Pre-winter copulation enhances overwintering success of *Orius* females (Heteroptera: Anthocoridae)

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Abstract
The majority of insects that overwinter in the adult stage copulate post-winter. Some such insects copulate not only post-winter, but also pre-winter and/or during winter. In *Orius* species, only females overwinter. As they have no chance to copulate post-winter, they do so pre-winter. We expected pre-winter copulation to be associated with increased survival of females during overwintering; therefore, we tested the effects of pre-winter copulation on winter survival in females of three *Orius* species, *O. sauteri*, *O. nagaii* and *O. strigicollis*. Under semi-field conditions, clear differences in overwintering survival were observed between copulated and virgin females in the late overwintering period. Overwintering success of copulated females was higher than that of virgin females; however, copulation frequency did not affect winter survival of females. With regard to the mechanism increasing winter survival, two possible explanations arose from this study. The first is the donation of nutrients from males through copulation, and the second is that copulation affects diapause syndrome and/or behavioral changes in females.

Key words: *Orius sauteri*; *Orius nagaii*; *Orius strigicollis*; winter survival; copulation frequency

INTRODUCTION

*Orius* species are minute multivoltine predatory bugs that are efficient natural enemies of agricultural pests such as aphids, thrips, and spider mites (Nagai, 1990, 1991; Yasunaga, 1993; Kakimoto et al., 2003; Toyoshima and Osakabe, 2005). On mainland Japan, *Orius sauteri* (Poppius), *Orius nagaii* Yasunaga, *Orius minutus* (Linnaeus), and *Orius strigicollis* (Poppius) commonly occur (Yasunaga, 1997). The majority of insects overwintering as adults copulate post-winter (Denlinger, 1985; Tauber et al., 1986). In contrast, because *Orius* males do not overwinter on the Japanese mainland (Yasunaga, 1993; Kohno, 1997; Ito and Nakata, 1998a, b; Shimizu and Kawasaki, 2001), *Orius* are likely to copulate only pre-winter. Although few reports of such female-biased overwintering exist, only the females successfully survive winter in social vespids (Danks, 1987) and blood-sucking mosquitoes (Danilevsky, 1961).

Some insects in which both males and females overwinter copulate both post-winter and pre-winter, or during winter. For example, a stink bug *Mendia scotti* (Puton) copulates post- and during winter (Koshiyama et al., 1997a, b), and damsel bugs belonging to Nabidae (Kott et al., 2000; Roth and Reinhardt, 2003), butterfly *Eurema hecabe* (Linnaeus) (Kato, 1986), ladybird beetle *Epilachna varivestis* Mulsant (Taylor, 1984), leaf beetle *Plagiogera versicolora* (Laicharting) (Stevens and McCauley, 1989), and fruit fly *Drosophila alboralis* Momma et Takada (Kimura, 1980) copulate post- and pre-winter. Previous studies have suggested that females that copulate pre-winter do not need to find mates prior to laying eggs in the spring, providing an advantage in oviposition (Kimura, 1980; Taylor, 1984; Kato, 1986; Stevens and McCauley, 1989; Kott et al., 2000; Roth and Reinhardt, 2003); however, no evidence exists to demonstrate the advantage of pre-winter copulation. In *M. scotti*, males transfer nutritive substances to females through copulation during winter (Koshiyama et al., 1993, 1996). Although such copulation with nutrient donation by males in winter results in a shorter pre-reproductive period...
for females the following spring, it is not effective in enhancing the winter survival of females (Koshiyama et al., 1997a).

Winter survival of females is lower in Orius species (Ito and Nakata, 1998a; Shimizu and Kawasaki, 2001) than in M. scotti (Koshiyama et al., 1997a) when individuals are provided with food. This reflects the difficulty of surviving winter in mainland Japan (Honshu) for Orius species. In fact, O. strigicollis does not inhabit the northern part of mainland Japan (Shimizu et al., 2001); therefore, if males are able to increase the overwinter survivorship of females via copulation, selection would favor pre-winter copulation in Orius species. In this study, we compared survival between copulated and virgin females of Orius species under starvation conditions and evaluated the effects of pre-winter copulation on the overwinter survival of females.

