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Superparasitism and host discrimination behavior of *Eretmocerus warrae* Naumann & Schmidt (Hymenoptera: Aphelinidae)

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Abstract: *Eretmocerus warrae* Naumann & Schmidt is a thelytokous parasitoid that attacks the greenhouse whitefly, *Trialeurodes vaporariorum*. Its host discrimination and superparasitism behavior was investigated at different host densities and time intervals between the first and second ovipositions under laboratory conditions (22 ± 1 °C, 60 ± 5% RH, a 16:8 light:dark photoperiod). At a density of between 10 and 15 hosts, significantly fewer ovipositions in parasitized nymphs were performed by the same experienced parasitoids (SEPs) or by different experienced parasitoids (DEPs) than by naïve parasitoids (NPs) 1 and 24 h after parasitization by the first female. However, at a host density of 15 nymphs, SEPs, NPs, and DEPs allocated significantly fewer eggs in the parasitized hosts than at that a density of 10 nymphs. Our results also showed that with the increase of host density from 20 to 140, the superparasitism rate of *E. warrae* significantly decreased. The findings from this study in relation to the mass rearing of *E. warrae* or field release are discussed.

Key words: *Eretmocerus warrae*, superparasitism, host discrimination, host density, time interval

1. Introduction

Host discrimination is the ability of a parasitoid to distinguish an unparasitized host from a parasitized one and to reject the latter for egg-laying (van Lenteren et al., 1978). Such an ability is considered to be useful for the survival of progeny (Doutt, 1959) because it reduces the risks associated with host defense, e.g., encapsulation (van Alphen and Visser, 1990). Although this ability is widespread among hymenopteran parasitoids, superparasitism is common in nature (van Lenteren et al., 1978; Bakker et al., 1985; van Alphen and Visser, 1990; Fatouros et al., 2005).

Superparasitism, previously considered to be nonadaptive by parasitoids, is now thought to be an adaptive behavior (Speirs et al., 1991). In solitary parasitoids, superparasitism can delay progeny development, increase larval mortality, and result in poor offspring fitness (Vet et al., 1994; Potting et al., 1997; Jones et al., 1999). However, the evolutionary stable strategy predicts that, under certain conditions, solitary parasitoids switch from rejecting parasitized hosts to superparasitizing them (Visser et al., 1992). This can be adaptive for conspecifics under a wider range of conditions due to the probability of eliminating the nonsibling competitors from the parasitized host (Visser et al., 1992). Nonetheless, in the case of superparasitism by the same female, siblings compete for the resources (Weisser and Houston, 1993). Therefore, the same female should always avoid superparasitization. Nevertheless, in host-depleted patches and in the presence of other conspecific females, it can be her adaptive strategy to superparasitize (Visser et al., 1992; Weisser and Houston, 1993). In that case, it is highly likely that the host parasitized by her can be attacked by another foraging parasitoid. Therefore, allocating more than one egg to the same host can enhance the possibility that the survivor would be of her own offspring (van Alphen and Visser, 1990).

In mass rearing programs, the parasitoids are often reared under crowded conditions (Waage and Godfray, 1985), resulting in frequent superparasitism. Prior to the present study, there was no published information on the superparasitism and host discrimination behavior of *Eretmocerus warrae* Naumann & Schmidt, making it difficult to develop strategies for effective mass rearing and field manipulation of this parasitoid. Therefore, the objectives of this study were to investigate superparasitism and host discrimination in *E. warrae*, which may lead to improving the use of this parasitoid for the management of whiteflies.

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2. Materials and methods

2.1. Plants

Tomato (*Lycopersicon esculentum* Mill. 'Money Maker') was used as a host plant for *Trialeurodes vaporariorum* (Westwood). The seeds were sown in 60-cell plastic trays with each cell (4.5 × 4.5 cm) filled with commercial potting mix (15% N, 8.4% P, and 10.8% K). After 3–4 weeks, seedlings were transplanted into plastic pots (10 cm in diameter × 8 cm in height) filled with potting mix. Plants or branches/leaves of 1.5 to 2 months old were used, depending on the experiment. To avoid wilting, a branch was placed in a water-filled transparent plastic container (3.5 cm in diameter × 5.5 cm in height) via a hole (0.5 cm) in the center of the lid.

