Host genotype affects the relative success of competing lines of aphid parasitoids under superparasitism

CHRISTOPH VORBURGER*, BETTINA EUGSTER†, JÖRG VILLIGER & CORINNE WIMMER‡

Institute of Zoology, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

Running title: Superparasitism in Lyisphlebus fabarum

*present address: Institute of Integrative Biology, ETH Zürich & EAWAG, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, 8600 Dübendorf, Switzerland

†present address: Limnological Station, Institute of Plant Biology, University of Zürich, Seestrasse 187, 8802 Kilchberg, Switzerland

‡present address: Palaeontological Institute and Museum, University of Zürich, Karl Schmid-Strasse 4, 8006 Zürich, Switzerland

Correspondence: Christoph Vorburger, Institute of Integrative Biology, ETH Zürich & EAWAG, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, 8600 Dübendorf, Switzerland. christoph.vorburger@eawag.ch

This is the accepted manuscript of the article. The definitive version is available at www.blackwell-synergy.com
Abstract. 1. In solitary parasitoids, only one individual can complete development in a given host. Therefore, solitary parasitoids tend to prefer unparasitised hosts for oviposition, yet under high parasitoid densities, superparasitism is frequent and results in fierce competition for the host's limited resources. This may lead to selection for the best intra-host competitors.

2. Increased intra-host competitive ability may evolve under a high risk of superparasitism if this trait exhibits genetic variation, and if competitive differences among parasitoid genotypes are consistent across environments, e.g. different host genotypes.

3. These assumptions were addressed in the aphid parasitoid *Lysiphlebus fabarum* (Hymenoptera: Braconidae: Aphidiinae) and its main host, the black bean aphid, *Aphis fabae* (Scopoli) (Hemiptera: Aphididae). Three parthenogenetic lines of *L. fabarum* were allowed to parasitise three aphid clones singly and in all pairwise combinations (superparasitism). The winning parasitoid in superparasitised aphids was determined by microsatellite analysis.

4. The proportions of singly parasitised aphids that were mummified were similar for the three parasitoid lines and did not differ significantly among host clones.

5. Under superparasitism, significant biases in favour of one parasitoid line were observed for some combinations, indicating that there is genetic variation for intra-host competitive ability. However, the outcome of superparasitism was inconsistent across aphid clones and thus influenced significantly by the host clone in which parasitoids competed.

6. Overall, this study shows that the fitness of aphid parasitoids under superparasitism is determined by complex interactions with competitors as well as hosts, possibly hampering the evolution of improved intra-host competitive ability.

Keywords. *Aphis fabae*, genotype-by-genotype interaction, *Hamiltonella defensa*, *Lysiphlebus fabarum*, parasitoids, superparasitism, symbiosis
Introduction

In solitary endoparasitoids of insects, only one individual can successfully complete development within a single host. Under superparasitism, i.e. when a host is attacked by more than one individual of the same species (Godfray, 1994), fierce competition among parasitoid larvae ensues for the host's limited resources. This may take the form of physical combat when larvae are similar in age and size (Mackauer, 1990; Marris & Casperd, 1996), or else the younger parasitoid may succumb because its development is suppressed physiologically by the older conspecific (Fisher, 1963). In either case, superparasitism entails a high risk of death and should be avoided if a sufficient number of unparasitised hosts is available (van Lenteren, 1981). This prediction is supported by a number of studies demonstrating that parasitoids can identify already parasitised hosts and discriminate against them in favour of unparasitised hosts (e.g. Salt, 1961; Hubbard et al., 1987; Bai, 1991). Aphid parasitoids, the subjects of the present study, also appear to possess such a host discrimination ability. The aphidiine wasp *Aphidius rhopalosiphi* (De Stefani Perez), for example, tends to avoid attacking previously parasitised aphids shortly after the first attack based on external cues (Outreman et al., 2001a). At longer time intervals after the first oviposition, it may avoid oviposition based on internal cues perceived during stabs (Outreman et al., 2001a). But in either case, host discrimination is far from perfect, resulting in a substantial rate of superparasitism (Outreman et al., 2001a; 2001b). Anyway, if unparasitised hosts are in short supply, most solitary parasitoids readily superparasitise, and superparasitism may even be adaptive in some situations (Janssen, 1989; van Alphen & Visser, 1990).