MATERIALS AND METHODS

**Bugs and rearing condition.** Orius sauteri was collected in 2002 and 2005, and O. nagaii was collected in 2003 from the field (mainly from Setaria viridis Beauv) in Kyoto, Japan (35°N, 135°E). O. nagaii and O. sauteri were identified from other Orius species on the basis of the characteristic whitish prostomium and the shape of genitalia following Yasunaga (1997), respectively. O. strigicollis, which was originally sampled in Kochi, Japan (33°N, 133°E), was provided by Sumitomo Chemical Co., Ltd. Bugs were reared on Ephestia kuehniella Zeller eggs (Koyo & Co., Tokyo, Japan) in plastic cases (1.7 L) in a laboratory at 25°C under a 16:8, light:dark cycle until overwintering experiments. To provide an oviposition substrate for females, kidney bean plants (Phaseolus vulgaris L.) were placed in each plastic case.

We conducted overwintering experiments for O. sauteri in 2002–2003 and 2005–2006, O. nagaii in 2003–2004, and O. strigicollis in 2004–2005. From September to October, bugs were reared in plastic cases in a screen house until they become third to fifth instar nymphs; for O. sauteri in 2002, third to fifth instar nymphs were collected in the field in October. Third to fifth instar nymphs were then individually transferred from plastic cases to 30-ml vials with a piece of wet cotton wool and E. kuehniella eggs, and reared in the screen house. Adults that emerged from late October to November were used for the following experiments.

**Preparation of adult females that had experienced different copulation frequencies.** To prepare females that had experienced different copulation frequencies, we divided females into virgin and copulated groups, taking the date of adult emergence into consideration to prevent a bias in the adult emergence period; the date may affect the overwintering success of Orius species (Shimizu and Kawasaki, 2001). For O. sauteri, we prepared virgin and once copulated females in autumn 2002, and virgin, once-copulated and multiply-copulated females (mean number of copulations=2.63±0.79 times [mean±SD]) in autumn 2005. Virgin and once-copulated O. nagaii females were prepared in autumn 2003, and virgin, once-copulated and multiply-copulated O. strigicollis females (2.29±0.46 copulations) in autumn 2004.

Pairing for copulation was conducted in a laboratory at 25°C. An adult female, which was within two weeks after adult emergence and had been individually reared, was moved into a 10-ml vial and copulated with a male which emerged under the same condition and more than 5 d previously. For multiple copulations, the female was allowed subsequent copulation with other males within three weeks after the first copulation. In these copulations treatments, if a pair had not begun to copulate within 10 min, the bugs were again allowed to copulate within one week. As a result, females were copulated 5 times at maximum. We fundamentally used virgin males; however, if virgin males were scarce, we also used males which had copulation experience but that had not copulated within a day before copulation treatment. After copulation, females and males were individually transferred into 30-ml vials and immediately brought back to the screen house. Both copulated and virgin adults were individually reared on E. kuehniella eggs until the end of November.

We monitored the survival of all females every day from 1 December to mid-March; bugs that died before December were omitted from data analysis. Starting on 1 December, the bugs were provided water but no food. Bugs that survived until mid-March were judged to have successfully overwintered because Orius females are likely to begin oviposition at that time (Shimizu and Kawasaki, 2001).
On 15 March, the effects of copulation on female survival were analyzed with Fisher’s exact probability test and an R×C test of independence using a G-test followed by unplanned tests of homogeneity.

**RESULTS**

**Copulation duration and male survival**

Although the difference was statistically marginal (Mann-Whitney U test, \( p=0.051 \)), the copulation duration of *O. sauteri* in autumn 2002 tended to be shorter than *O. nagaii* in autumn 2003 (Table 1). Copulation duration of *O. sauteri* in autumn 2005 was shorter than *O. strigicollis* in autumn 2004 (two-way ANOVA, \( p=0.046 \); Table 2). Two-way ANOVA also revealed that copulation duration of one copulation was shorter than the total copulation duration of multiple copulations (\( p=0.0001 \); Table 2). However, no significant difference was found between the total duration of multiple copulations and the duration of one copulation in *O. strigicollis* (Mann-Whitney U test, \( p>0.05 \)), while the total duration of multiple copulations was significantly longer than the duration of one copulation in *O. sauteri* (Mann-Whitney U test, \( p<0.001 \)).

