Putative sex pheromone and mating behaviour in the whitefly parasitoid *Eretmocerus eremicus* Rose & Zolnerowich

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We studied the response of males to volatile and non-volatile components of sex pheromone and the mating behaviour in the whitefly parasitoid *Eretmocerus eremicus* (Hymenoptera: Aphelinidae). Males of *E. eremicus* reacted to volatile pheromones emitted by conspecific females in a wind-tube set-up: all males tested walked upwind in a tangential longitudinal spiral path along the tube, and reached the females in 30(S) compared to 70(S) in the control. When males were released into a patch that was previously exposed to virgin females, they showed an arrestment response: males spent more time in and around the contaminated patch. After encountering a female, male courtship behaviour consists of various sequential elements including vibration of the antennae, wing fanning and following of the female in an accelerated manner. Courtship and mating is clearly affected by the age of the female and the presence of whitefly hosts. The consequences of mate-allocation on population dynamics and biological control releases are discussed.

*Keywords*: *Eretmocerus eremicus*, courtship behaviour, sex pheromones

Hymenopterous parasitoids reproduce either parthenogenetically or bisexualy. In a natural situation a bisexual parasitoid must be able to find a mate and hosts for producing a next generation. Mating at emergence sites is likely to occur in gregarious parasitoids because the sexes emerge in proximity to each other (Pompanon *et al.*, 1997). In such circumstances, mate finding is done through tactile and visual stimuli (van den Assem & Jachmann, 1982). In solitary parasitoids searching for mates poses a challenge, especially when densities are low and mates may not be available at the emergence site (van den Assem, 1996). To find a mate, a female parasitoid may produce volatile and/or non-volatile sex pheromones. Volatile pheromones enable mate finding by attracting males over long distances to the females, and non-volatile pheromones mediate close-range courtship behaviour (Quicke, 1997). If a male parasitoid perceives volatile pheromones he may increase its antennal movement, vibrate wings, and follow the female in an accelerated manner. While if a male perceives non-volatile pheromones on patches previously visited by a virgin female, it more frequently visits or stays longer on substrates.

Many sexual pheromones used by parasitoids are volatile (Quicke, 1997). However among gregarious species with mating at the site of emergence, emission of long-range sexual pheromones is not common (Godfray, 1994). In arrhenotokous parasitoids, unmated females produce only male progeny. Therefore, if mating does not occur rapidly, females search for hosts and only lay male eggs. In some parasitoids, females can mate only once (e.g. Ruther *et al.*, 2000), in other species, females allow multiple mating (e.g. Mohan *et al.*, 1992), but the sex-ratio decreases in favour of males over time. When mating ability does not decrease over time, this would allow further production of female offspring (Pompanon *et al.*, 1997).

*Eretmocerus eremicus* Rose & Zolnerowich is a parasitoid of whiteflies that is released to control *Bemisia tabaci* (Gennadius), and *Trialeurodes vaporariorum* (Westwood) in greenhouses. Male courtship and mating behaviour in *E. eremicus* has been described in detail by Hunter *et al.*, (1996, as *Er. near californicus*). However, it is still not clear whether females produce any volatile or non-volatile pheromones to attract males. In addition there is no report of any overt behaviour associated with the release of sex pheromones in whitefly parasitoids. Moreover, the ability and capacity of a female to mate more than once is unknown. Here we present first evidence that sexual pheromones in the whitefly parasitoid *E. eremicus* exist and that it is able to mate more than once over a period of two days.
MATERIAL AND METHODS

We used commercially available parasitoids (ErCal®, Koppert Biological Systems, The Netherlands). Unmated females and males were collected immediately after hatching and kept isolated until used for experiments. As whitefly hosts larval instars of *B. tabaci* were used. All experiments were done in a climate room at 25°C, 65% RH, and artificial light (3000 lux). For statistical evaluations, ANOVA analyses were done.

**Experiment 1: Occurrence of sex pheromones**

1.1. Reaction to volatile substances

To test whether males react to volatile substances, five virgin females, (2<age<10 h), were confined in the upper part of a glass tube (11*0.6 cm Ø, 1 litre air flow per minute). After 10 minutes a virgin male was introduced at the opposite part (n=11). The experiment was done without females as control (n=9). Each experiment was finished when a male reached the end of the tube (the females’ location), or up to 240 s maximum. To see how long females remained attractive to males, we offered them hosts and kept them until the next day. One-day-old mated and virgin females were compared (n=10).

