

Defensive symbionts mediate species coexistence in phytophagous insects

Corinne Hertäg^{1,2} and Christoph Vorburger^{1,2,*}

¹ Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland

² D-USYS, Department of Environmental Systems Science, ETH Zürich, Zürich, Switzerland

Article type: Research Article

Running head: Endosymbionts and species coexistence

* correspondence:

Christoph Vorburger, Eawag – Eco, Überlandstrasse 133, 8600 Dübendorf, Switzerland

christoph.vorburger@eawag.ch, phone +41 58 765 51 96

Abstract

1. Competition of two species for the same resource is expected to result in competitive exclusion of the inferior competitor. In natural communities, however, other antagonists and symbionts moderate competition. Thus we have to go beyond studying pairwise interactions.

2. Natural enemies may facilitate coexistence if they affect the superior competitor more strongly, or they can hinder coexistence via apparent competition. Less well studied is the role of symbionts, which may influence species coexistence in conjunction with enemies.

3. Eukaryotes commonly harbor microbial endosymbionts that provide protection against natural enemies, but are costly in their absence. Such defensive symbionts could thus mediate coexistence of species competing for the same resource, both in the presence and in the absence of enemies, but as yet there is little evidence for this claim.

4. We addressed this proposed role of defensive symbionts in replicated simple communities consisting of two aphid species sharing the same host plant and the same natural enemy, a parasitoid wasp. Both, one, or neither species were infected with a resistance-conferring symbiont, and they competed in the absence as well as the presence of parasitoids.

5. The symbiont had significant effects in the absence of parasitoids by lowering competitive ability especially in one species, but the effects were more dramatic in the presence of parasitoids. With both species protected by the symbiont, parasitoid densities remained low and both aphid species persisted. When neither species was protected, parasitoids drove both species to extinction. Surprisingly, the same outcome was observed when only one species was protected. The susceptible species supported high densities of parasitoids that also killed the resistant aphids via mechanisms other than parasitism, presumably by disturbing them to the point of starvation. This is an intriguing form of apparent competition.

6. Our results demonstrate an important role of defensive symbionts in insect communities through modifying species interactions. This highlights the need for experimental data when

27 studying species coexistence in competitive networks. Furthermore, the observation that a
28 susceptible host can negatively affect a resistant host via a shared parasitoid is an instructive
29 insight for biological control.

30

31 Key words: aphids, defensive symbionts, insect communities, interspecific competition,
32 parasitoids, population dynamics, resistance

33

1 | INTRODUCTION

How species relying on the same resource can coexist has been a long-standing problem in ecology (Hardin 1960). Natural enemies like predators are often implicated, but their influence can go either way. They can facilitate coexistence if they have more impact on the stronger competitor, thus reducing or equilibrating competition (predator-mediated coexistence) (Caswell 1978; van Veen, van Holland & Godfray 2005), but they can also impede coexistence if high predator densities supported by one species negatively affect the other, even if the competing species have different resource requirements (apparent competition) (Holt 1977). A yet underappreciated factor that may mediate species coexistence in conjunction with predators is infection with microbial endosymbionts, which are highly prevalent in eukaryotes, particularly in insects (Duron & Hurst 2013). Some of these endosymbionts, referred to as defensive symbionts, have evolved the ability to protect their hosts against natural enemies (White & Torres 2009; Clay 2014), although this protection often comes at a cost to the host (Oliver *et al.* 2008; Vorburger & Gouskov 2011). Based on such observations, it has been proposed that defensive symbionts may be important mediators of community structure (McLean *et al.* 2016). They have the potential to alter interspecific competition, both in the absence of natural enemies (via costs) and in their presence (via protection), but as yet there is little empirical evidence supporting this claim.

Aphids and their heritable bacterial endosymbionts represent an excellent system to address this issue. Aphids have been used extensively for the study of food web dynamics and community interactions in the field (e.g. Müller *et al.* 1999; Morris, Müller & Godfray 2001; van Veen, Brandon & Godfray 2009), as well as in a laboratory setting (e.g. Herzog, Müller & Vorburger 2007; Sanders, Sutter & van Veen 2013), and research over the last two decades has shown that aphid ecology is strongly influenced by endosymbionts (reviewed in Oliver *et al.* 2010; Oliver, Smith & Russell 2014). In addition to the obligate endosymbiont *Buchnera*