During the overwintering experiment, although one virgin *O. nagaii* male survived until 2 January and one copulated male survived until 19 January, remaining *O. nagaii* males and *O. sauteri* and *O. strigicollis* males died during December. Copulation experience did not affect the survival of males, at least in *O. sauteri* and *O. nagaii*. We did not monitor the effect of copulation experience on *O. strigicollis* males.

**Effects of copulation on female survival**

The survival of virgin *O. sauteri* females in 2002–2003 began to decrease in the beginning of mid-December, whereas all once-copulated females survived at least until the end of January (Fig. 1A). Consequently, the overwinter survival of *O. sauteri* was higher in once-copulated females (52.6% on 15 March) than in virgin females (10.0%; Fisher’s exact probability test, \( p<0.005 \); Fig. 1A). In *O. nagaii* females, the difference in overwintering survival between copulated and virgin females became obvious in mid-February in 2004 (Fig. 1B). Consequently, the overwintering success of *O. nagaii* in 2002–2003 was higher in once-copulated females (52.0%) than in virgin females (25.9%; Fisher’s exact probability test, \( p<0.05 \)).

**Effects of copulation frequency on female survivorship**

Overwinter survival of once-copulated (81.8%) and multiple-copulated females (68.4%) was higher than that of virgin females (33.3%) in *O. sauteri* in 2005–2006 (G-test, \( p<0.01 \); Fig. 2A); however, the survival of multiple-copulated females was not significantly different from that of once-copulated females (G-test, \( p>0.05 \); Fig. 2A).

In *O. strigicollis*, survival declined more quickly than in other species, whose survival clearly decreased during March; however, the overwintering survival of once- (38.9%) and multiple-copulated
(19.0%) *O. strigicollis* females was higher than that of virgin females (2.6%) in 2004–2005 (*G*-test, \( p < 0.05 \); Fig. 2B). We found no significant difference in survival between once- and the multiple-copulated females (*G*-test, \( p > 0.05 \); Fig. 2B).

**DISCUSSION**

Pre-winter copulation affected the overwinter survival of adult *Orius* females. The bugs were not supplied with any food from 1 December through...
mid-March. In *M. scotti* provided with food, regardless of whether they had copulated, more than 80% of females successfully overwintered (Koshiyama et al., 1997a). In this study, if some food had been provided, the survival of *Orius* females to the late overwintering period might have been higher. If that was the case, the effects of copulation experience on overwinter survival of females might have been obscured. A considerable number of males successfully survive winter when they are provided with food (Shimizu and Kawasaki, 2001), although males are not found from late winter to early spring in the field (Yasunaga, 1993). We consider that it might be difficult for *Orius* to seek prey in winter and that starving is therefore a more realistic simulation of winter than eating sufficiently; however, cold might also affect the mortality of *O. strigicollis* females since their distribution is limited in northern Japan (Shimizu et al., 2001) and mortality in January and February is higher in this study.

In a stink bug and butterflies, males donate nutrients in their ejaculate to females during copulation, and those nutrients are incorporated into oocytes and/or the somatic tissue of females (Boggs and Gilbert, 1979; Koshiyama et al., 1996). Such nutrient donation reduces the survival of copulated males (Shapiro, 1982; Koshiyama et al., 1997b). Life-span benefits for copulated females of a bruchid beetle *Callosobruchus maculatus* (Fabricius) (Fox, 1993) and a cricket *Gryllus lineaticeps* Stål (Wagner et al., 2001) are likely reaped from male-derived nutrients, and the effect is speculated to be increased via accumulation through repeated copulation; however, in *Orius* species, no evidence indicated that males donate nutrients to females through copulation, and we did not find a cost of pre-winter copulation for males. Moreover, the overwintering success of multiple-copulated females was not higher than that of once-copulated females.

These results are not sufficiently conclusive to exclude the possibility that females receive nutrients from males through pre-winter copulation; therefore, two possible mechanisms may increase the overwinter survival of *Orius* females after pre-winter copulation. The first explanation is still the donation of nutrients from males through copulation. In this case, donated nutrients from the first copulation might be sufficient for females to increase their overwinter survival. The second explanation is that copulation affects diapause syndrome (e.g. accumulation of lipid content) and/or behavioral changes to ensure insemination before overwintering because females have no chance to copulate after winter. Further studies are needed to clarify the mechanism by which pre-winter copulation increases the overwinter survival of *Orius* females.

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