Host selection for self-superparasitism by *Diaeretiella rapae* (M’Intosh) (Hymenoptera: Aphidiidae)

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**Abstract** Superparasitism (repeated oviposition in same host) indicates either a fitness benefit for the parasitoid, or an inability of ovipositing females to discriminate parasitised versus unparasitised hosts. Superparasitism in *Diaeretiella rapae*, the solitary endoparasitoid of cabbage aphid *Brevicoryne brassicae*, was studied in the laboratory. When given a choice of parasitised and unparasitised aphids, 70% of females made their attack on unparasitised hosts first, even though already-parasitised aphids were less defensive to parasitoid attack and ovipositing females had shorter host-handling time. The female superparasitised hosts during repeated attack and the large hosts were superparasitised more often than the small ones. Although superparasitised hosts carried two to five parasitoid larvae, only one larva developed into an adult. The body size of parasitoid larvae, as measured 4 days after oviposition, was affected by the total number of larvae in the host. Superparasitism strategies of *D. rapae* are discussed in the context of reproductive fitness.

**Keywords** superparasitism, host defence, *Brevicoryne brassicae, Diaeretiella rapae*.

**INTRODUCTION**

Reproductive fitness of female parasitoids depends on their ability to assess the suitability of hosts as resources for successful development of their larvae. Superparasitism is a common phenomenon (Van Alphen & Visser 1990; Jaramillo et al. 2006) in which female parasitoids lay egg(s) in already-parasitised hosts, and the larvae developing inside the host compete for the limited resources (Bai & Mackauer 1992). Self-superparasitism (oviposition in a host previously parasitised by the individual) could reflect an opportunity for fitness gain to the parasitoid or indicate that the ovipositing female is unable to discriminate between already-parasitised and unparasitised hosts (Van Lenteren et al. 1978).

In gregarious parasitoids, where more than one individual emerges from a single host, superparasitism could increase fitness by increasing the total number of emergences per host (Silva-Torres et al. 2009). In solitary parasitoids, however, only one individual per host will emerge, irrespective of the number of eggs laid or the number of larvae developing in a host. Ovipositing more than one egg per host by solitary parasitoids means death of all but one offspring, which means the self-superparasitising females lose the extra egg(s) laid (Volkl & Mackauer 1990; Tracy Reynolds & Hardy 2004). Nevertheless, superparasitism could be advantageous when unparasitised hosts
are not readily available (Van Alphen & Visser 1990). Superparasitism may also increase the chance of survival of at least one larva, as two or more larvae probably depress the host defence system more effectively than one (Mackauer & Chau 2001).

This study looked at the superparasitism strategy of Diaeretiella rapae, a solitary endoparasitoid of cabbage aphid Brevicoryne brassicae and several other aphids of cruciferous and non-cruicerous plants (Pike et al. 1999). There is little information on superparasitism in this cosmopolitan wasp, although Lester & Holtzer (2002) suggested superparasitism in D. rapae occurs at low host density. Previous studies on D. rapae showed that females prefer larger hosts for oviposition, when given a choice of different sizes of hosts (Kant et al. 2008). It is important to understand the superparasitism behaviour in terms of fitness consequences for the parasitoid, as this may improve biological control programmes at the level of mass rearing and/or during augmentative release (Hamelin et al. 2007). The following questions were addressed in the present study to understand the superparasitism strategy of D. rapae: (1) Is the female able to distinguish between parasitised and unparasitised host? (2) Does the female show any preference for host size during superparasitism? (3) Does defensive behaviour of already-parasitised and unparasitised aphids differ during parasitoid attack? (4) What are the effects of superparasitism on growth of parasitoid larvae?

**MATERIALS AND METHODS**

**Insect culture**

Cultures of D. rapae and its host, B. brassicae, were established from individuals collected in a cabbage field in Palmerston North (Manawatu, New Zealand). Aphids were maintained on 8-week-old cabbage Brassica oleracea var. capitata (cv. Summer Globe Hybrid) at 20±2°C under 65% RH and 16 h light regime. Diaeretiella rapae were reared on large (5-7 days old) cabbage aphid nymphs.