aphidicola, which supplies them with essential nutrients (Douglas 1998), aphids may harbour a variety of facultative or secondary endosymbionts (Zytynska & Weisser 2016; Guo *et al.* 2017). These occur at variable frequencies in different aphid species (Henry *et al.* 2015) and they are not strictly required for aphid survival, but they can provide their hosts with significant ecological benefits such as increased thermal tolerance or defense against natural enemies (Oliver *et al.* 2010). At least four species of facultative symbionts, namely *Hamiltonella defensa*, *Serratia symbiotica*, *Regiella insecticola* (Moran *et al.* 2005) and a symbiont referred to as X-type (Guay *et al.* 2009), include strains that increase aphid resistance to parasitoid wasps (Oliver *et al.* 2003; Vorburger, Gehrler & Rodriguez 2010; Heyworth & Ferrari 2015). Parasitoids are important natural enemies of aphids (Schmidt *et al.* 2003), hence the possession of resistance-conferring symbionts can be under strong positive selection (Herzog, Müller & Vorburger 2007; Oliver *et al.* 2008; Käch *et al.* 2017). However, this selective advantage may be lost or even reversed in the absence of parasitoids, because the possession of defensive symbionts can also entail costs to the host. For example, pea aphids (*Acyrtosiphon pisum*) infected with *H. defensa* are outcompeted by uninfected conspecifics in mixed populations (Oliver *et al.* 2008), and black bean aphids (*Aphis fabae*) infected with *H. defensa* show a reduction in lifespan and lifetime reproduction (Vorburger & Gouskov 2011).

Here we investigate the proposed role of defensive symbionts in mediating species coexistence using replicated simple communities consisting of two aphid species exploiting the same resource (host plant), both in the presence and absence of a shared parasitoid. Our results indeed demonstrate significant effects of the symbionts on interspecific competition, including indirect effects via the parasitoids. Surprisingly, symbiont-conferred resistance against co-occurring parasitoids only translated into long-term species persistence if the competing aphid species was also infected with the symbiont.

2 | MATERIALS AND METHODS

2.1 | Study system

The experimental communities consisted of broad bean plants (*Vicia faba*, var. "Fuego") as the single food resource for the two aphid species *Aphis fabae* (black bean aphid, hereafter *Aphis*) and *Myzus persicae* (green peach aphid, hereafter *Myzus*) as well as the parasitoid wasp *Aphidius colemani*, a natural enemy of both aphid species that is commonly employed for biological control of pest aphids in greenhouse crops (Boivin, Hance & Brodeur 2012). Both aphid species occur naturally on broad beans, but this plant is a more important host for *Aphis* than for the very generalist *Myzus* in the field (Blackman & Eastop 2000). Parasitoids were purchased from a commercial supplier (Andermatt Biocontrol, Grossdietwil, Switzerland). We used a single clone of each aphid species. Clone 5.3 of *Myzus* was collected in 2003 in Bacchus Marsh, Australia (Herzog, Müller & Vorburger 2007), and clone 405 of *Aphis* was collected in 2006 in St. Margrethen, Switzerland (Vorburger *et al.* 2009). We used two different sublines of each clone, i.e. a subline uninfected with any facultative, heritable endosymbionts (their natural state), and a subline experimentally infected with *Regiella insecticola* strain R5.15 (hereafter *Regiella*). These lines were designated as 5.3^{R5.15} and 405^{R5.15}, respectively. Strain R5.15 strongly increases the resistance of both aphid species to the parasitoid *A. colemani* (Vorburger, Gehrler & Rodriguez 2010).

2.2 | Cage experiment

The experiment consisted of eight treatments with six replicates per treatment. All communities contained both aphid species. There were four symbiont-aphid combinations: (i) neither species infected with *Regiella*; (ii) only *Aphis* infected with *Regiella*; (iii) only *Myzus*