If superparasitism occurs frequently in a parasitoid population, selection is expected to favour the best within-host contestants. Improved competitive ability under superparasitism may evolve if there is sufficient genetic variation for traits affecting this ability, and if
competitive differences among parasitoid genotypes are relatively consistent under different environmental conditions, which are largely determined by the host and its physiology. It would be difficult for increased intra-host competitive ability to evolve if the relative success of competing genotypes changed in different hosts or even host genotypes. Here, these issues are addressed in the black bean aphid, *Aphis fabae*, and its parasitoid *Lysiphlebus fabarum*. Although little is known about natural rates of superparasitism in *L. fabarum*, it is clear that this species readily superparasitises in laboratory cultures (C. Vorburger, personal observation). This system is uniquely suited for such a study because unlike most other parasitoids of aphids, *L. fabarum* reproduces by thelytokous parthenogenesis in the majority of populations (Belshaw et al., 1999; Starý, 1999; Vorburger et al., 2009). Given that aphids are also capable of parthenogenesis, it is possible to work with genetically homogeneous lines of both host and parasitoid, and thus to replicate contests among the exact same parasitoid genotypes and observe their outcome in several host genetic backgrounds (i.e. aphid clones). This study took advantage of this possibility to address the following two questions: (i) do different parthenogenetic lines of *L. fabarum* differ in their intra-host competitive ability under superparasitism? and (ii) is the relative success of competing parasitoid lines consistent across different genotypes of their host, *Aphis fabae*?

**Material and methods**

**Study organisms**

The black bean aphid, *Aphis fabae*, is very common in temperate regions of the northern hemisphere. Based on the range of secondary host plants used, four subspecies of *Aphis fabae* are distinguished (Heie, 1986; Raymond et al., 2001), but only the nominal subspecies *A. f.*
Aphids may harbour facultative or secondary bacterial endosymbionts that can affect their susceptibility to parasitoids (Oliver et al., 2003). This was not the case for clones A and B, but clone C was infected with a strain of the endosymbiotic bacterium *Hamiltonella defensa* which provides some, albeit limited, protection against parasitoids (Vorburger et al., 2009).

*Lysiphlebus fabarum* is the most important parasitoid of *A. f. fabae* (Starý, 2006). After oviposition of a single egg by the female wasp, the parasitoid larva develops inside the still active aphid. Upon completion of its larval development, the parasitoid kills the host and pupates in a cocoon spun inside the aphid's exoskeleton. At this stage, parasitised aphids are easily recognisable as 'mummies', from which the adult wasps emerge after several days.

Three different, parthenogenetic lines of *L. fabarum* referred to as lines 1, 2 and 3 were used for the experiments described below. All were founded by a single female for which collection details and microsatellite genotypes are provided as supplementary material in Table S1. Unlike parthenogenesis in aphids, which is apomictic and thus results in truly clonal progeny, parthenogenesis in *L. fabarum* occurs by central fusion automixis (Belshaw & Quicke, 2003). Therefore, isofemale lines of parthenogenetic *L. fabarum* should not be termed clones. Nevertheless, they can be regarded as genetically uniform, because central fusion
automixis rapidly leads to homozygosity distal to chiasmata, while leaving nonrecombining regions of the genome unaffected. This is evidenced by the fact that the microsatellite genotypes of the three experimental lines remained unchanged since their collection (C. Sandrock & C. Vorburger, unpubl. data).

Experimental procedures

The experiment consisted in exposing all three aphid clones to all three parasitoid lines singly to obtain estimates of mummification rates in the absence of superparasitism, and to stage pairwise contests in all three aphid clones by letting aphids be attacked twice by different parasitoid lines to determine the outcome of intra-host competition.

