of predatory activity due to low temperature and/or from an induction of reproductive diapause provoked by short photoperiod. However, little is known about the life history characteristics of *Orius* species in Japan to date. One reason for this is that the classification of Japanese *Orius* species had been confused until the revision of *Orius* by YASUNAGA (1993).

In this report, the photoperiodic reaction of the Kurume (33°18'N and 130°33'E) population of *O. sauteri* and *O. minutus* in the laboratory and seasonal changes in the ratio of the individuals that exhibited reproductive diapause in the field were examined.

MATERIALS AND METHODS

Laboratory experiments. The offspring of the field-collected females were tested. Each of the individuals tested was reared under constant conditions of 22 ± 1°C and a photoperiod of 10L–14D, 12L–12D,

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12.5L–11.5D, 13L–11D, 14L–10D or 15L–9D throughout the period of the experiment.

Insects were reared in a plastic container (55 mm in diameter and 10 mm in depth). Field-collected females were kept individually. Nymphs were kept together with some siblings. Each emerged female adult was kept with a male. A piece of filter paper (55 mm in diameter) was set at the bottom of the container. The mold mite, *Tyrophagus putrescentiae* (SCHRANK), and the stamina of the dishcloth gourd, *Luffa cylindrica* ROEM., were amply supplied as the diet. One piece of cut eggplant leaf including leaf vein (ca. 2 cm² in area) was put on the filter paper. Water was given at the base of the leaf vein and on the filter paper ad-lib. The leaf and the diet were exchanged every two or three days. The filter paper was exchanged ad-lib. The male was replaced when he died before the female started oviposition.

Whether or not each female laid eggs was examined for 20 days after copulation. Females that laid eggs before the 20th day were treated as non-diapausing females. Those that laid no eggs by the 20th day or that died before the 20th day without oviposition were dissected and the ovaries investigated under a binocular microscope. Females that had mature eggs in the ovary when dissected were treated as non-

diapausing females. Females that had resorbed eggs when they died were treated as non-diapausing females, because the ovary had developed before dissection. Females that died before the 10th day and had no mature eggs nor resorbed eggs at the time of death were excluded from the calculation. Other females were treated as diapausing ones.

The copulating partner for the female tested was reared under the same photoperiod as the female. Since males reared under short photoperiod exhibited active copulative behavior and the copulation by such males was effective for fertilization (KOHNO, unpublished), the effect of the difference in the photoperiod for the rearing of males was neglected.

Because field-collected females cannot be correctly identified (YASUNAGA and KASHIO, 1993), they were identified by observing the genitalia of their male progenies under a binocular microscope.

Field-collected females. The incidence of the oviposition of the females collected in the field along with the seasonal changes was examined for seven days at $22 \pm 1^\circ\text{C}$ and various photoperiodic regimes between 10L–14D and 15L–9D. The effect of photoperiod during the test was treated as negligible, because non-diapausing females continued to lay eggs when transferred to short photoperiodic conditions (8L–16D or

Table 1. Incidence of oviposition and ovarian maturation at death in *O. sauteri* and *O. minutus* females when reared under various photoperiodic conditions

		No. of individuals					% Diapause	
		Photoperiod	Oviposition	No oviposition				Total
				Mature eggs	Resorbed eggs	No eggs		
<i>O. sauteri</i> ^a	10L–14D	0	0	0	46	46	100.0	
	12L–12D	3	1	0	17	21	81.0	
	12.5L–11.5D	8	1	0	5	14	35.7	
	13L–11D	16	2	0	7	25	28.0	
	14L–10D	26	1	1	2	30	6.7	
	15L–9D	12	0	0	5	17	29.4	
<i>O. minutus</i> ^b	10L–14D	0	0	0	5	5	100.0	
	12L–12D	2	0	0	28	30	93.3	
	12.5L–11.5D	32	3	0	16	51	31.4	
	13L–11D	6	2	0	8	16	50.0	
	14L–10D	10	0	0	0	10	0.0	
	15L–9D	4	0	0	0	4	0.0	

^a Ratio of *O. sauteri* females in diapause differed significantly between photoperiods (PEARSON test, $\chi^2 = 85.1$, $p < 0.01$).

