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Evaluation for Risk of Interspecific Mating among Native and Exotic *Orius* Species (Heteroptera: Anthocoridae)

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Abstract

Importation of exotic natural enemies is a common approach in biological control but they may threaten native natural enemies through interspecific interactions. *Orius laevigatus*, which is native to Europe, is a commercially available, effective biocontrol agent of pest thrips. The present study was undertaken to assess the effects of exotic *O. laevigatus* on two *Orius* species native to Japan, i.e., *O. sauteri* and *O. strigicollis*. Specifically, the possibility of interspecific cross and harassment was evaluated in the laboratory. Laboratory tests showed that mating between *O. laevigatus* and *O. sauteri* or *O. strigicollis* was unlikely to occur. Males mostly did not respond to interspecific females, and mounting on interspecific females was rarely observed. No insertion of male genitalia took place even when mounting had occurred. Thus, harassment by interspecific males is not likely to take place in the field. Reproductive isolation appears perfect at least against the two native species. There are however a number of points to be examined in order to assess the potential risk of the importation and use of *O. laevigatus*. Given the presence of promising native *Orius* species, importation of exotic species should be decided with great caution.

Keywords: Biological control; Reproductive interference; Predatory bugs; IPM

Introduction

The use of natural enemies is a main tactic in Integrated Pest Management (IPM) [1,2]. General approaches to biological control are importation, augmentation and conservation of natural enemies [2-4]. The importation and use of commercially available, non-native natural enemies are often on strong demand when native natural enemies are not available or when they do not provide satisfactory control. However, attention should carefully be paid when generalist natural enemies are to be imported. Imported generalists may attack non-target native organisms and have a serious negative impact on the native species, if they escape, are established and become abundant in the field [4-6]. Intraguild predation may also take place and reduce the effectiveness of biological control [5,7]. In addition, native natural enemies may be at risk because of competition and interspecific cross with imported natural enemies [6]. Thus, environmental risks must be assessed before the importation of exotic natural enemies [8].

Orius spp. (Heteroptera: Anthocoridae) are a group of insect predators that play an important role in suppressing pest thrips, aphids, and spider mites [9-11]. *Orius laevigatus* (Fieber) is a commercially available biocontrol agent and has been used in greenhouses to control thrips widely in Eurasia [7,12-15]. The recent success of biological control with *O. laevigatus* evokes the demand of importing this predator from Europe to Japan for biological control in greenhouses.

Although *O. laevigatus* would be used in inoculative and inundative releases in greenhouses, it may escape to the field. In fact, this predator bug is found established outside its natural range in the Netherlands [16]. Because several *Orius* species native to Japan are widely found in agricultural and nonagricultural fields [17], detrimental sexual interactions and reproductive interference may occur between exotic and native *Orius* predators. Although hybridization is rather unusual among insect species natively inhabiting a shared area, this may not be the case when an exotic species is introduced. If reproductive isolation is imperfect, interspecific mating and reproductive hazard (sexual harassment, mating disruption, hybrids, genetic introgression, etc.) should take place.

Here we focus on sexual interactions between the exotic and native *Orius* species. *Orius* sauteri (Poppius) and *O. strigicollis* (Poppius) are native to Japan, and are widely distributed in the country [17]. They are commonly abundant in agricultural fields, and are important natural enemies of thrips [18-21]. There is no clear evidence that the habitats and prey range of the two native species differ from those of exotic *O. laevigatus*. *Orius sauteri* and *O. strigicollis* may thus be affected by the occurrence of *O. laevigatus*.

In the present study, we examine whether interspecific mating and sexual interaction can take place between the three predators. For this purpose, direct behavioral observations are made in the laboratory. We discuss the benefits and risks of using *O. laevigatus* for biological control in Japan.

Materials and Methods

The thrips Thrips palmi Karny, as prey, was originated from wild populations collected at Hisayama, Fukuoka Prefecture, and was reared on kidney bean. Stock cultures of *Orius sauteri* and *O. strigicollis* were originated from wild populations collected in Kawanishi City, Hyogo Prefecture and Fukuoka City, Fukuoka Prefecture, respectively, whereas *O. laevigatus* was obtained from Syngenta Bioline Ltd. The culture of both predators and prey are kept at 25 ± 0.5 °C with a 16: 8 L: D photoperiod.

Orius predators were reared in plastic cages (10 × 15 × 5.3 cm) and were fed eggs of Ephestia kuehniella Zeller. Young shoots of kidney

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bean were placed in the cages as a substrate for oviposition. Prey eggs and oviposition substrates were replaced 3 times a week. Rearing cages were kept at 25 ± 0.5 °C with a 16: 8 L: D photoperiod. Nymphs of the last instar, i.e., 5th instar, were collected from rearing cages and were individually placed in glass vials. A piece of paper (1.5 × 1.5 cm) and that of kidney bean leaf (1.5 × 1.5 cm) were placed in each glass vial. E. kuehniella eggs were given as food. Glass vials were checked every day, and when adult moulting was observed, the day was recorded. Adult predators were reared in glass vials until used for testing.