Aphid nymphs (48-72 h old, mostly 2\textsuperscript{nd} instar) were exposed to wasps in 3 cm Petri dishes and monitored. When an attack by the parasitoid was observed, the aphids were immediately removed from the dish and either placed on a plant (singly parasitised treatment) or moved to another dish containing wasps of a different parasitoid line (superparasitised treatment). When the aphids had suffered a second attack, they were also transferred to plants. The order of the first and second parasitoid line to attack the aphids was alternated, although survival of same-aged larvae was found to be independent of oviposition sequence (Mackauer \textit{et al.}, 1992). The goal was to have 50 replicate aphids of each clone attacked by each parasitoid line and each pairwise combination of parasitoid lines. To keep the daily workload manageable, the experiment had to be temporally staggered such that approx. 10 replicates of each combination were done per day over five consecutive days. The five days were treated as temporal blocks in all analyses.

The attacked aphids were reared at 20°C and a 16 h photoperiod on seedlings of \textit{Vicia faba} (var. Scirocco) covered with cellophane bags. When the mummies had formed, they were
isolated in gelatine capsules until the parasitoids emerged. The wasps were then dried at 56°C for 22 h and weighed to the nearest microgram on a Mettler MX5 microbalance (Mettler-Toledo GmbH, Greifensee, Switzerland) to obtain an estimate of body size. Which parasitoid won the larval competition in superparasitised aphids was determined by genotyping the wasps at six microsatellite loci, Lysi02, Lysi03, Lysi05, Lysi06, Lysi07 and Lysi08 (Sandrock et al., 2007), amplified in one multiplex PCR reaction. DNA extractions and PCR conditions followed the protocols described in Sandrock et al. (2007).

Because of the generally low rates of mummification observed in the experiment, a small follow-up experiment was conducted to test whether parasitoid attacks in the rather artificial environment of a Petri dish indeed resulted in oviposition. For this, 2nd instar nymphs of A. fabae were placed singly into a Petri dish containing approx. 15 female L. fabarum and removed after they were observed to have been attacked either once or twice. Nine singly and nine doubly attacked aphids were subsequently dissected under a microscope at 100× magnification to search for parasitoid eggs. The aphids used in this follow-up experiment belonged to clone B, and the parasitoids we used belonged to a parthenogenetic line of L. fabarum that was not included in the main experiment (line 06-533, collected on 2 July 2006 in Hessen, Germany).

Statistical analyses

Statistical analyses were carried out in R 2.7.1 (R Development Core Team, 2008). Mummification rates of singly parasitised aphids were analysed at the level of individual aphids (1 = mummified, 0 = not mummified), using a generalised linear model with a logit link and binomial errors, testing for the effects of block, aphid clone, parasitoid line and the aphid × parasitoid interaction. Mummification rates of superparasitised aphids were analysed
with a similar model, but testing for the effect of pairwise combinations rather than individual lines of parasitoids.

For each host clone and pairwise combination of parasitoid lines, the frequencies of wasps of each line emerging from superparasitised aphids were compared with expected frequencies using $\chi^2$-tests. Two different tests were carried out. One simply compared the observed frequencies with a 1:1 ratio (Test 1 in Table 1), which was justified given that the variation among parasitoid lines in mummification rates of singly parasitised aphids was non-significant (see Results). The second test (Test 2 in Table 1) compared the observed frequencies with expected frequencies when nevertheless accounting for the (nonsignificant) variation in mummification rates of singly parasitised aphids on the different aphid clones.

From these, the probabilities that only the larva of the first parasitoid line developed ($p_1$), that only the larva of the second line developed ($p_2$), and that both larvae developed initially in the host ($p_{1+2}$) were calculated. The expected numbers for each line under the null hypothesis that the two lines are equal competitors when both larvae develop was then obtained as follows:

$$N_1 = \frac{p_1 + 0.5p_{1+2}}{p_1 + p_2 + p_{1+2}} \times N$$

$$N_2 = \frac{p_2 + 0.5p_{1+2}}{p_1 + p_2 + p_{1+2}} \times N$$

where $N$ is the total number of wasp obtained from each pairwise combination, and $N_1$ and $N_2$ are the expected numbers of these belonging to the first and second line, respectively.

