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

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Running title: Superparasitism in *Lyisphlebus fabarum*

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This document is the accepted manuscript version of the following article:
Vorburger, C., Eugster, B., Villiger, J., & Wimmer, C. (2010). Host genotype affects the relative success of competing lines of aphid parasitoids under superparasitism. Ecological Entomology, 35(1), 77-83.
http://doi.org/10.1111/j.1365-2311.2009.01159.x

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.*

fabae is considered here. In central Europe, *A. f. fabae* reproduces by cyclical parthenogenesis. The parthenogenetic summer generations can cause major damage on broad bean (*Vicia faba*) and sugar beet (*Beta vulgaris*) crops. In autumn, black bean aphids migrate back to the primary host, the European spindle tree (*Euonymus europaeus*), where sexual reproduction takes place. In the present study, three different, genetically distinct clones of *A. f. fabae* were used: A06-404, A06-407 and Af6. All clones were collected in Switzerland and used previously in a published study of susceptibility to parasitoids (Vorburger *et al.*, 2009), where more detailed collection information is available. For simplicity, they are referred to as clone A (A06-404), B (A06-407) and C (Af6) hereafter.

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 2nd 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 *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 *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 χ^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:

$$N_1 = \frac{p_1 + 0.5p_{1+2}}{p_1 + p_2 + p_{1+2}} \times N \qquad N_2 = \frac{p_2 + 0.5p_{1+2}}{p_1 + p_2 + p_{1+2}} \times N$$

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.

222

223 *Parasitoid size*

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225 The analysis of wasp dry masses indicated a highly significant effect of aphid clone and a
226 marginally non-significant effect of parasitoid line, but there was no significant difference in
227 mass between wasps emerging from singly or superparasitised aphids (Table 2). On average,
228 wasps were heaviest when emerging from aphid clone C (Fig. 2). There was also a significant
229 aphid clone × parasitoid line interaction, indicating that the relative sizes of wasps from the
230 three lines depended on which aphid clone they developed in. To a limited extent, these size
231 differences reflected the relative success under superparasitism. Parasitoid line 1, which was a
232 poor intra-host competitor in aphid clone C, also produced the smallest wasps in this clone
233 (Fig. 2). On the other hand, parasitoid line 3 produced by far the heaviest wasps on aphid
234 clone A, but it was not more successful than the other lines in parasitising this clone (Figs. 1
235 & 2).

236

237 **Discussion**

238

239 This study examined the outcome of superparasitism between parthenogenetic lines of the
240 parasitoid *L. fabarum* in different clones of their host, *A. f. fabae*. It showed that although
241 parasitoid genotypes appear to vary in their intra-host competitive ability, the outcome of
242 superparasitism in any given instance is difficult to predict, because it may be influenced
243 substantially by the host clone within which parasitoids compete. Thus, at least under a high
244 risk of superparasitism, the genotypic composition of hosts as well as competitors may
245 strongly influence a parasitoid's fitness. Similar to other forms of genotype × environment or
246 genotype × 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, becaused 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 als 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

297 the point that relative fitness of parasitoid genotypes in this system is determined by complex
298 interactions with their hosts. The effect that superparasitism might have on parasitoid body
299 size is somewhat difficult to predict. On one hand, the winner might have incurred costs from
300 having to compete with a conspecific and therefore emerge smaller, on the other hand, there is
301 some evidence that the growth potential is higher in superparasitised aphids because they
302 ingest more food than singly parasitised ones (Bai & Mackauer, 1992; Mackauer & Chau,
303 2001). The present study detected no significant mass difference between wasps emerging
304 from singly or superparasitised aphids, suggesting that these effects have limited importance
305 in *L. fabarum*, or else that they counteract each other.
306 In summary, this study suggests that the aphid parasitoid *L. fabarum* exhibits genetic

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317 **Acknowledgements**
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319 We thank Christoph Sandrock for advice on microsatellite analyses and Alexandre Gouskov
320 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

Bai, B. (1991) Conspecific superparasitism in two parasitoid wasps, *Aphidius ervi* Haliday and *Aphelinus asychis* Walker - Reproductive strategies influence host discrimination. *Canadian Entomologist*, **123**, 1229-1237.

Bai, B. & Mackauer, M. (1992) Influence of superparasitism on development rate and adult size in a solitary parasitoid wasp, *Aphidius ervi*. *Functional Ecology*, **6**, 302-307.

Belshaw, R. & Quicke, D.L.J. (2003) The cytogenetics of thelytoky in a predominantly asexual parasitoid wasp with covert sex. *Genome*, **46**, 170-173.

Belshaw, R., Quicke, D.L.J., Völkl, W., & Godfray, H.C.J. (1999) Molecular markers indicate rare sex in a predominantly asexual parasitoid wasp. *Evolution*, **53**, 1189-1199.