**Discrimination between parasitised and unparasitised host**

In order to get parasitised aphids, 1-day old mated D. rapae females were each offered five cabbage aphid nymphs in a Petri dish. There were two groups of females (n=35 in each group), and these were offered either small (1-2 days old) or large (6-7 days old) nymphs. Two sizes of aphids were used in order to make easy visual reference of unparasitised versus already-parasitised aphids when recording behaviour. Parasitism was observed and the parasitised aphids were transferred to cabbage leaves for 1 h for later use in the host discrimination test; the unparasitised aphids were discarded.

The discrimination ability of D. rapae females was examined in a two-part experiment. In the first part, each of the 35 females previously used to parasite 1–2-day-old aphids was offered a small (1-2 days old) self-parasitised aphid nymph and a large (6-7 days old) unparasitised nymph in a Petri dish. In the second part of the experiment, the other 35 females (those used to parasite 6–7-day-old nymphs) were each offered a large self-parasitised aphid and a small unparasitised aphid. The first encounter of the female (with unparasitised or already-parasitised host) and defensive behaviour of aphids were recorded. After parasitism or superparasitism, the aphids were transferred to cabbage seedlings for their development, for 4 days, and subsequently dissected under a stereomicroscope (Olympus SZX12). The number of larvae found in each host was recorded and the larval body lengths were measured using Motic imaging software (version 2.0).

**Host size preference for superparasitism**

The host size preference for superparasitism was tested by offering four aphid nymphs: 1, 3, 5 and 7 days old (age of aphid nymphs is directly correlated to their size, Kant et al. 2008) to a 1-day-old mated D. rapae female in a small Petri dish. The female was allowed to oviposit for 20 min and the behavioural responses of the female and the hosts were recorded for that period. Twenty-five females were tested in this experiment.
After parasitism, the aphids were dissected and parasitoid larvae were counted and body lengths were measured as in the previous experiment.

**Parasitoid attack and host defence behaviour**
During parasitism and superparasitism, duration of the host-parasitoid encounter and the number of times each female *D. rapae* inserted her ovipositor into the host during the encounter were recorded. Defensive behaviours (kicking, abdomen shaking and walk-away from oviposition site) of unparasitised and already-parasitised aphid nymphs were recorded and quantified.

**Data analysis**
Logistic regression (PROC LOGISTIC, SAS 9.2) was used to analyse the first attack and oviposition by *D. rapae* in the unparasitised versus already-parasitised host choice test, walk-away by host during the attack, and for analysis of superparasitism incidences in different size hosts. Chi-square tests were used to compare incidence between different size hosts. Linear regression was used to express the relationship between the size of host and the size of developing larvae. Analysis of variance (PROC GLM, SAS 9.2) was used to analyse the mean number of eggs found in the different size hosts, time spent and the number of ovipositor probing activities performed by *D. rapae* during host attack and the abdomen shaking and kicks by *B. brassicae* nymphs during parasitoid attack. A T-test was used to compare the difference in the size of larvae resulting from parasitism and superparasitism.

**RESULTS**

**Discrimination between parasitised and unparasitised host**
When given a choice of parasitised and unparasitised aphids, females of *D. rapae* were able to recognise the parasitised aphids, and 70% attacked the unparasitised ones first (*P<0.002*) (Table 1). The previously demonstrated preference for large host size contributed significantly to the female choice, as the females attacked the already-parasitised larger aphids significantly more often than smaller aphids (*P<0.001*). The unparasitised small and already-parasitised large aphids were equally preferred for attack (*P=0.06*).

When females made their first attack on unparasitised hosts, larger hosts were attacked more frequently than smaller hosts (*P=0.031*), and 90% of the attacks on large hosts resulted in oviposition compared to 71% of attacks on small hosts (*P=0.098*) (Table 1). When females first attacked already-parasitised hosts, the attacks resulted in oviposition significantly more often in large hosts than in small hosts (*P<0.04*). The females superparasitised large hosts more often than small hosts (*P<0.001*). The least preferred for oviposition was small already-parasitised hosts.

**Host size preference for superparasitism**
When given a choice of different sizes (ages) of cabbage aphid nymphs, larger aphids were more often superparasitised than the smaller ones (*P<0.03*; Figure 1). Overall, the mean number of eggs laid in different size hosts was not significantly different.