112 infected with *Regiella*; and (iv) both species infected with *Regiella*. Each combination was
113 reared in the presence and in the absence of *A. colemani*. Communities were reared in 25 × 25
114 × 25 cm insect cages (BugDorm 4020F, MegaView Science, Taichung, Taiwan) in a
115 climatized room with a 16 h photoperiod at 22 °C. Due to space constraints, the experiment
116 was carried out in two series, each with three replicates per treatment. Cages were arranged on
117 three adjacent shelves in the room with one cage per treatment on every shelf in randomized
118 positions (randomized complete blocks). Each cage contained four potted broad bean plants.
119 Cages were first inoculated with 13 adult females of *Myzus* and 8 days later with 5 adult
120 females of *Aphis*. *Myzus* was given a 'head start' with more individuals because we knew it to
121 establish on the plants more slowly than *Aphis*. Another 8 days later, when both aphid species
122 had established sizeable populations, we added 10 female and 5 male *A. colemani* to all cages
123 assigned to parasitoid treatments and began the quantification of population densities, which
124 was done twice weekly for a total of 8 weeks. For this we removed one of the four plants from
125 each cage and replaced it with a fresh, 2 week-old plant. Total stem length of the old plant
126 was measured as a rough estimate of plant size, and all live individuals of both aphid species
127 were counted on this plant. In cages with parasitoids, we also counted the mummies of both
128 aphid species. Mummies are aphids that were successfully parasitized and killed by a
129 parasitoid. They are easily recognizable as inflated aphid husks containing the pupating wasp.
130 The counts divided by the plant stem length provided our estimates of aphid and mummy
131 densities for both species (individuals per cm plant stem length). After counting, the cut plant
132 was returned to the cage so that aphids could migrate to other plants and parasitoid mummies
133 could hatch. Once all of the initial plants in the cages had been replaced, the density
134 estimations continued by always removing and replacing the oldest plants, which had been in
135 the cages for 2 weeks. By the 4th week of the experiment, aphid densities in the treatments
136 without parasitoids became very high, such that the oldest plants in the cages deteriorated and
137 aphids began leaving the plants before they were counted. At this point we shifted to

harvesting one plant already after 1.5 weeks in the cage and continued the experiment with three plants per cage for all treatments.

2.3 | Statistical analyses

Aphid and mummy densities of each species were cube root-transformed to improve normality of residuals and analyzed with linear mixed models, testing for the effects of *Regiella* infection in *Aphis*, *Regiella* infection in *Myzus*, and Time (day of count), as well as their interactions. Treatments with and without parasitoids were analyzed separately. Cage was included as a random effect to account for the non-independence of successive counts from the same cage. Block was not significant for any response variable and therefore omitted. Analyses were executed with the lme4 library in R v. 3.1.3 (Bates *et al.* 2015; R Core Team 2016), and the lmerTest library was used for significance tests of fixed and random effects in the models (Kuznetsova, Brockhoff & Christensen 2015).

For cages without parasitoids, in which aphid population densities exhibited exponential growth for the first approx. 20 days of the experiment, we estimated the intrinsic rate of increase (r) per day over the first 18 days of the experiment by fitting a linear regression to the natural log-transformed density estimates from the first six counts (day 0 to 18). We compared these estimates between species, and we assessed potential influences of the symbiont using ANOVA on the r estimates of each focal species, testing for the effects of *Regiella* in the focal species, of *Regiella* in the competing species, as well as their interaction.

3 | RESULTS

When *Aphis* and *Myzus* populations developed in the absence of parasitoids, both species persisted until the end of the experiment and reached very high densities (Fig. 1A-1D).

Initially *Aphis* populations grew faster than *Myzus* populations but they began to plateau after about 20 days, whereas populations of *Myzus* grew more slowly but continued to increase throughout the experiment. The difference in population growth between the two species was supported by significantly higher estimates of the intrinsic rate of increase for *Aphis* than for *Myzus* over the first 18 days in the parasitoid-free cages (*Aphis*: 0.286 ± 0.013 ; *Myzus*: 0.137 ± 0.007 ; paired *t*-test, $t_{23} = 11.88$, $P < 0.001$). However, the development of population densities differed between treatments and was therefore influenced by *Regiella*. When neither species was infected with *Regiella*, *Myzus* densities only caught up with those of *Aphis* towards the end of the experiment (Fig. 1A), and the pattern was similar when only *Myzus* carried the symbiont (Fig. 1B). When only *Aphis* harbored *Regiella*, on the other hand, densities of *Myzus* reached those of *Aphis* after one month and began to exceed them in the second month, such that the average density of *Myzus* was 3.7 times higher than that of *Aphis* on the last count (Fig. 1C). Densities of *Myzus* overtook those of *Aphis* also when both species carried the symbiont, albeit not as early, and the density of *Myzus* was about 2-fold higher on average than that of *Aphis* on the last count (Fig. 1D). *Aphis* was thus handicapped by the infection with *Regiella*, and this effect was particularly strong when the competitor was uninfected. This was reflected by a significant (negative) main effect of *Regiella* infection on *Aphis* densities, and a significant (positive) effect of *Regiella* infection in the competitor *Myzus*, as well as a significant interaction between these effects (Table 1A). The densities of *Myzus*, on the other hand, were not affected significantly by its own infection with *Regiella*, but by the infection of *Aphis*, both as a main effect and in interaction with time, the latter seemingly reflecting a stronger increase of *Myzus* densities in the second half of the experiment in cages where *Aphis* carried *Regiella* (Table 1A; Figs. 1A-D). There was also a significant interaction between the infection status of *Aphis* and that of *Myzus* on *Myzus* densities (Table 1A). Despite its effect on the relative population densities in the different parasitoid-free treatments, there was no obvious influence of *Regiella* on the intrinsic rate of