Carius, H.J., Little, T.J., & Ebert, D. (2001) Genetic variation in a host-parasite association: Potential for coevolution and frequency-dependent selection. *Evolution*, **55**, 1136-1145.

Degnan, P.H. & Moran, N.A. (2008) Evolutionary genetics of a defensive facultative symbiont of insects: exchange of toxin-encoding bacteriophage. *Molecular Ecology*, **17**, 916-929.

Fisher, R.C. (1963) Oxygen requirements and physiological suppression of supernumerary insect parasitoids. *Journal of Experimental Biology*, **40**, 531-540.

Godfray, H.C.J. (1994) Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press, Princeton.

344 Heie, O.E. (1986) The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. III. Faily
345 Aphididae: subfamily Pterocommatinae & tribe Aphidini of subfamily Aphidinae. *Fauna*
346 *Entomologica Scandinavica*, **17**, 314 pp.
347 Hubbard, S.F., Marris, G., Reynolds, A., & Rowe, G.W. (1987) Adaptive patterns in the
348 avoidance of superparasitism by solitary parasitic wasps. *Journal of Animal Ecology*, **56**,
349 387-401.
350 Janssen, A. (1989) Optimal host selection by *Drosophila* parasitoids in the field. *Functional*
351 *Ecology*, **3**, 469-479.
352 Mackauer, M. (1990). Host discrimination and larval competition in solitary endoparasitoids.
353 In Critical Issues in Biological Control (eds M. Mackauer, L.E. Ehler & J. Roland), pp. 41-
354 62. Intercept, Andover, .
355 Mackauer, M., Bai, B., Chow, A., & Danyk, T. (1992) Asymmetric larval competition
356 between two species of solitary parasitoid wasps - the influence of superparasitism.
357 *Ecological Entomology*, **17**, 233-236.
358 Mackauer, M. & Chau, A. (2001) Adaptive self superparasitism in a solitary parasitoid wasp:
359 the influence of clutch size on offspring size. *Functional Ecology*, **15**, 335-343.
360 Marris, G.C. & Casperd, J. (1996) The relationship between conspecific superparasitism and
361 the outcome of in vitro contests staged between different larval instars of the solitary
362 endoparasitoid *Venturia canescens*. *Behavioral Ecology and Sociobiology*, **39**, 61-69.
363 Maynard Smith, J. & Hoekstra, R. (1980) Polymorphism in a varied environment: how robust
364 are the models? *Genetical Research*, **37**, 45-57.
365 Niklasson, M., Tomiuk, J., & Parker, E.D. (2004) Maintenance of clonal diversity in *Dipsa*
366 *bifurcata* (Fallen, 1810) (Diptera : Lonchopteridae). I. Fluctuating seasonal selection
367 moulds long-term coexistence. *Heredity*, **93**, 62-71.

368 Oliver, K.M., Moran, N.A., & Hunter, M.S. (2005) Variation in resistance to parasitism in
369 aphids is due to symbionts not host genotype. *Proceedings of the National Academy of*
370 *Sciences of the United States of America*, **102**, 12795-12800.
371 Oliver, K.M., Russell, J.A., Moran, N.A., & Hunter, M.S. (2003) Facultative bacterial
372 symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National*
373 *Academy of Sciences of the United States of America*, **100**, 1803-1807.
374 Outreman, Y., Le Ralec, A., Plantegenest, M., Chaubet, B., & Pierre, J.S. (2001a)
375 Superparasitism limitation in an aphid parasitoid: cornicle secretion avoidance and host
376 discrimination ability. *Journal of Insect Physiology*, **47**, 339-348.
377 Outreman, Y., Le Ralec, A., Wajnberg, E., & Pierre, J.S. (2001b) Can imperfect host
378 discrimination explain partial patch exploitation in parasitoids? *Ecological Entomology*, **26**,
379 271-280.
380 R Development Core Team (2008) R: a language and environment for statistical computing.
381 http://cran.r-project.org.
382 Raymond, B., Searle, J.B., & Douglas, A.E. (2001) On the processes shaping reproductive
383 isolation in aphids of the *Aphis fabae* (Scop.) complex (Aphididae : Homoptera).
384 *Biological Journal of the Linnean Society*, **74**, 205-215.
385 Salt, G. (1961) Competition among insect parasitoids. *Symposia of the Society for*
386 *Experimental Biology*, **15**, 96-119.
387 Sandrock, C., Frauenfelder, N., Von Burg, S., & Vorburger, C. (2007) Microsatellite DNA
388 markers for the aphid parasitoid *Lysiphlebus fabarum* and their applicability to related
389 species. *Molecular Ecology Notes*, **7**, 1080-1083.
390 Seppälä, O., Karvonen, A., Valtonen, E.T., & Jokela, J. (2009) Interactions among co-
391 infecting parasite species: a mechanism maintaining genetic variation in parasites?
392 *Proceedings of the Royal Society B-Biological Sciences*, **276**, 691-697.