1.2. Reaction to non-volatile substances

To study the presence of non-volatile substances, five females (1<age<10 h) were confined in a glass vial (1*11 cm). One-day-old mated and virgin females were compared (n=10). The vial was covered by a poinsettia leaf and females were allowed to walk on the exposed leaf patch. After 30 minutes, the leaf was removed and a male was released on it. The male tracks on the leaf were recorded using the Etho-vision® program (Noldus Information Technology), and compared with clean ones (n=10). To verify whether searching was affected by the substrate, the experiment was repeated by using a glass Petri dish (4.5 cm Ø) instead of a leaf.

1.3. Substrate effects

To find out whether female pupa would produce pheromone and affect male mate-allocation, five female pupae were put on one part and five male pupae in another part of a Petri dish. A male was introduced in the Petri dish and its behaviour observed (n=10).

1.4. Reaction to parasitized pupae

Female pupae that had a small emergence hole were removed from the card and put on a poinsettia leaf 45 to 60 minutes prior to emergence. Three males were released within the same arena at a distance of 3-10 cm, and their reaction until locating the female and mating was recorded (n=10).

**Experiment 2: Mating**

2.1. Mating behaviour, multiple mating

The courtship and mating behaviour of *E. eremicus* was observed with pairs of parasitoids (1<age<3 hrs) enclosed in a gelatin capsule (0.5*1.5 cm). The duration of mating was recorded via a stereomicroscope and The Observer (Noldus Information Technology) program. To verify the ability for multiple mating, a male was replaced by another one after each mating (n=15).

2.2. Mating persistence

To test for mating persistence, females were kept in Petri dishes (10 in each) and provided with either honey or a host plant with or without host. When females were exposed to hosts, we offered a piece of a poinsettia leaf infested with at least 20 larvae (L1-L3) of *B. tabaci* on agar in a Petri dish (upside down). Females were kept in a climate room for 1 or 2 days.

**RESULTS**

**Experiment 1: Occurrence of sex pheromones**

1.1. Reaction to volatile substances

There was a significant difference between males walking in a wind-tube with and without virgin females. When virgin females were exposed, males went upwind, spiraling in a tangential
longitudinal path along the tube, and reached the site where virgin females were enclosed in less than 30 s. Males reacted similarly to newly emerged or to one-day-old virgin females. When exposed to one-day-old mated females, males showed a similar behaviour as if no females were present: males walked a random, tangential and non-directed path. If it reached the upper part at all, it took more then 70 s. (Fig. 1).

1.2. Reaction to non-volatile substances
When males were exposed to non-volatile substances on Poinsettia leaves, they spent more time on and just around the patch, and turned back by changing their direction outside the patch (for more than 4 minutes). They extended their searching area around the patch in a circular shape (Fig. 2). When a male was introduced to a clean part of a leaf or of a Petri dish on the other hand, he either moved out quickly or stood still for a moment and then ‘jumped’ out (Fig. 3).

1.3. Substrate effects
On poinsettia leaves a newly emerged female stood still for about 5 minutes after hatching before it started to walk. Males (3 per female) did initially not respond to her presence, but after 12 to 20 minutes they raised their antennae, fanned their wings and run towards the female. They found the female in about 20 s (17.3 ± 3.7 s). The female mated successfully with one or two males, never all three; results similar to other laboratory experiments we did.

Figure 1. Mean time (s) response (± s.d) of *Eretmocerus eremicus* males to volatiles emitted by females in a wind tube during a maximum period of 240 s. Treatments (n=10) indicate mating condition and age (days) of the females.

Figure 2. Walking patterns of a male exposed to a contaminated Poinsettia leaf patch (left), a contaminated glass Petri dish (middle) or a clean Poinsettia leaf (right) (● = point of release).
1.4. Reaction to parasitised pupae

When males were exposed to conspecific pupae, males either walked or stood still and preened in the Petri dish, but they did not show any response towards the pupae, male or female.

**Experiment 2: Mating**

2.1. Mating behaviour

When a male meets a female, the female walks away at the first encounter, but slows down her walking speed, while the male increases his speed. After a second or third encounter, the female is standing still and she allows mating. The male usually contacts the female from the side. If the female remains standing still, the male mounts the female, moves forward, puts the fore leg on the head near the eyes, grasps her by the middle legs and puts his hind legs on the female wings. At that time the males starts antennation for 20-30 seconds and then rubs his mid tibia on the above edge of the female thorax three times. Then the female puts her antenna down and the male bends backwards for mating, by putting fore and middle feet on the female’s wings, and hind feet around her abdomen. When the female does not accept mating, she either moves forward or does not give up her abdomen for mating and she tries to push away the male using her hind right leg. In that condition the male moves back again in mounted position and starts antennation, whereas the female begins cleaning her genitalia by her hind right leg. The average time for such a sequence was less than two minutes: 20-30 seconds courtship behaviour, 3-6 seconds mating time and 40-50 seconds post-mating behaviour. In three cases a male mated after a 4-minute courtship period, but the actual mating and post-mating time were the same as in other males. When a female did not accept a male, courtship time lasted 5 up to 15 minutes. While the female was standing still, she was only moving the antennae, while the male was antennating her head and predominantly her antennae. Sometimes the male moved around on the female’s back and antennated other parts of the female body too. Occasionally a male moved away, came back and remounted the female. Males did not manage to mate after 5 to 15 minutes antennation and then left the female.