increase (first 18 days) in either species, neither directly (*Aphis*: $F_{1,20} = 1.235$, $P = 0.280$; *Myzus*: $F_{1,20} = 0.503$, $P = 0.486$), nor indirectly via the competing species (*Aphis*: *Regiella* in *Myzus* $F_{1,20} = 0.383$, $P = 0.543$; *Regiella* in *Aphis* \times *Regiella* in *Myzus* $F_{1,20} = 0.534$, $P = 0.474$; *Myzus*: *Regiella* in *Aphis* $F_{1,20} = 0.243$, $P = 0.627$; *Regiella* in *Aphis* \times *Regiella* in *Myzus* $F_{1,20} = 0.182$, $P = 0.674$), in line with the observation that effects of *Regiella* on population densities mainly occurred at later stages of the experiment.

In the presence of parasitoids, the outcomes were very different. When neither species harbored *Regiella*, parasitoids increased rapidly over the first 30 days, as shown by the increasing number of mummies on the plants (Fig. 1E). Parasitoids eventually drove both aphid species to extinction or near-extinction (2 out of 6 cages still contained a very small number of *Myzus* at the end of the experiment), resulting in their own extinction due to a lack of hosts (Fig. 1E). When both species harbored the defensive symbiont, on the other hand, parasitism remained very low such that both aphid species persisted and increased over the course of the experiment, although their densities remained lower than in the treatments without parasitoids (Fig. 1H). The high resistance of aphids even resulted in the complete extinction of parasitoids in 3 of the 6 replicate communities. When only one aphid species carried an infection with *Regiella*, we expected a selective advantage for the infected species. Resistance conferred by *Regiella* against parasitoids was indeed very strong, as evidenced by the very small number of mummies forming in the protected species (Figs. 1F & 1G), and supported by a significant main effect of *Regiella* infection as well as a significant *Regiella* \times Time interaction on the densities of mummies in both species (Table 1C). However, this only delayed the decline relative to when the species were unprotected. Eventually, parasitoids decimated both species until they were completely or nearly extinct (Figs. 1F & 1G), which also resulted in the loss of parasitoids from the communities in the majority of cases (very few parasitoids were still present in three cages with protected *Aphis* and unprotected *Myzus* at the end of the experiment). The benefit of *Regiella*-conferred protection was thus contingent on

the other aphid species also being protected. Accordingly, the analyses showed that each focal species' population density over time was influenced by *Regiella* infection in both aphid species, i.e. there were highly significant *Regiella* in *Aphis* \times Time, *Regiella* in *Myzus* \times Time and *Regiella* in *Aphis* \times *Regiella* in *Myzus* \times Time interactions on the density of both species (Table 1B). The symbiont's effects are mainly expressed as interactions with time because both species' temporal trajectories of population densities were totally different when they were infected, but only when the other species was infected as well (steady increase vs. growth and collapse: Fig. 1H vs. Figs 1E-G).

4 | DISCUSSION

Competition and predation act simultaneously in natural communities (Gurevitch, Morrison & Hedges 2000; Chase *et al.* 2002), and studies on phytophagous insects provide excellent examples to illustrate that direct as well as indirect effects via predators have to be considered to understand species coexistence (reviewed in van Veen, Morris & Godfray 2006; van Veen & Godfray 2012). More recent is the realization that microbial endosymbionts of insects could play an important role in these food webs. It has been suggested that they can be regarded as "communities affecting communities" (Ferrari & Vavre 2011), because such symbionts have the potential to modify species interactions (McLean *et al.* 2016). Using replicated simple communities supported by a single resource, we could show that a defensive endosymbiont indeed affects the interaction of two aphid species, both in the presence and in the absence of the natural enemy.