Finally, it was tested whether the relative numbers of wasps of the two lines emerging from superparasitised aphids were independent from the aphid clone in which they developed, using Fisher's exact test (Test 3 in Table 1).
Results

Proportion mummified

Overall, the rates of successful parasitism were rather low in our experiment. Of totally 824 aphid nymphs that were attacked either once or twice, only 200 were mummified (24%). From these, 170 wasps emerged. No adult parasitoid emerged from 30 mummies, but in all but two of these cases it was possible to identify the line that pupated (i.e. the ‘winner’ in superparasitised aphids) by genotyping the mummy.

The low rates of mummification are only partially explicable by observed attacks that did not result in oviposition. Of nine singly attacked aphids dissected in the follow-up experiment, two parasitoid eggs were found in one individual, one egg in five individuals and no egg was detected in three individuals. In the nine aphids attacked twice, two contained three eggs, two contained two eggs and five contained one egg. Based on these numbers, a crude estimate can be derived that about 30% of observed attacks may not have resulted in oviposition in our main experiment (33% when calculated from the singly attacked aphids, 28% from the doubly attacked aphids). This is likely to be an upper bound, because it cannot be excluded that some of the small and unpigmented eggs were overlooked in dissections. The follow-up experiment also showed that what looks like a single attack may sometimes result in the deposition of more than one egg.

The proportions of singly parasitised aphids that were mummified are illustrated in Figure 1a. These proportions did not differ significantly among the three aphid clones used (GLM, $\chi^2_2 = 1.66, P = 0.44$), nor among the three parthenogenetic lines of *L. fabarum* ($\chi^2_2 = 0.34, P = 0.85$). The aphid clone × parasitoid line interaction was also not significant ($\chi^2_4 = 4.42, P = 0.35$), but there was a marginally significant block effect ($\chi^2_4 = 9.48, P = 0.05$).
In superparasitised aphids, mummification rates were slightly but not significantly higher \( (\chi^2_1 = 3.16, P = 0.08) \) (Fig. 1b). Again, there was no significant variation among aphid clones \( (\chi^2_2 = 0.20, P = 0.90) \), nor was there a significant difference among the three pairwise combinations of parasitoid lines \( (\chi^2_2 = 1.18, P = 0.55) \). However, there was a significant interaction between host clone and parasitoid combination \( (\chi^2_4 = 17.30, P = 0.002) \), mainly because very few wasps emerged from aphid clone A when it was simultaneously attacked by the wasp lines 2 and 3 (Fig. 1b). The block effect on mummification was not significant in superparasitised aphids \( (\chi^2_4 = 6.94, P = 0.14) \).

**Outcome of superparasitism**

In three out of nine different superparasitism assays (three pairwise combinations of wasp lines × three aphid clones), the relative frequencies of competing lines among the emerging wasps differed significantly from the expectation under the null hypothesis, independent of whether variation in mummification rates of singly parasitised aphids was accounted for or whether simply tested against a 1:1 ratio (Table 1, tests 1 and 2). All three significant cases concerned aphid clone C (Table 1, Fig. 1b). Wasp line 1 was outcompeted by both other lines when larval competition took place within clone C, but this was not the case in the other two aphids. When wasp line 2 and 3 competed within C, most emerging adults belonged to line 3 (Table 1, Fig. 1b).

Generally, the outcome of larval competition in superparasitised aphids appeared relatively inconsistent across the three host clones. At least in one case, line 1 vs. line 2, this was supported by a significant test result from Fisher's exact test of independence (Table 1), indicating that the relative success of parasitoid lines under superparasitism depends on the host in which larval competition takes place.
Parasitoid size

The analysis of wasp dry masses indicated a highly significant effect of aphid clone and a marginally non-significant effect of parasitoid line, but there was no significant difference in mass between wasps emerging from singly or superparasitised aphids (Table 2). On average, wasps were heaviest when emerging from aphid clone C (Fig. 2). There was also a significant aphid clone $\times$ parasitoid line interaction, indicating that the relative sizes of wasps from the three lines depended on which aphid clone they developed in. To a limited extent, these size differences reflected the relative success under superparasitism. Parasitoid line 1, which was a poor intra-host competitor in aphid clone C, also produced the smallest wasps in this clone (Fig. 2). On the other hand, parasitoid line 3 produced by far the heaviest wasps on aphid clone A, but it was not more successful than the other lines in parasitising this clone (Figs. 1 & 2).