2.2. Insects

The colony of *T. vaporariorum* was started with 500–600 pupae obtained from Bioforce Ltd. (New Zealand). A tomato plant was placed in an aluminum-framed cage (60 × 45 × 40 cm) with a fine metal screen (aperture diameter = 0.2 mm) on the back and both sides and Perspex on the top and front for egg-laying. After 24 h, the plant was removed and kept in another rearing cage (90 × 60 × 60 cm) with a fine metal mesh on two sides and the back. The top, bottom, and front were made of steel with a 30 × 30 cm window of fine metal mesh. When whiteflies pupated, the infested branches were cut off from the plant and placed in the aforementioned transparent plastic containers with water to avoid wilting. Those containers were kept in the aforementioned aluminum framed cages for maintenance of the colony.

The colony of *E. warrae* was initiated with 400–500 parasitized pupae obtained from Bioforce Ltd. These pupae were kept in plastic petri dishes (5.5 cm in diameter × 1.3 cm in height) and transparent glass vials (1.5 cm diameter × 5 cm height) with a 0.5-cm mesh-covered hole in lids. As *E. warrae* is thelytokous in nature (Hanan et al., 2009), no males were found in the colony. The emerging adult parasitoids were directly released onto the plants/branches infested with the second- and third-instar nymphs of *T. vaporariorum* kept in cages (30 × 30 × 30 cm) with Perplex and a fine metal screen (aperture diameter = 0.2 mm) on top for ventilation for parasitization. When parasitized nymphs reached the pupal stage, they were harvested and placed individually into transparent glass vials or plastic petri dishes for emergence and used for experiments and colony maintenance.

2.3. Environmental conditions

All experiments were carried out at 22 ± 1 °C and 60 ± 5% RH, with a 16:8 light:dark photoperiod (lights on from 0900 to 0100 hours and off from 0100 to 0900 hours). Lighting was provided by high frequency broad-spectrum Biolux tubes (Osram, Germany).

2.4. Host discrimination and superparasitism behavior recording

To determine whether *E. warrae* individuals recognized hosts parasitized by herself or by a different female, an experiment was set up with 6 treatments (Table 1) with host densities of 10 or 15 hosts/parasitoid. Only the second-instar nymphs were used in this experiment, with 10 parasitoids for each treatment. For each treatment, a leaf/leaflet infested with the test number of host nymphs was placed into a petri dish and a map was drawn to describe the distribution of the hosts. Subsequently, a naïve parasitoid (<12 h) was released into the petri dish and was observed until she probed five nymphs (first oviposition). Parasitoid behavior was recorded with a system consisting of a camera (JVC, Japan) attached to a stereomicroscope (Leica MZ12, Germany), which was connected to a Samsung video cassette recorder (DVD-V530, Korea). The images were viewed on a Panasonic color monitor (TC-21T1Z, Japan). When a parasite probed a nymph, the location of the nymph was marked on the map. After the female had probed five nymphs, she was removed and all the probed nymphs were gently turned over to confirm the

Table 1. Six treatments used in the experiment.

First oviposition	Second oviposition	Time interval between 1st and 2nd ovipositions
NP	NP	After 1 h
NP	SEP	After 1 h
NP	DEP	After 1 h
NP	NP	After 24 h
NP	SEP	After 24 h
NP	DEP	After 24 h

SEP = Same experienced parasitoid; NP = naïve parasitoid; DEP = different experienced parasitoid.

presence of an egg. Nymphs with eggs underneath were then marked on the same map. After 1 or 24 h, the same experienced parasitoid (SEP, 1 day old), a naïve parasitoid (NP, <12 h), or a different experienced parasitoid (DEP, 1 day old) was released into a petri dish to record its ability to discriminate parasitized hosts in the first oviposition. The parasitoid was observed until she probed five nymphs. At the end of the experiment, all the probed nymphs were turned over to assess the host discrimination and superparasitism of *E. warrae*.

The following behaviors of the parasitoids in the first and second ovipositions were recorded:

1) Encounter: the parasitoid meets a host physically.
2) Rejection of host: the parasitoid walks away from a host after encounter without probing.
3) Superparasitism: the parasitoid lays eggs under the parasitized hosts.
4) Total time: time taken by parasitoids to probe five nymphs.