Behavioral Observations

Mating behavior of *Orius* predators was directly observed in the laboratory. Adult *Orius* of 2 or 3 days old, which had have no sexual experience, were used. One male or female *O. laevigatus* and one *O. sauteri* or *O. strigicollis* of the opposite sex were carefully transferred into a small arena (glass Petri dishes, 2.8 cm in diameter), in which a piece of kidney bean leaf had been placed. An adult female was carefully placed first onto the leaf piece. Two min later, a male was released into the arena, and the behavioral observation was initiated. The number of contact between individuals, the time taken for a male to start mating with a female, and the number of mountings were recorded. Care was taken to observe whether or not the male genitalia were successfully inserted to female abdomen. Successful insertion of male genitalia was regarded as "successful mating". The observations were terminated when successful mating occurred or after 10 min of male introduction. When no successful mating was taken place within 10 min of observations, it was regarded that mating was unsuccessful. As control, mating behavior was observed for intraspecific pairs. The data were analyzed with the aid of JMP version 8.0 [22].

Results and Discussion

Successful mating was observed for the majority of intraspecific pairs in the control observation. The percentages of males successfully mounted on a female were 100% for three *Orius* species tested, indicating that males readily recognized the presence of intraspecific females and located them to mate. The percentages of successful mating, i.e., successful genitalia insertion, were 77.8% (n=27), 88.9% (n=18) and 75.0% (n=20) for *Orius laevigatus*, *O. sauteri*, and *O. strigicollis*, respectively. Failure of genitalia insertion occurred when females had actively walked around or when they had resisted to mating trials by males. The results demonstrated that a 10 min observation was enough to examine mating behavior of the three *Orius* species.

Striking differences were observed for interspecific pairs. Mounting and genitalia insertion were rarely found (Table 1). All exotic *O. laevigatus* males did not responded to interspecific females. Curiously, males of two native species occasionally responded to exotic females by chasing and mounting on them. Hence, the percentages of males mounted on an interspecific female differed among the four test pair groups (chi-squared test; df=3, χ^2=12.85, P=0.005). The mean numbers of mounting observed during each trial were also significantly different (Wilcoxon's test; df=3, χ^2=10.29, P=0.016). Because of the small spatial scale of experimental arenas, males frequently encountered a female during the observations. Males of the exotic species approached female's probably in response to their physical features (i.e., size, color, movement) but mostly neglected the females. As the result, mounting behavior was rarely observed, suggesting the presence of discrimination upon encounter. Males of two native *Orius* did not show perfect discrimination upon encounter, however. Nevertheless, no genitalia insertion took place even when the males had mounted on an interspecific female (Table 1). Additional discrimination therefore appeared to occur at this stage.

	Test pairs (M=male; F=female)			
	O. laevigatus M × *O. sauteri* F	*O. laevigatus* M × *O. strigicollis* F	*O. sauteri* M × *O. laevigatus* F	*O. strigicollis* M × *O. laevigatus* F
N	19	18	18	19
Mean no of mounting	0	0	0.33 ± 0.14	0.16 ± 0.09
% successful mating	0	0	0	0

The mean numbers (with ± SE) of mounting trials made by males differ significantly as a whole (Wilcoxon's test; P <0.05). For mating success observed for intraspecific pairs, see the results.

Table 1: Mating trail (mounting) and success in interspecific pairs of three Orius species.

The results strongly suggest that interspecific mating do not occur between *O. laevigatus* and *O. sauteri* or *O. strigicollis*. Reproductive isolation thus appears perfect between the species at the level of pre-mating process. The recent molecular phylogenic study suggests that *O. laevigatus* can be placed relatively far from the native *Orius* species [23]. This may explain why reproductive isolation is rather perfect. Behavioral observations evidently suggest that males of the three *Orius* species discriminate their mating partners upon encounter and do not try to mount on them. This discrimination contributes to strong sexual isolation. Cues that are present on female body surface may play a dominant role in mate discrimination by *Orius* males. It is known that female O. insidiosus produce both volatile and non-volatile trail sex pheromones [24,25]. The absence of such pheromones may not evoke the motivation of male *Orius* to actively chase non-conspecific females.

We did not test whether males could be attracted to the trail of interspecific females. Given the spatial scale of confined environments in our laboratory test, it may be difficult to make definitive conclusions on sexual interactions between native and exotic *Orius* predators but even so, sexual interaction occurs only rarely even when they are in close proximity or even when they encounter each other. Female trails might affect the behavior of interspecific males but the effects would be minimized because the majority of males neglect interspecific females in the vicinity. We thus conclude that sexual interactions are not the matter between exotic *O. laevigatus* and native *O. sauteri* or *O. strigicollis*.

Many aspects with respect to interactions remain unsolved, however. Intra-guild predation is known for *Orius laevigatus* [7], and this may negatively affect native species. Also, the side effects on non-target insects are very unclear; this must be difficult to evaluate for tiny generalist predators such as *O. laevigatus*. In Japan, native species of *Orius* predators have the promising potential to suppress populations of target thrips such as Thrips palmi and Frankliniella occidentalis both in fields and greenhouses [21,22,26]. Given this, introduction of exotic *Orius* predators should be made with great caution or should ideally not be made unless non-native species show apparent benefits that cannot be given by native species.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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