Predation is most likely to aid species coexistence if there is a trade-off between competitive ability and susceptibility to the predator. Although infection with *R. insecticola* did induce a trade-off – resistance to *A. colemani* came at the cost of a slightly reduced competitive ability – the outcome of our treatments including parasitoids was dominated by

other effects. Most striking was that in both species, protection against parasitoids only resulted in long-term persistence when the competitor harbored the protective symbiont as well. Under these conditions, the high level of resistance in both species kept parasitoid densities very low or even resulted in parasitoid extinction - an effect comparable to herd immunity (Anderson & May 1985). Experimental evolution studies in the laboratory have shown that given enough time and a conducive experimental setup, aphid parasitoids have the potential to adapt to the presence of defensive symbionts in their aphid hosts (Dion *et al.* 2011; Rouchet & Vorburger 2014; Dennis *et al.* 2017). Under the conditions of the present experiment, adaptation did not occur fast enough for parasitoids to maintain themselves.

When only one aphid species was protected by the symbiont, we observed an intriguing form of apparent competition. The unprotected species supported such high densities of parasitoids that they were able to suppress the protected species as well. In pea aphids (*A. pisum*) with another protective symbiont, *H. defensa*, parasitoids are sometimes able to overcome the symbiont-conferred resistance by multiple oviposition into the same aphid (Oliver *et al.* 2012), as would occur under high parasitoid densities. However, the low number of mummies from protected hosts in the mixed populations make this an unlikely scenario in the present case (Figs. 1F, G). Parasitoids must have affected the resistant aphids via mechanisms other than parasitism, either by disturbing them to the point of starvation or by stabbing-induced injuries. It is possible that the strong negative effect of parasitoids on resistant aphids was a consequence of studying these communities in a closed experimental system. The aphids could not move away to avoid attack and disturbance by parasitoids, and such high densities of parasitoids may not build up in the field because parasitoids would also disperse to avoid competition. The selective advantage of the species possessing the defensive symbiont could thus have been curbed by the confinement in cages. That said, the observation that high parasitoid densities can also control resistant, symbiont-protected aphids could nevertheless be important in the context of crop protection. Inoculative or inundative releases

of parasitoids normally take place in protected crops (Boivin, Hance & Brodeur 2012). These are also confined systems, albeit of a larger scale, and there are concerns that symbiont-conferred resistance could compromise biological control of pest aphids in greenhouses (Vorburger 2017). If sufficiently high densities of parasitoids can also control symbiont-protected aphids, this could be exploited, for example by using banker plant systems containing an unprotected non-pest aphid to sustain high numbers of a shared parasitoid (Frank 2010).

In our simple food web with two hosts sharing the same parasitoid, the increased resistance ultimately did not translate into a competitive edge for the symbiont-protected species because both species were extirpated by the parasitoids. We do not think that this outcome can be generalized. Apart from the above caveat that this outcome could be an artefact of using closed systems, we believe that the effect of protection will also depend on the topology of the food web. This is illustrated by an interesting study by Sanders et al. (2016), which also took place in closed experimental cages. They studied communities of three aphid species in which each aphid species had its own, specialized parasitoid. In those communities, introducing a defensive symbiont to one species did provide a competitive advantage. The protected species escaped control by its parasitoid and hence outcompeted the other aphid species on the same host plant, resulting in a cascade of extinctions of the competing species and their parasitoids (Sanders *et al.* 2016).

The defensive symbiont in our communities also affected the species interaction in the absence of parasitoids. Interestingly, the symbiont's effect on the focal species was again contingent on the infection status of the competitor, although less dramatically than in the treatments with parasitoids. The more specialized broad bean feeder *Aphis* had the higher growth rate initially, while the generalist *Myzus* coped better with the crowded conditions developing in cages without parasitoids, such that population densities caught up with those of *Aphis* toward the end of the experiment. When *Aphis* harbored *Regiella*, *Myzus* even began

to outcompete the other species, suggesting that *Aphis* is handicapped by the possession of *Regiella*, even though *Regiella* had no obvious effect on the initial growth rate. Infection with *Regiella* may thus become more of a liability when plants get very crowded. The symbiont appears to be a weak liability for *Myzus* as well, since it outcompeted *Aphis* more quickly when uninfected. That *Aphis* is more affected than *Myzus* could be related to the fact that *Regiella* strain R5.15 was originally discovered in another clone of *M. persicae* (Vorburger, Gehrler & Rodriguez 2010), although *Regiella* occurs naturally in *A. fabae* (Vorburger *et al.* 2009; Henry *et al.* 2015), and strain R5.15 is fully heritable and functional in *A. fabae* as well.