Discussion

This study examined the outcome of superparasitism between parthenogenetic lines of the parasitoid *L. fabarum* in different clones of their host, *A. f. fabae*. It showed that although parasitoid genotypes appear to vary in their intra-host competitive ability, the outcome of superparasitism in any given instance is difficult to predict, because it may be influenced substantially by the host clone within which parasitoids compete. Thus, at least under a high risk of superparasitism, the genotypic composition of hosts as well as competitors may strongly influence a parasitoid's fitness. Similar to other forms of genotype $\times$ environment or genotype $\times$ genotype interactions, this is likely to contribute to the maintenance of genotypic
diversity (Maynard Smith & Hoekstra, 1980; Weeks & Hoffmann, 1998; Carius et al., 2001; Niklasson et al., 2004; Tétard-Jones et al., 2007; Seppälä et al., 2009).

The strongest biases in genotype ratios of wasps emerging from superparasitised aphids occurred in clone C, the only aphid clone infected with the secondary endosymbiont *H. defensa*. Although the effects of aphid genotype and endosymbiont cannot be separated here, it is worth considering that *H. defensa* might influence intra-host competition of parasitoids.

In the pea aphid (*Acyrthosiphon pisum*) as well as in the black bean aphid used here, *H. defensa* can strongly increase resistance to parasitoids (Oliver et al., 2003; Vorburger et al., 2009). This protective effect not only differs among isolates of *H. defensa* (Oliver et al., 2005; Degnan & Moran, 2008), it is also differentially effective against different parasitoid lines (Vorburger et al., 2009, R. Rouchet & C. Vorburger, unpubl. data), indicating that parasitoids exhibit genetic variation their ability to overcome symbiont-conferred resistance. The strain of *H. defensa* harboured by C clearly does not provide complete protection, as all three lines of *L. fabarum* used here were able to parasitise it in the absence of competitors (Fig. 1a). Nevertheless, it is possible that the presence of *H. defensa* might affect the parasitoid lines unequally and thus put one competitor at a disadvantage under superparasitism. This could have been the case for line 1 in our experiment. It was outcompeted in clone C and also achieved the lowest mummification rates when parasitising this *H. defensa*-bearing clone singly (Fig. 1). However, the generally low proportions of aphids mummified precluded a decision whether the latter was a meaningful difference.

Mummification rates in this study were substantially lower than in another study on the same system (Vorburger et al., 2009), but they cannot be directly compared, as in Vorburger et al. (2009), the aphids are likely to have suffered multiple rather than just single or double attacks by wasps. The small follow-up experiment suggested that up to a third of observed attacks in the main experiment would not have resulted in oviposition by parasitoids. This
cannot fully explain the low rates of mummification, especially not in the superparasitism
treatment, because there the vast majority of aphids must have received at least one
parasitoid egg. Most survivors thus seem to have successfully resisted the parasitoids. The
follow-up experiment also showed that a substantial fraction of doubly attacked aphids would
only have received one parasitoid egg, which means that some apparent ‘winners’ in the
superparasitism treatment did in fact not have to compete with another parasitoid larva. While
this has to be acknowledged, it is unlikely to explain the observed patterns in the results.
Aphid parasitoids can use the presence of aphid cornicle secretions on an aphid’s body as a
cue of previous attacks and may thus have been less inclined to sting already parasitised
aphids in the experiment (Outreman et al., 2001a). Yet attacks were visually observed in this
experiment, and sting rejection, i.e. stinging but refraining from oviposition based on internal
cues of superparasitism, is only expected to occur at longer time intervals after the first
oviposition (Outreman et al., 2001a). Even if sting rejections had occurred, they should not
have biased the results because the order of parasitoid lines attacking the hosts was alternated
in the superparasitism treatment. Therefore, the conclusion remains that the host clone
somehow modified the interaction between parasitoid lines attacking the same individual.