^b Ratio of *O. minutus* females in diapause differed significantly between photoperiods (PEARSON test, $\chi^2 = 48.6$, $p < 0.01$).

Table 2. Preovipositional periods of non-diapausing female *O. sauteri* and *O. minutus* under various photoperiodic regimes at 22°C

	Photoperiods				
	12L-12D	12.5L-11.5D	13L-11D	14L-10D	15L-9D
<i>O. sauteri</i> ^a					
No. days ± SE	12.0 ± 1.2	9.1 ± 1.3	7.1 ± 0.8	8.8 ± 1.0	7.3 ± 1.1
Range	10-14	5-14	4-16	4-19	4-14
n	3	8	16	26	12
<i>O. minutus</i> ^b					
No. days ± SE	13.0 ± 3.0	8.4 ± 0.7	8.8 ± 1.8	7.4 ± 1.0	5.5 ± 0.5
Range	10-16	4-19	5-17	4-14	5-7
n	2	32	6	10	4

^a Preovipositional periods of *O. sauteri* did not differ significantly between photoperiods (KRUSKAL-WALLIS test, $\chi^2 = 5.21$, df = 4, $p > 0.27$).

^b Preovipositional periods of *O. minutus* did not differ significantly between photoperiods (KRUSKAL-WALLIS test, $\chi^2 = 6.38$, df = 4, $p > 0.17$).

12L-12D, KOHNO, unpublished). Other conditions were the same as the test for laboratory-reared females.

RESULTS AND DISCUSSION

The photoperiod affected the incidence of reproductive diapause in *O. sauteri* and *O. minutus* (Table 1). The incidence of diapause induction of both species exhibited a long-day response. The critical photoperiod inducing reproductive diapause of both species fell between 12L-12D and 13L-11D. Although the ratio of individuals of *O. minutus* that exhibited reproductive diapause under 13L-11D was higher than that under 12.5L-11.5D, this might have been due to the sampling error. The proportion of individuals that exhibited reproductive diapause may be underestimated in all the photoperiodic regimes, because all living individuals at the 20th day were dissected. This may prevent misjudging overall tendencies.

The preovipositional period of the individuals of both species that laid eggs during the experimental period varied considerably and exhibited no significant difference with various photoperiods (Table 2). However, the preovipositional period of the individuals of both species reared under 12L-12D exhibited a tendency to be elongated. The preovipositional period may be underestimated for the same reason as mentioned above. This may also prevent misjudging overall tendencies.

Most females collected in the field before mid September laid eggs. However, the proportion of the egg-laying females decreased rapidly from late Sep-

tember to mid October (Table 3). Although the number of identified individuals was small, there was no significant difference in the incidence of diapause induction between the two species.

From the results described above, *O. sauteri* and *O. minutus* appeared to overwinter as adults in reproductive diapause, as do most *Orius* species in temperate regions (e.g. COLLYER, 1953).

The ratio of individuals of both species that exhibited reproductive diapause did not change drastically at a threshold, but changed gradually along with differences in the photoperiod (Table 1). This is similar to the case of *O. insidiosus* (SAY) that is distributed in North America (RUBERSON et al., 1991; MEIRACKER, 1994). This may be due to the genetic diversity in the induction of reproductive diapause by photoperiod. In the case where *Orius* species is used as a natural enemy in greenhouses during winter, the trait whereby reproductive diapause is not induced even in short photoperiod is advantageous. The diversity of both species in the reaction against photoperiod may indicate that a non-diapausing strain can be selected.

Some individuals of *O. sauteri* did not lay eggs even under long photoperiods (14L-10D or 15L-9D). Such cases are also known in *O. insidiosus* (RUBERSON et al., 1991; MEIRACKER, 1994). This might be because the copulation was unsuccessful, however, it may also be possible that the induction of reproductive diapause had nothing to do with photoperiod as in the case of *O. tristicolor* (WHITE) (MEIRACKER, 1994). This must be clarified by further examination under

Table 3. Seasonal changes in the proportion of egg-laying *Orius* females collected in the field and the result of identification