To conclude, we demonstrated strong direct and indirect effects of a defensive symbiont on species interactions in simple communities of aphids, both in the presence and absence of their natural enemy. These results indicate that in natural communities, where a significant proportion of many aphid species is infected (Henry *et al.* 2015; Zytynska & Weisser 2016), such invisible passengers can be important mediators of species coexistence. This conclusion is by no means restricted to aphids. Endosymbionts are best studied in arthropods, for which recent estimates suggest that the vast majority harbors bacterial endosymbionts (Duron & Hurst 2013), but this may well apply to other animals as well (McFall-Ngai *et al.* 2013). The number of endosymbionts that can be linked to protection against natural enemies has been increasing rapidly over the last years (Florez *et al.* 2015), suggesting that defensive symbioses are a common phenomenon. Since defensive symbionts tend to be facultative associates of their hosts, populations are often just partially infected and infection prevalence can differ among populations (e.g. Hansen *et al.* 2007; Vorburger & Rouchet 2016). This is an important consideration for food web studies. The experiment by Sanders *et al.* (2016) has shown impressively that if just a single link in a complex food web is weakened by the presence of a defensive symbiont, a complete change in the community structure can result, including the extinction of species. Knowledge of the presence or absence of defensive

319 symbionts could thus be crucial for understanding predation- and competition-mediated
320 effects in food webs.

322 **ACKNOWLEDGMENTS**

323
324 We are grateful to P. Rodriguez for the skillful maintenance of insect stocks used in this
325 experiment, and we thank the Associate Editor A. Biere and the reviewers for their
326 constructive comments on our manuscript. This research was supported by the Swiss National
327 Science Foundation (SNSF Professorship nr. PP00P3_146341 and Sinergia grant nr.
328 CRSII3_154396 to C.V.).

330 **AUTHOR CONTRIBUTIONS**

331
332 C.H. and C.V. designed the study, C.H. carried out the experiment, C.V. and C.H. analyzed
333 the data and wrote the manuscript.

335 **DATA ACCESSIBILITY**

336
337 Data deposited in the Dryad Digital Repository: <http://doi.org/10.5061/dryad.pm620>, (Hertäg
338 & Vorburger 2018).

340 **REFERENCES**

341
342 Anderson, R.M. & May, R.M. (1985) Vaccination and herd immunity to infectious diseases.
343 *Nature*, 318, 323-329.

344 Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models
 345 using lme4. *Journal of Statistical Software*, 67, 1-48.

346 Blackman, R.L. & Eastop, V.F. (2000) *Aphids on the World's Crops: An Identification and*
 347 *Information Guide*, 2nd edn. John Wiley and Sons, Chichester.

348 Boivin, G., Hance, T. & Brodeur, J. (2012) Aphid parasitoids in biological control. *Canadian*
 349 *Journal of Plant Science*, 92, 1-12.

350 Caswell, H. (1978) Predator-mediated coexistence - non-equilibrium model. *American*
 351 *Naturalist*, 112, 127-154.

352 Chase, J.M., Abrams, P.A., Grover, J.P., Diehl, S., Chesson, P., Holt, R.D., Richards, S.A.,
 353 Nisbet, R.M. & Case, T.J. (2002) The interaction between predation and competition: a
 354 review and synthesis. *Ecology Letters*, 5, 302-315.

355 Clay, K. (2014) Defensive symbiosis: a microbial perspective. *Functional Ecology*, 28, 293-
 356 298.

357 Dennis, A.B., Patel, V., Oliver, K.M. & Vorburger, C. (2017) Parasitoid gene expression
 358 changes after adaptation to symbiont-protected hosts. *Evolution*, 71, 2599-2617.

359 Dion, E., Zele, F., Simon, J.C. & Outreman, Y. (2011) Rapid evolution of parasitoids when
 360 faced with the symbiont-mediated resistance of their hosts. *Journal of Evolutionary*
 361 *Biology*, 24, 741-750.