The host clone did not only influence the outcome of intra-host competition, it also had a
highly significant influence on the dry mass of emerging parasitoids. This is not surprising
and might simply reflect host size. It is known that A. f. fabae exhibits clonal variation for
adult mass, and there is some evidence that infection with H. defensa has a positive effect on
aphid body size (Vorburger et al., 2009), which might explain why wasps were heaviest on
average when they developed in clone C. One would also expect an effect of the wasp's own
genotype on adult mass, which was only supported to a limited extent, because the variation
among wasp lines was marginally non-significant. More interesting was the finding that the
aphid clone × parasitoid line interaction had a significant effect on wasp dry mass, re-iterating
the point that relative fitness of parasitoid genotypes in this system is determined by complex
interactions with their hosts. The effect that superparasitism might have on parasitoid body
size is somewhat difficult to predict. On one hand, the winner might have incurred costs from
having to compete with a conspecific and therefore emerge smaller, on the other hand, there is
some evidence that the growth potential is higher in superparasitised aphids because they
ingest more food than singly parasitised ones (Bai & Mackauer, 1992; Mackauer & Chau,
2001). The present study detected no significant mass difference between wasps emerging
from singly or superparasitised aphids, suggesting that these effects have limited importance
in *L. fabarum*, or else that they counteract each other.

In summary, this study suggests that the aphid parasitoid *L. fabarum* exhibits genetic
variation for intra-host competitive ability under superparasitism. Despite that, it appears that
a strategy to always superparasitise based on superior competitive ability would be difficult to
evolve, because the success of superarasitism depends on complex interactions with the
competing genotype as well as the genotype of the host in which competition takes place.
Furthermore, time delays as they are likely to occur in a natural situation will put
superparasitising wasps at a disadvantage (Bai, 1991; Visser, 1993; but see Marris & Casperd,
1996), whereas here the two wasp lines were allowed to oviposit simultaneously. The
potential influence of defensive endosymbionts such as *H. defensa* on the outcome of
superparasitism deserves further attention.

**Acknowledgements**

We thank Christoph Sandrock for advice on microsatellite analyses and Alexandre Gouskov
for his help with aphid rearing. Two anonymous reviewers and an associate editor provided
helpful comments on the manuscript. This work was supported through a grant from the Swiss National Science Foundation (nr. 3100A0-109266) to CV.

References


Aphididae: subfamily Pterocommatinae & tribe Aphidini of subfamily Aphidinae. Fauna  
Entomologica Scandinavica, 17, 314 pp.

avoidance of superparasitism by solitary parasitic wasps. Journal of Animal Ecology, 56,  
387-401.

Ecology, 3, 469-479.

In Critical Issues in Biological Control (eds M. Mackauer, L.E. Ehler & J. Roland), pp. 41- 
62. Intercept, Andover, .

between two species of solitary parasitoid wasps - the influence of superparasitism.  
Ecological Entomology, 17, 233-236.

the influence of clutch size on offspring size. Functional Ecology, 15, 335-343.

the outcome of in vitro contests staged between different larval instars of the solitary  

are the models? Genetical Research, 37, 45-57.

bifurcata (Fallen, 1810) (Diptera : Lonchopteridae). I. Fluctuating seasonal selection  


Table 1. Numbers of mummies produced by competing parasitoid lines under superparasitism for each pairwise combination, and tests against different null expectations (see Methods).

