The reproductive biology of the omnivorous bug *Orius laevigatus*

Thesis

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Abstract

The study of mating behavior of natural enemies is of great importance for the management of biological control programs. Since every reproductive event can have a decisive effect upon fitness, a thorough understanding of the entire mating system of a biocontrol agent is necessary for the development of techniques for mass production, release, and establishment in the field. Several studies have examined the biology of the omnivorous bug *Orius laevigatus*, which serves as a biological control agent of a number of agricultural pests. Nevertheless, very little is known about the reproductive habits of these bugs. This study is aimed at clarifying the courtship and mating behavior, male and female mating systems and mate selection patterns of this important natural enemy.

Among all the courtship behaviors observed, mate quality assessment appears to begin with frontal encounters between males and females, which include wing waving and antennal contact. Mate assessment may continue after the male mounts the female, since the female remains in motion and can therefore estimate male weight as a quality parameter. Female resistance to unwanted copulations, manifested either by walking away or by removing the mounted male, indicates another component of female choice. During copulation, the male holds the female's thorax with both his forelegs, passes the tip of his abdomen to her right side, and presses his genitalia to the ventral side of the female's abdomen, thus adjusting for the left-leaning asymmetry of the male abdomen and genitalia.

The male mating system and insemination capacity were studied by allowing a virgin male to copulate sequentially with three virgin females during a single day or with 1 or 2 days between copulations. Males proved to be polygamous: they successfully inseminated all the females. The total number of eggs laid by the females who were third to mate was significantly lower than the number laid by previous females. This decline in female fitness could be ascribed to insufficiency of sperm, or of accessory gland fluids, which affect fecundity when transferred to the female at copulation, or both. These possible causes were not, however, affected by time elapsed between copulations. It therefore seems that the refractory period between copulation does not increase males' sperm or accessory fluids capacity. Moreover, time elapsed between copulations had no significant effect on egg hatching success.

Female receptivity was evaluated by testing the willingness of a mated female to copulate with a virgin male 1, 7, or 14 days after first mating. Results show females to be monandrous; mated females avoided any additional copulations, regardless of time elapsed between mating attempts. The high cost of multiple copulations or receptivity suppression caused by males could be responsible for the evolution of a monandrous mating system in *Orius* females.

A further experiment was conducted to determine whether the presence of virgin males during oviposition reduces female fitness. In this experiment, total oviposition and hatching rate were observed for females in the presence of 0, 3, or 5 virgin males during oviposition. Controls consisted of five virgin females in the presence of the ovipositional females, in order to ensure that interference, if present, is caused by interactions between the sexes and not by increased *Orius* density. Results support the hypothesis tested: association of the female with males reduces her fitness: as the number of males near the female increased, her total oviposition significantly decreased. In addition, females in the control treatment laid nearly twice as many eggs as did females associated with five males. The reduction in female fitness thus appears to be caused mainly by continuous mating attempts by males, and not by bug density. Since hatching success was not affected by the number of males associated with the female, it is reasonable to conclude that the presence of males influenced the oviposition process itself, and not the quality of fertilization or the vitality of laid eggs.

Mate selection patterns were studied by providing virgin females with a choice between two males of the same age but differing in their mating status (virgins vs. twice-mated males) or their diet (corn pollen vs. moth eggs). In spite of our findings showing a significant decline in insemination capacity after two copulations, the females did not demonstrate a preference for virgin males. This lack of preference could be the result of a female-biased sex ratio in nature and thus the low probability of encountering a virgin male.

The bugs' diet had a significant effect on their mate selection decisions: prey-fed females differed from pollen-fed females in their choice of mates. Pollen-fed females mated more frequently with prey-fed males than with pollen-fed ones. This tendency could compensate for the reduced fecundity previously found to result from pollen feeding, as opposed to prey feeding, among females. In contrast, prey-fed

females preferred to mate with pollen-fed males, although this tendency was not statistically significant. It is possible that females acquire most of their nutritional requirements for egg maturation by feeding on prey, and therefore show a low level of selectiveness for their mates' nutritional background.

In conclusion, the contrasting mating systems of *O. laevigatus* males and females could have important implications for their mass rearing protocol and establishment in the field. After copulating once, the females are unwilling to mate again and actively remove males that attempt to mate with them; these efforts decrease fecundity. In addition, the decline in insemination capacity after the male's second copulation, and the female's failure to distinguish between virgin and mated males, can cause reduced fecundity among the monandrous females. Therefore, in order to maximize reproduction by this natural enemy, the sex ratio should remain 1:1 in mass rearing and upon release in the field. Moreover, these results show potential benefits from removal of males after the first mating when the bugs are mass reared, in order to minimize detrimental effects on fecundity and reduce rearing costs.

The effect of diet on the reproductive potential of *Orius* males was tested for the first time in this study. The results show that mate selection is influenced by both male and female nutritional status, indicating the importance of considering diet when integrating *Orius* into biological control programs. Reducing the cost of mass rearing by feeding the bugs on a pollen-based diet, and improving our ability to predict the success of *Orius* populations in the field when prey is scarce, will be possible only after we understand the effect of nutritional ecology *Orius* fitness.