362 Douglas, A.E. (1998) Nutritional interactions in insect-microbial symbioses: Aphids and their
 363 symbiotic bacteria *Buchnera*. *Annual Review of Entomology*, 43, 17-37.

364 Duron, O. & Hurst, G.D.D. (2013) Arthropods and inherited bacteria: from counting the
 365 symbionts to understanding how symbionts count. *BMC Biology*, 11, 45.

366 Ferrari, J. & Vavre, F. (2011) Bacterial symbionts in insects or the story of communities
 367 affecting communities. *Philosophical Transactions of the Royal Society B-Biological*
 368 *Sciences*, 366, 1389-1400.

369 Florez, L.V., Biedermann, P.H.W., Engl, T. & Kaltenpoth, M. (2015) Defensive symbioses of
 370 animals with prokaryotic and eukaryotic microorganisms. *Natural Product Reports*, 32,
 371 904-936.

372 Frank, S.D. (2010) Biological control of arthropod pests using banker plant systems: Past
 373 progress and future directions. *Biological Control*, 52, 8-16.

374 Guay, J.F., Boudreault, S., Michaud, D. & Cloutier, C. (2009) Impact of environmental stress
 375 on aphid clonal resistance to parasitoids: Role of *Hamiltonella defensa* bacterial symbiosis
 376 in association with a new facultative symbiont of the pea aphid. *Journal of Insect*
 377 *Physiology*, 55, 919-926.

378 Guo, J., Hatt, S., He, K., Chen, J., Francis, F. & Wang, Z. (2017) Nine facultative
 379 endosymbionts in aphids. A review. *Journal of Asia-Pacific Entomology*, 20, 794-801.

380 Gurevitch, J., Morrison, J.A. & Hedges, L.V. (2000) The interaction between competition and
 381 predation: A meta-analysis of field experiments. *American Naturalist*, 155, 435-453.

382 Hansen, A.K., Jeong, G., Paine, T.D. & Stouthamer, R. (2007) Frequency of secondary
 383 symbiont infection in an invasive psyllid relates to parasitism pressure on a geographic
 384 scale in California. *Applied and Environmental Microbiology*, 73, 7531-7535.

385 Hardin, G. (1960) The competitive exclusion principle. *Science*, 131, 1292-1297.

386 Henry, L.M., Maiden, M.C.J., Ferrari, J. & Godfray, H.C.J. (2015) Insect life history and the
 387 evolution of bacterial mutualism. *Ecology Letters*, 18, 516-525.

388 Hertäg, C. & Vorburger, C. (2018) Data from: Defensive symbionts mediate species
 389 coexistence in phytophagous insects. Dryad Digital Repository.
 390 <http://doi.org/10.5061/dryad.pm620>.

391 Herzog, J., Müller, C.B. & Vorburger, C. (2007) Strong parasitoid-mediated selection in
 392 experimental populations of aphids. *Biology Letters*, 3, 667-669.

393 Heyworth, E.R. & Ferrari, J. (2015) A facultative endosymbiont in aphids can provide diverse
 394 ecological benefits. *Journal of Evolutionary Biology*, 28, 1753-1760.

395 Holt, R.D. (1977) Predation, apparent competition, and structure of prey communities.
 396 *Theoretical Population Biology*, 12, 197-229.

397 Käch, H., Mathé-Hubert, H., Dennis, A.B. & Vorburger, C. (2017) Rapid evolution of
 398 symbiont-mediated resistance compromises biological control of aphids by parasitoids.
 399 *Evolutionary Applications*, in press.

400 Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2015) lmerTest: Tests in Linear
 401 Mixed Effects Models. R package version 2.0-29. [http://CRAN.R-](http://CRAN.R-project.org/package=lmerTest)
 402 [project.org/package=lmerTest](http://CRAN.R-project.org/package=lmerTest).

403 McFall-Ngai, M., Hadfield, M.G., Bosch, T.C.G., Carey, H.V., Domazet-Loso, T., Douglas,
 404 A.E., Dubilier, N., Eberl, G., Fukami, T., Gilbert, S.F., Hentschel, U., King, N., Kjelleberg,
 405 S., Knoll, A.H., Kremer, N., Mazmanian, S.K., Metcalf, J.L., Nealson, K., Pierce, N.E.,
 406 Rawls, J.F., Reid, A., Ruby, E.G., Rumpho, M., Sanders, J.G., Tautz, D. & Wernegreen,
 407 J.J. (2013) Animals in a bacterial world, a new imperative for the life sciences.
 408 *Proceedings of the National Academy of Sciences of the United States of America*, 110,
 409 3229-3236.