2.5. Superparasitism at different host densities

To determine how host density affected superparasitism, we set up seven host densities: 20, 40, 60, 80, 100, 120, and 140 second-instar nymphs of *T. vaporariorum*. Ten parasitoids (ten replicates) were used for each treatment. For each replicate, one parasitoid (<12 h) was released into a petri dish with a fresh leaf infested by a test number of nymphs, allowed to stay for 24 h, and then moved into another petri dish containing the same number of nymphs. This process was repeated until she died. As *E. warrae* place their eggs between the nymph's venter and the leaf surface (Hanan et al., 2009, 2010, 2012), all nymphs were turned over to determine the presence or absence of eggs under the stereomicroscope. Superparasitism was determined when two or more eggs were found under the same nymph.

2.6. Statistical analyses

A goodness-of-fit test was used to test the distribution of data prior to analysis. Data for superparasitism rates after 1 h at a density of 10 hosts, number of encounters, rejection rate, and total time spent by each parasitoid to probe five nymphs were normally distributed and were thus analyzed using ANOVA followed by Tukey's studentized range test. All other data were not normally distributed, even after transformation, and were thus analyzed using the nonparametric Kruskal–Wallis test followed by Dunn's procedure for multiple comparisons (Zar, 1999).

3. Results

3.1. Host discrimination and superparasitism behavior recording

At a density of 10 hosts, SEPs superparasitized a significantly lower proportion of parasitized nymphs than NPs and DEPs (after 1 h: $F = 8.64$; $df = 2, 27$; $P < 0.001$; after 24 h: $\chi^2 = 11.19$; $df = 2$; $P < 0.01$) (Table 2). Similarly, at a density of 15 hosts, a significantly lower proportion of parasitized nymphs were superparasitized by the SEPs and DEPs than by NPs at 1 and 24 h after parasitization ($\chi^2 = 8.89$ and 9.44 for 1 h and 24 h, respectively; $df = 2$; $P < 0.01$) (Table 2).

Host density had a significant effect on the host discrimination and superparasitism of *E. warrae*, with a significantly lower proportion of parasitized nymphs being superparasitized by the SEPs, NPs, and DEPs at a host density of 15 nymphs than at 10 nymphs (after 1 h: $\chi^2 = 5.18$, 12.01, and 12.14 for the SEPs, NPs, and DEPs, respectively, $df = 1$, $P < 0.05$; after 24 h: $\chi^2 = 7.32$, 10.56, and 11.56 for the SEPs, NPs, and DEPs, respectively, $df = 1$, $P < 0.001$) (Table 2).

Table 2. Superparasitism (%) of *E. warrae* at different host densities.

Time interval	Parasitoid type	Host density	
		10 hosts	15 hosts
1 h	SEP	22.00 ± 3.09b	10.50 ± 3.53b
	NP	44.50 ± 3.37a	24.00 ± 1.94a
	DEP	35.50 ± 3.76a	14.50 ± 2.91b
24 h	SEP	24.50 ± 1.89b	12.50 ± 3.44b
	NP	45.50 ± 3.69a	25.50 ± 2.52a
	DEP	39.00 ± 4.98a	16.00 ± 3.82b

For time intervals of 1 or 24 h, means ± SE followed by the same letters in columns are not significantly different ($P > 0.05$). Data from the time intervals of 1 and 24 h were analyzed separately. SEP = Same experienced parasitoid; NP = naïve parasitoid; DEP = different experienced parasitoid.

At a density of 10 hosts, SEPs, NPs, and DEPs rejected significantly more hosts in the second oviposition than in the first oviposition (after 1 h: F = 20.86, 18.35, and 12.56 for the SEPs, NPs, and DEPs, respectively, df = 1, 18, P < 0.002; after 24 h: F = 13.89, 8.62, and 6.76 for the SEPs, NPs, and DEPs, respectively; df = 1, 18, P < 0.01) (Figure 1a). Consequently, the parasitoids encountered significantly more hosts in the second oviposition than in the first oviposition (after 1 h: F = 23.92, 17.31, and 13.20 for the SEPs, NPs, and DEPs, respectively; df = 1, 18, P < 0.001; after 24 h: F = 15.97, 19.89, and 13.20 for the SEPs, NPs, and DEPs, respectively, df = 1, 18, P < 0.01) (Figure 1b). Eventually, parasitoids spent significantly more time in probing five nymphs during the second oviposition than in the first oviposition (after 1 h: F = 7.52, 7.31, and 8.87 for the SEPs, NPs, and DEPs, respectively, df = 1, 18, P < 0.01; after 24 h: F = 6.83, 8.70, and 6.58 for SEPs, NPs, and DEPs, respectively; df = 1, 18, P < 0.01) (Figure 1c).