2.2. Multiple mating

When exposed to a male, a female was never observed to mate twice with one male in a row. When a male encountered a female that he already mated with, he would leave her. In contrast, a female could mate with two different males within 10 minutes, but when a third male was introduced she did not accept him anymore (n=15).
Table 1. Mating success in *Eretmocerus eremicus* males at different conditions.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Conditions</th>
<th>Mating (n)</th>
<th>No mating (n)</th>
<th>Total (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no honey – no hosts</td>
<td>13</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>1</td>
<td>with honey – no host</td>
<td>0</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>with hosts</td>
<td>7</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>day 1 hosts, day 2 honey</td>
<td>7</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>day 1 honey, day 2 hosts</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
</tbody>
</table>

2.3. Mating persistence

Females and males started mating shortly after emergence. However, the presence of the hosts had a very significant effect on the mating success in older females (Table 1). Virgin females that were exposed to whitefly hosts, would still mate when contacting a male after two days. However, when no hosts were available, they would not mate after the first day.

**DISCUSSION**

Mating can be separated into three parts: pre-mating, mating and post-mating. During the pre-mating period sex pheromones help female parasitoids to attract a mate (Pompanon *et al.*, 1997): volatile pheromones to attract males over long distances, and non-volatile pheromones to mediate arrestment of males when nearby the female. In the whitefly parasitoid *Eretmocerus eremicus*, males respond to both volatile substances emitted by conspecific virgin females and to patches contaminated by virgin females thus revealing the presence of putative sex pheromones.

During the mating-period, other close range substances may play a role. After an encounter, the male mounts the female, and mates. Multiple matings occur, but take place only during a very short interval and females were unattractive to males the next day once they had mated. The number of times that a female mates, could be a direct control of the sex ratio of the offspring produced during her lifespan. Whether initial multiple mating with different males occur to avoid sperm-depletion by *E. eremicus* females, and thus causing a bias in sex-ratio towards males at the end of the reproductive life, or contribute to spreading of her genes, remains to be studied.

Once *E. eremicus* has mated, males are not attracted anymore. Although the role of post-mating behaviour is explained as mate guarding in gregarious species (*e.g.* Ruther *et al.*, 2000), post-mating behaviour in solitary species like *E. eremicus* might have a different explanation. Biological and ecological characteristics make it likely that effective mating at an early stage of life secure a life-long bisexual offspring production. *E. eremicus* is pro-ovigenic, so females eclose with a large egg complement (Asplen *et al.*, 2002). When deprived of host meals, eggs are soon resorbed for maintenance. Multiple mating at the beginning of their reproductive life might therefore be beneficial to a female. Dispersal studies (Bellamy & Byrne, 2001) indicate an effect of gender and mating status in the dispersal behaviour of *E. eremicus*: females fly longer and disperse further than males, and unmated parasitoids fly longer than mated parasitoids. Because of her relatively short adult lifespan (8-10 days), which suggests that it is largely limited reproductively by host encounter rate rather than egg load (Asplen *et al.*, 2002), the likelihood of encountering a male will sharply decrease during adult life. The presence of volatile sex pheromones mediates males to unmated females when present at low densities.

The moment of release of sex pheromones differs among parasitoid species. In some species, like *Anisopteromalus calandrae* (How.), it is released at the pupal stage, in others it takes a few hours after hatching, like in *A. listronoti* (Cormier *et al.*, 1998). The males of *E. eremicus* did not show any reaction towards unhatched pupa, or to those where females started to bite their way out. Males perceived the sex pheromone of newly emerged females just a few minutes after the female had emerged. The response of males to one-day-old virgin females indicates that the latter are still releasing volatile sex pheromones. The ability of releasing pheromones is influenced by mating, as mated females did not attract males.

From a biological control viewpoint, mate-searching efficiency could affect the probability of parasitoids to establish a population (Hopper & Roush, 1993). In inundative control programmes, where releases are made in greenhouses at weekly or at regular intervals, this is less relevant than...
for those agents that are seasonally released in the field. When released from cards or containers, mating likely may have occurred there or nearby the release site. The presence of putative sex pheromones in *E. eremicus* could have important consequences for sex allocation strategies in field conditions.

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**REFERENCES**