Collected on		No. of individuals				% Oviposition
		Oviposition			No oviposition	
		<i>O. sauteri</i>	<i>O. minutus</i>	Unidentified	Unidentified	
1994						
July	22	17	1	3	0	100
August	1	7	6	4	1	94
	2	6	6	1	2	87
	8	2	2	1	0	100
September	19	2	1	1	0	100
	8	7	6	1	1	93
	14	2	9	1	1	92
	26	14	2	2	0	100
October	4	1	0	1	1	67
	6	0	0	1	1	50
	7	1	0	2	4	43
	13	4	0	0	6	40
	19	0	0	0	4	0
	26	0	0	0	1	0
1995						
May	24	9	0	1	0	100
	30	8	0	2	0	100
June	1	3	1	2	0	100
	5	1	1	0	0	100
	20	3	0	0	0	100
August	17	10	2	4	0	100
September	4	1	1	3	0	100
	11	7	2	2	0	100
	25	9	4	1	1	93
October	6	1	1	0	1	67
	17	1	1	0	5	29
	22	0	0	0	1	0
	24	0	0	0	2	0

diapause-breaking conditions.

The preovipositional period varied considerably (Table 2). A preovipositional period exceeding 20 days could not be examined in this experiment. Several individuals did not lay eggs until more than 10 days after copulation under most of the test conditions. This might suggest that other individuals start oviposition after the 20th day. A relatively longer preovipositional period may be explained by the fact that weak reproductive diapause is provoked and broken within a relatively short period. Further tests are required to clarify this, as well.

No considerable differences were detected between

the incidence of reproductive diapause induction by photoperiod of the Kurume population of *O. sauteri* and that of *O. minutus*. In the case of *O. insidiosus*, the critical photoperiod for the induction of the reproductive diapause in the population from Arkansas (RUBERSON et al., 1991), which is located in a higher latitude, was shorter than that in the population from Georgia (MEIRACKER, 1994), which is located in a lower latitude. Both *O. sauteri* and *O. minutus* are widely distributed. Since the critical photoperiod for the induction of reproductive diapause may differ more remarkably between different local populations of the same species than between different species in

the same area, that of the other local populations should be tested.

The incidence of reproductive diapause in the field population was not incompatible with the laboratory experiments. The photoperiod in Kurume becomes 12.5L–11.5D around September 10. Considering that the sensitive period for the photoperiodic reaction is presumed to be the nymphal stage, that twilight hour may function as day-time for the reproductive diapause induction, and that non-diapausing females survive and lay eggs even in short photoperiods, the result that the proportion of egg-laying females decreased abruptly in early October is fairly compatible with the laboratory experiments.

Although both *O. sauteri* and *O. minutus* exhibited a dull response to photoperiod in the laboratory, the field population exhibited an abrupt response. Gradually decreasing photoperiod and temperature in the field might have affected the incidence of diapause induction.

Both the Kurume populations of *O. sauteri* and *O. minutus* exhibited reproductive diapause when reared under short photoperiod. Most females of the field population of these species exhibited reproductive diapause by mid October. Therefore, avoiding the induction of reproductive diapause appears to be very important for using these species as biological control agents in greenhouses under short photope-

riod in winter. Artificial lighting may have a good effect on this. Selection for a non-diapausing strain should be considered in the future, as well.

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Attraction of Males to Females in the Rice Leaf Bug, *Trigonotylus caelestialium* (KIRKALDY) (Heteroptera: Miridae)¹

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The rice leaf bug, *Trigonotylus caelestialium*, is one of the major pests causing a pecky rice, and also injures wheat and gramineous forage grasses in northern parts of Japan.

In Pentatomidae, Alydidae, Rhopalidae, Reduviidae and Coreidae, it is known that males secrete an aggregation pheromone or a sex pheromone (ALDRICH, 1988). Some of these pheromones have been chemically identified as attractants (ALDRICH et al., 1991; KOCHANSKY et al., 1989; LEAL et al., 1995; SUGIE et al., 1996). In Miridae, it was reported that females attract males of the same species, e.g., *Lygus lineolaris* (SCALES, 1968), *Ligocoris communis* (BOIVIN and STEWART, 1982), *Campylomma verbasici* (THISTLEWOOD et al., 1989), and five other species. However, the mating behavior in *T. caelestialium* has not been reported.

In this paper, we describe mating behavior and

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