3.2. Superparasitism at different host densities

As host density increased from 20 to 140, the superparasitism rate of *E. warrae* decreased significantly (F = 82.34; df = 6, 63; P < 0.0001) (Figure 2).

4. Discussion

In solitary parasitoids, host discrimination is never absolute (van Lenteren et al., 1978; Bakker et al., 1985). In the present study, the *E. warrae* superparasitism rate was significantly higher at lower host densities than at the higher

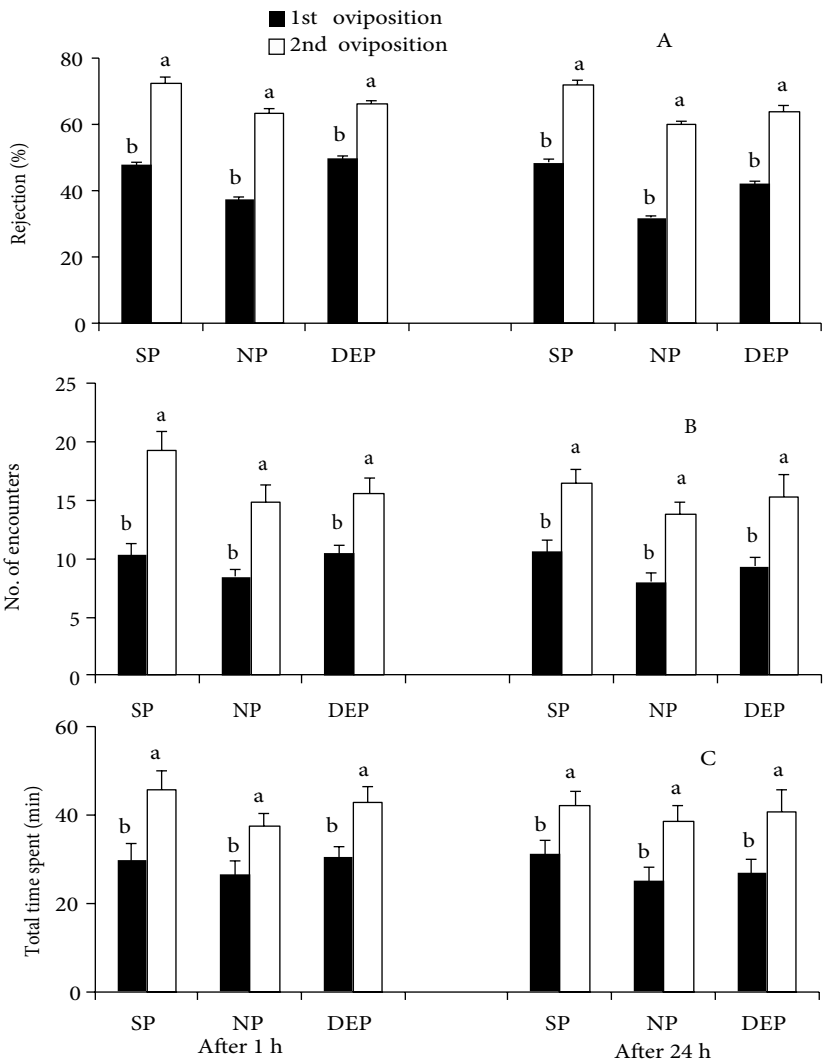

Figure 1. Rejection rate **(A)**, number of encounters **(B)**, and total time spent **(C)** by *E. warrae* in the first and second oviposition at a density of 10 hosts after 1 and 24 h of oviposition. SEP = Same experienced parasitoid; NP = naïve parasitoid; DEP = different experienced parasitoid. Columns with the same letters in each category are not significantly different (P > 0.05).