Starý, P. (1999) Biology and distribution of microbe-associated thelytokous populations of aphid parasitoids (Hym., Braconidae, Aphidiinae). *Journal of Applied Entomology*, **123**, 231-235.

Starý, P. (2006) Aphid Parasitoids of the Czech Republic (Hymenoptera: Braconidae, Aphidiinae). Academia, Praha.

Tétard-Jones, C., Kertesz, M.A., Gallois, P., & Preziosi, R.F. (2007) Genotype-by-genotype interactions modified by a third species in a plant-insect system. *American Naturalist*, **170**, 492-499.

van Alphen, J.J.M. & Visser, M.E. (1990) Superparasitism as an adaptive strategy for insect parasitoids. *Annual Review of Entomology*, **35**, 59-79.

van Lenteren, J.C. (1981). Host discrimination by parasitoids. In Semiochemicals, Their Role in Pest Control (eds D.A. Nordlund, R.L. Jones & W.J. Lewis), pp. 153-180. John Wiley, New York.

Visser, M.E. (1993) Adaptive self-superparasitism and conspecific superparasitism in the solitary parasitoid *Leptopilina heterotoma* (Hymenoptera, Eucoilidae). *Behavioral Ecology*, **4**, 22-28.

Vorburger, C., Sandrock, C., Gouskov, A., Castañeda, L.E., & Ferrari, J. (2009) Genotypic variation and the role of defensive endosymbionts in an all-parthenogenetic host-parasitoid interaction. *Evolution*, **63**, 1439-1450.

Weeks, A.R. & Hoffmann, A.A. (1998) Intense selection of mite clones in a heterogeneous environment. *Evolution*, **52**, 1325-1333.

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

Line 1 vs. Line 2:

	Parasitoid			
Aphid clone	Line 1	Line 2	Test 1 (H_0 = 1 : 1 ratio)	Test 2 (H_0 = equal competitors)
A	6	7	$\chi^2_1 = 0.077$, $P = 0.782$	$\chi^2_1 = 0.169$, $P = 0.681$
B	5	7	$\chi^2_1 = 0.333$, $P = 0.564$	$\chi^2_1 = 2.503$, $P = 0.114$
C	0	13	$\chi^2_1 = 13.00$, $P < 0.001$	$\chi^2_1 = 8.556$, $P = 0.003$
Overall	11	27		
Test 3 (H_0: Parasitoid success host-independent)	Fisher's exact test $P = 0.015$			

Line 1 vs. Line 3

	Parasitoid			
Aphid clone	Line 1	Line 3	Test 1 (H_0 = 1 : 1 ratio)	Test 2 (H_0 = equal competitors)
A	8	11	$\chi^2_1 = 0.474$, $P = 0.491$	$\chi^2_1 = 2.998$, $P = 0.083$
B	7	5	$\chi^2_1 = 0.333$, $P = 0.564$	$\chi^2_1 = 0.803$, $P = 0.370$
C	1	8	$\chi^2_1 = 5.444$, $P = 0.020$	$\chi^2_1 = 3.423$, $P = 0.064$
Overall	16	24		
Test 3 (H_0: Parasitoid success host-independent)	Fisher's exact test $P = 0.096$			

Line 2 vs. Line 3

	Parasitoid			
Aphid clone	Line 2	Line 3	Test 1 (H_0 = 1 : 1 ratio)	Test 2 (H_0 = equal competitors)
A	2	2	$\chi^2_1 = 0.000$, $P = 1.000$	$\chi^2_1 = 0.155$, $P = 0.693$
B	4	10	$\chi^2_1 = 2.571$, $P = 0.109$	$\chi^2_1 = 0.087$, $P = 0.768$
C	2	14	$\chi^2_1 = 9.000$, $P = 0.003$	$\chi^2_1 = 9.936$, $P = 0.002$
Overall	8	26		
Test 3 (H_0: Parasitoid success host-independent)	Fisher's exact test $P = 0.178$			

Table 2. Analysis of variance results for parasitoid dry mass.

Effect	d.f.	MS (× 1000)	*F*	*P*
Block	4	0.061	1.024	0.397
Aphid clone	2	0.430	7.269	< 0.001
Parasitoid line	2	0.165	2.792	0.065
Treatment (single vs. super)	1	0.014	0.236	0.628
Aphid clone × parasitoid line	4	0.168	2.840	0.026
Aphid clone × treatment	2	0.022	0.379	0.686
Parasitoid line × treatment	2	0.141	2.388	0.095
Aphid × parasitoid × treatment	4	0.056	0.946	0.439
Residual	148	0.059		

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*.