**Table 1** First attack and oviposition of female *Diaeretiella rapae* in two choice tests: (1) Small unparasitised versus Large already-parasitised and (2) Large unparasitised versus Small already-parasitised *Brevicoryne brassicae* nymphs. The percentage value in the oviposition column indicates percentage of attacks that resulted in parasitism.

<table>
<thead>
<tr>
<th>Choice experiments</th>
<th>First attack</th>
<th>Oviposition</th>
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<tbody>
<tr>
<td></td>
<td>Unparasitised</td>
<td>Already-parasitised</td>
</tr>
<tr>
<td>Small unparasitised vs Large</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>already-parasitised</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large unparasitised vs Small</td>
<td>29</td>
<td>6</td>
</tr>
<tr>
<td>already-parasitised</td>
<td></td>
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The size of the parasitoid larvae was also dependent on whether they developed as a single larva or as multiple larvae in superparasitised hosts (P<0.001). In larger hosts, the larvae were significantly larger when developed as a single larva than when developed as multiple larvae (P<0.0001) (Table 2). However, the size of larvae developed in smaller hosts did not differ significantly between single larva and multiple larvae (P=0.25) (Table 2).

Table 2 Size of *Diaeretiella rapae* larvae in single larva and multiple larvae hosts. Same letter within a column indicates no significant difference (α=0.05).

<table>
<thead>
<tr>
<th>Development condition of the <em>D. rapae</em> larvae</th>
<th>Larval size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larger host</td>
<td>Smaller host</td>
</tr>
<tr>
<td>Single larva</td>
<td>841±56 a</td>
</tr>
<tr>
<td>Multiple larvae</td>
<td>549±19 b</td>
</tr>
</tbody>
</table>

The intensity of superparasitism (number of larvae ≥ 2 per host) did not influence the size of developing larvae significantly (P=0.71). The major difference in the size of larvae (10%) was found between the larvae developing in pairs (594.2±41.2 µm) and the larvae developing at five per host (539.7±34.1 µm).

**Parasitoid attack and host defence behaviour**

Ovipositing females of *D. rapae* had shorter host-handling time (P<0.001) and were less aggressive to the hosts (P<0.003) during superparasitism than at first parasitism. Already-parasitised and unparasitised aphids defended themselves from the parasitoid attack, although defensive behaviour involved significantly more kicking in unparasitised aphids than in the already-parasitised ones (P<0.01) (Table 3). The number of times unparasitised and already-parasitised aphids shook their abdomen during the parasitoid attack did not differ significantly (P>0.06). Aphids also defended themselves by walking away from the parasitoid attack, and this behaviour was displayed by 83% of unparasitised...
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aphids and by 51% of already-parasitised ones during the *D. rapae* attack (*P*<0.005).

**DISCUSSION**

Female *D. rapae* were able to discriminate the unparasitised hosts from the already-parasitised ones, and showed clear preference for laying eggs in unparasitised hosts when making oviposition decisions. In solitary parasitoids, after intrinsic competition among the larvae, only one adult individual emerges per host, irrespective of the number of eggs laid in that host (Godfray 1994). Therefore, the ability of a female to distinguish unparasitised hosts from already-parasitised ones would allow her to save eggs from direct loss through superparasitism, and would probably gain reproductive success by increasing the number of parasitised hosts.

Female *D. rapae* self-superparasitised hosts when they had repeated encounters with the same host. Repeated encounters would be expected when the number of hosts is limited. When the number of aphids available to a parasitoid is far less than the ovipositing capacity of the female, it might be advantageous to the female to lay more than one egg per host (Godfray 1994; Zhang et al. 2010). Female *D. rapae* used in this research were young, naïve and mated, and *D. rapae* females emerge with developed eggs (R. Kant, unpublished data). Therefore, egg loads of these females could have encouraged them to perform repeated oviposition in the available hosts, as high egg-loads in parasitoids encourage superparasitism (Keasar et al. 2006; Silva-Torres et al. 2009). Laying multiple eggs in one host increases the chance of survival and successful emergence of at least one individual (Rosenheim & Hongkham 1996; Zhang et al. 2010).