<table>
<thead>
<tr>
<th>Aphid clone</th>
<th>Line 1</th>
<th>Line 2</th>
<th>Test 1 (H₀ = 1 : 1 ratio)</th>
<th>Test 2 (H₀ = equal competitors)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>7</td>
<td>χ² = 0.077, P = 0.782</td>
<td>χ² = 0.169, P = 0.681</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>7</td>
<td>χ² = 0.333, P = 0.564</td>
<td>χ² = 2.503, P = 0.114</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>13</td>
<td>χ² = 13.00, P &lt; 0.001</td>
<td>χ² = 8.556, P = 0.003</td>
</tr>
<tr>
<td>Overall</td>
<td>11</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 3</td>
<td>Fisher's exact test</td>
<td>P = 0.015</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aphid clone</th>
<th>Line 1</th>
<th>Line 3</th>
<th>Test 1 (H₀ = 1 : 1 ratio)</th>
<th>Test 2 (H₀ = equal competitors)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8</td>
<td>11</td>
<td>χ² = 0.474, P = 0.491</td>
<td>χ² = 2.998, P = 0.083</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>5</td>
<td>χ² = 0.333, P = 0.564</td>
<td>χ² = 0.803, P = 0.370</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>8</td>
<td>χ² = 5.444, P = 0.020</td>
<td>χ² = 3.423, P = 0.064</td>
</tr>
<tr>
<td>Overall</td>
<td>16</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 3</td>
<td>Fisher's exact test</td>
<td>P = 0.096</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aphid clone</th>
<th>Line 2</th>
<th>Line 3</th>
<th>Test 1 (H₀ = 1 : 1 ratio)</th>
<th>Test 2 (H₀ = equal competitors)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>2</td>
<td>χ² = 0.000, P = 1.000</td>
<td>χ² = 0.155, P = 0.693</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>10</td>
<td>χ² = 2.571, P = 0.109</td>
<td>χ² = 0.087, P = 0.768</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>14</td>
<td>χ² = 9.000, P = 0.003</td>
<td>χ² = 9.936, P = 0.002</td>
</tr>
<tr>
<td>Overall</td>
<td>8</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 3</td>
<td>Fisher's exact test</td>
<td>P = 0.178</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Analysis of variance results for parasitoid dry mass.

<table>
<thead>
<tr>
<th>Effect</th>
<th>d.f.</th>
<th>MS ($\times$ 1000)</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>4</td>
<td>0.061</td>
<td>1.024</td>
<td>0.397</td>
</tr>
<tr>
<td>Aphid clone</td>
<td>2</td>
<td>0.430</td>
<td>7.269</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Parasitoid line</td>
<td>2</td>
<td>0.165</td>
<td>2.792</td>
<td>0.065</td>
</tr>
<tr>
<td>Treatment (single vs. super)</td>
<td>1</td>
<td>0.014</td>
<td>0.236</td>
<td>0.628</td>
</tr>
<tr>
<td>Aphid clone $\times$ parasitoid line</td>
<td>4</td>
<td>0.168</td>
<td>2.840</td>
<td>0.026</td>
</tr>
<tr>
<td>Aphid clone $\times$ treatment</td>
<td>2</td>
<td>0.022</td>
<td>0.379</td>
<td>0.686</td>
</tr>
<tr>
<td>Parasitoid line $\times$ treatment</td>
<td>2</td>
<td>0.141</td>
<td>2.388</td>
<td>0.095</td>
</tr>
<tr>
<td>Aphid $\times$ parasitoid $\times$ treatment</td>
<td>4</td>
<td>0.056</td>
<td>0.946</td>
<td>0.439</td>
</tr>
<tr>
<td>Residual</td>
<td>148</td>
<td>0.059</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure legends

**Fig. 1.** Proportions of aphids mummified by each line of *Lysiphlebus fabarum* in (a) singly parasitised and (b) superparasitised aphids belonging to three different clones of *Aphis fabae fabae*. Numbers above bars indicate the number of aphids tested.

**Fig. 2.** Mean dry mass (± SE) of adult *Lysiphlebus fabarum* emerging from mummies of the three clones of *Aphis fabae fabae*. 
Fig. 1. Proportions of aphids mummified by each line of Lysiphlebus fabarum in (a) singly parasitised and (b) superparasitised aphids belonging to three different clones of Aphis fabae fabae. Numbers above bars indicate the number of aphids tested.

259x262mm (600 x 600 DPI)
Fig. 2. Mean dry mass (± SE) of adult Lysiphlebus fabarum emerging from mummies of the three clones of Aphis fabae fabae.