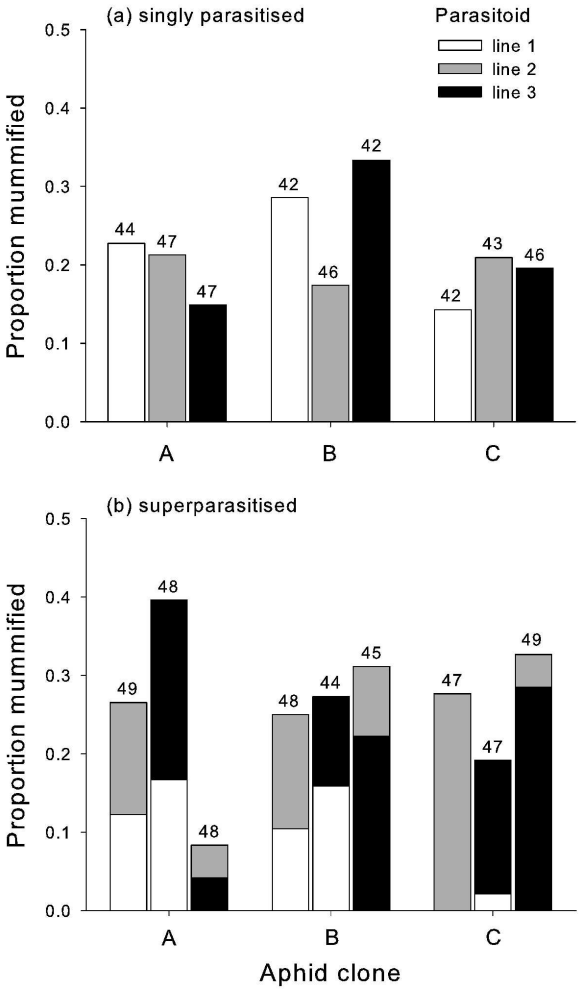

Fig. 1. Proportions of aphids mummified by each line of Lysiphlebus fabarum in (a) singly parasitised and (b) superparasitised aphids belonging to three different clones of Aphis fabae fabae. Numbers above bars indicate the number of aphids tested.
259x262mm (600 x 600 DPI)

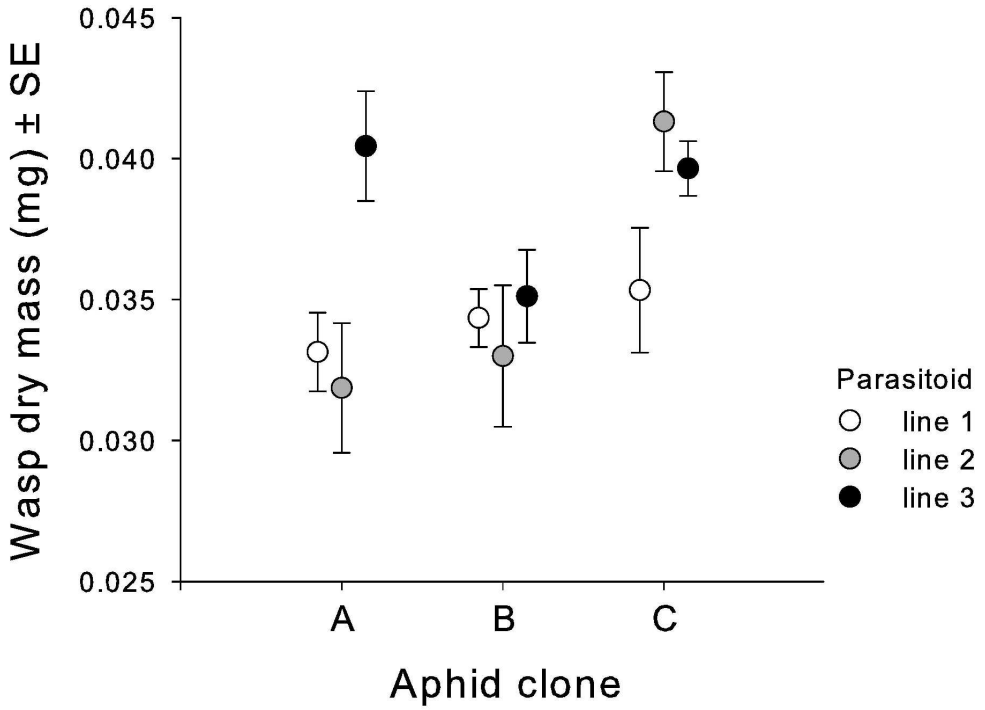

Fig. 2. Mean dry mass (± SE) of adult Lysiphlebus fabarum emerging from mummies of the three clones of Aphis fabae fabae.
158x123mm (600 x 600 DPI)