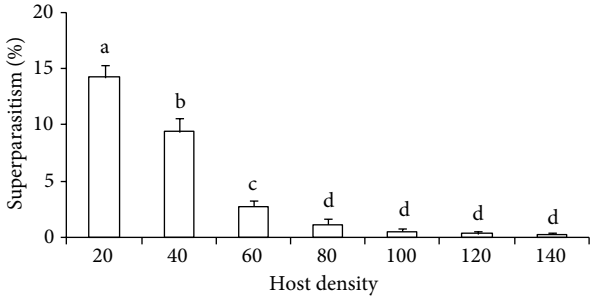

Figure 2. Superparasitism rates in *E. warrae* at different host densities. Columns with the same letters are not significantly different (P > 0.05).

ones (Table 2; Figure 2). This suggests that superparasitism is common in situations when a limited number of hosts is available to the parasitoid for oviposition. Moreover, at lower host densities, *E. warrae* self-superparasitism might be adaptive and advantageous to the parasitoid when the probability of a host being attacked by another parasitoid is high. Laying two or more eggs in a host parasitized by herself increases the possibility of survival of her offspring from that host (van Alphen and Visser, 1990). However, it is always an evolutionary stable strategy for a parasitoid to employ conspecific (different) superparasitism when she senses the presence of other parasitoids in the same patch (van Alphen and Visser, 1990).

The superparasitism rate in the naïve *E. warrae* was significantly higher than the in experienced ones in the present study (Table 2), indicating that the naïve parasitoids have a limited capacity to discriminate between the parasitized and unparasitized hosts. Ardeh (2004) also reported that naïve *E. eremicus* and *E. mundus* frequently lay eggs under parasitized hosts. Godfray (1994) suggested that if the number of hosts available for oviposition is less than the potential egg load of the parasitoid, it can be advantageous for the parasitoid to superparasitize the host. *E. warrae* is a pro-synovigenic species, which emerges with a high number (30–35) of mature eggs (Hanan et al., 2010). Therefore, the higher egg load of naïve parasitoids may encourage them to lay eggs under the parasitized hosts, as the higher egg load in the parasitoids increases the probability of superparasitism (Keasar et al., 2006). It is also possible that when they find only parasitized hosts at the first encounter, they secure at least some of the offspring in the first visiting patch by superparasitization (Bakker et al., 1985). Therefore, the significantly higher superparasitism rate of *E. warrae* at lower host densities may be due to the higher probability of females encountering parasitized hosts.

Many parasitoid species often deposit marking pheromones as an indication to themselves and other females that the host has been parasitized (van Alphen and Visser, 1990; Ardeh, 2004; Buckner and Jones, 2005). Our study also suggests that *E. warrae* females mark the host after oviposition. Buckner and Jones (2005) reported that *E. mundus* applies chemicals (dimethyl alkanes) to its host, *B. argentifolii*, which prevents other parasitoids from using the same host.

In most parasitoids, time is a limiting factor, which can be applied to determine the parasitoid's willingness to accept a host for oviposition (Bakker et al., 1985; Drost et al., 1999). In the present study, *E. warrae* spent significantly more time in the second oviposition than in the first oviposition (Figure 1c). The longer searching time in the second oviposition may be attributed to the higher rejection rate and greater number of hosts being encountered by the parasitoids (Figures 1a and 1b). It is possible that when the density of unparasitized hosts is lower, the second parasitoids spend a longer time searching for a host rather than accepting the parasitized hosts for oviposition. Several studies have also suggested that when parasitoids are forced to stay in a patch with a low density of unparasitized hosts for a long time, they utilize the patch with lower marginal value, resulting in a longer search time and higher superparasitism (van Lenteren et al., 1978; Bakker et al., 1985; van Alphen and Visser, 1990; Montoya et al., 2000).

Although parasitoids of the genus *Eretmocerus* avoid superparasitism when provided with abundance of hosts (Headrick et al., 1995), they superparasitize when confined to a limited number of hosts (van Lenteren et al., 1978; Montoya et al., 2000). In the present study, self-superparasitism significantly increased with the decrease in host density (Figure 2). This suggests that self-superparasitism could be more common in situations where the unparasitized hosts are depleted in the absence of immediate local competition. Under that situation, allocating two or more eggs to the same host increases the probability of survival of the offspring if one egg/larva fails to survive (Visser et al., 1990). Host shortage is likely to occur in the field and laboratory, which can affect the efficiency of a parasitoid as a biological control agent. Therefore, this behavior should be considered before estimating the release rates of *E. warrae* for field application and mass rearing. For example, when a sufficient number of hosts is available for oviposition, host discrimination can be an adaptive strategy of *E. warrae*, enabling the female to avoid superparasitism and thus reducing the food competition among her offspring, which ensures high-quality progeny being available for a biological control program. However, when a limited number of hosts is available, particularly when the host patches have been utilized by other females, superparasitism will allow at least some of her offspring to survive.

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