The number of eggs laid by *D. rapae* females in a host was far fewer than the number of times the females probed with their ovipositor. This suggests that some of these ovipositor probing activities of a female lead to oviposition and the rest may use for assessing the quality of the host before oviposition and injection of venom to paralyse host appendages to facilitate oviposition. Host paralysis in parasitoids is common. Idiobiont parasitoids permanently paralyse and stop growth and development of their hosts, while koinobiont parasitoids, including *D. rapae*, temporarily paralyse their hosts (Godfray 1994) and allow them to grow, develop and reproduce (Zhang & Hassan 2003). Earlier studies suggest that during attack, parasitoids not only target the main body but also the appendages (Phillips 2002; Kant et al. 2008). The amount of time *D. rapae* females spent per encounter and the number of ovipositor probings they administered in already-parasitised hosts were fewer compared to unparasitised hosts. This weaker defence in already-parasitised aphids during superparasitism could be due to the temporary paralysis caused when these aphids were first attacked. Since the second parasitism was done just about an hour after the first parasitism, it is possible that the effects of paralysis from the first parasitism persisted in the aphids.

Growth and development of parasitoid larvae were influenced by both the size of hosts and the number of larvae developing per host. A direct effect of the host size on development of larvae is expected, because more resources are available in larger hosts compared to smaller ones (Godfray

### Table 3

Behavioural responses in parasitoid-host encounters during first parasitism and superparasitism. Parasitoid (*Diaeretiella rapae*) expenditure per host was measured as time spent and the number of probes (insertions of the ovipositor) administered by the female. Host (*Brevicoryne brassicae*) defence behaviour per encounter was measured as the number of abdomen shakings and the number of kicks.

<table>
<thead>
<tr>
<th>Oviposition</th>
<th>Parasitoid expenditure</th>
<th>Host defence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time spent (s)</td>
<td>Probes</td>
</tr>
<tr>
<td>First parasitism</td>
<td>29.7±3.9 a</td>
<td>7.2±0.6 a</td>
</tr>
<tr>
<td>Superparasitism</td>
<td>8.8±1.1 b</td>
<td>4.7±0.5 b</td>
</tr>
</tbody>
</table>

The offspring of *D. rapae* developed in older, and also larger, *B. brassicae* are of relatively large size (Kant et al. 2009). This suggests that larger cabbage aphids offer more resources to developing parasitoid larvae. Since the host resources for developing parasitoid larvae are fixed, the more eggs laid in a host, the smaller share an individual larva gets during growth (Bai & Mackauer 1992).

The size of hosts *D. rapae* females encountered significantly affected their superparasitism decisions. The females preferred to superparasitise larger hosts. Some of the superparasitised small aphids died and those that survived had no more than two larvae in them. In contrast, up to five larvae were found in living larger hosts. Larger (older) hosts have stronger immune defence than the younger or smaller ones (Jervis et al. 2005). Therefore, laying more than one egg in a host could be an adaptive strategy of *D. rapae* females to weaken the defence system of the large host.

In addition, self-superparasitism may provide extra nutrition to the surviving parasitoid larva under low host density conditions. In aphid nymphs superparasitised by *D. rapae*, only one larva successfully develops into an adult after winning the intrinsic competition, and the winning larva consumes all the resources including the siblings (cannibalism) (Goubault et al. 2003) and thereby gains more nutrients. This study shows that when more than two larvae (up to five) of *D. rapae* developed in a host, they all gained equal mean body size, which suggests that the larvae utilised the available resources faster when the number of larvae in a host increased. When the resources in superparasitised host deplete quicker, the host may need more resources for its own survival and could eventually increase feeding. As *D. rapae* is a koinobiont parasitoid, which allows the parasitised host to live and develop, the multiple larvae developing in a superparasitised host may enhance the feeding efficiency of the host.

This study suggests that females of *D. rapae* maximise parasitism by avoiding superparasitism. However, superparasitism occurs at a low host density (Lester & Holtzer 2002), which may be a strategy to enhance provisioning to the surviving offspring. Furthermore the superparasitism could also be a strategy to utilise the host resources more efficiently, which could maximise the quality (if not quantity) of the progeny.

ACKNOWLEDGEMENTS

The research is supported by a Massey University Doctoral Research Scholarship. We thank Ms Philippa Stevens and Dr Sue Zydenbos for their insightful comments on the manuscript. We are also grateful to Ms Kay Kitchen, Massey University, Palmerston North, for her help in growing plants and establishment of insect colonies.

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