410 McLean, A.H.C., Parker, B.J., Hrčák, J., Henry, L.M. & Godfray, H.C.J. (2016) Insect
 411 symbionts in food webs. *Philosophical Transactions of the Royal Society B-Biological*
 412 *Sciences*, 371.

413 Moran, N.A., Russell, J.A., Koga, R. & Fukatsu, T. (2005) Evolutionary relationships of three
 414 new species of *Enterobacteriaceae* living as symbionts of aphids and other insects. *Applied*
 415 *and Environmental Microbiology*, 71, 3302-3310.

416 Morris, R.J., Müller, C.B. & Godfray, H.C.J. (2001) Field experiments testing for apparent
 417 competition between primary parasitoids mediated by secondary parasitoids. *Journal of*
 418 *Animal Ecology*, 70, 301-309.

419 Müller, C.B., Adriaanse, I.C.T., Belshaw, R. & Godfray, H.C.J. (1999) The structure of an
 420 aphid-parasitoid community. *Journal of Animal Ecology*, 68, 346-370.

421 Oliver, K.M., Campos, J., Moran, N.A. & Hunter, M.S. (2008) Population dynamics of
 422 defensive symbionts in aphids. *Proceedings of the Royal Society B-Biological Sciences*,
 423 275, 293-299.

424 Oliver, K.M., Degnan, P.H., Burke, G.R. & Moran, N.A. (2010) Facultative symbionts in
 425 aphids and the horizontal transfer of ecologically important traits. *Annual Review of*
 426 *Entomology*, 55, 247-266.

427 Oliver, K.M., Noge, K., Huang, E.M., Campos, J.M., Becerra, J.X. & Hunter, M.S. (2012)
 428 Parasitic wasp responses to symbiont-based defense in aphids. *BMC Biology*, 10, 11.

429 Oliver, K.M., Russell, J.A., Moran, N.A. & Hunter, M.S. (2003) Facultative bacterial
 430 symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National*
 431 *Academy of Sciences of the United States of America*, 100, 1803-1807.

432 Oliver, K.M., Smith, A.H. & Russell, J.A. (2014) Defensive symbiosis in the real world -
 433 advancing ecological studies of heritable, protective bacteria in aphids and beyond.
 434 *Functional Ecology*, 28, 341-355.

435 R Core Team (2016) R: A language and environment for statistical computing. R Foundation
 436 for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org>.

437 Rouchet, R. & Vorburger, C. (2014) Experimental evolution of parasitoid infectivity on
 438 symbiont-protected hosts leads to the emergence of genotype-specificity. *Evolution*, 68,
 439 1607-1616.

440 Sanders, D., Kehoe, R., van Veen, F.J.F., McLean, A., Godfray, H.C.J., Dicke, M., Gols, R. &
 441 Frago, E. (2016) Defensive insect symbiont leads to cascading extinctions and community
 442 collapse. *Ecology Letters*, 19, 789-799.

443 Sanders, D., Sutter, L. & van Veen, F.J.F. (2013) The loss of indirect interactions leads to
 444 cascading extinctions of carnivores. *Ecology Letters*, 16, 664-669.

445 Schmidt, M.H., Lauer, A., Purtauf, T., Thies, C., Schaefer, M. & Tschamntke, T. (2003)
 446 Relative importance of predators and parasitoids for cereal aphid control. *Proceedings of*
 447 *the Royal Society of London Series B-Biological Sciences*, 270, 1905-1909.

448 van Veen, F.J.F., Brandon, C.E. & Godfray, H.C.J. (2009) A positive trait-mediated indirect
 449 effect involving the natural enemies of competing herbivores. *Oecologia*, 160, 195-205.

450 van Veen, F.J.F. & Godfray, H.C. (2012) Consequences of trait changes in host–parasitoid
 451 interactions in insect communities. *Trait-Mediated Indirect Interactions: Ecological and*
 452 *Evolutionary Perspectives* (eds O. Schmitz, R.D. Holt & T. Ohgushi), pp. 28-46.
 453 Cambridge University Press, Cambridge.

454 van Veen, F.J.F., Morris, R.J. & Godfray, H.C.J. (2006) Apparent competition, quantitative
 455 food webs, and the structure of phytophagous insect communities. *Annual Review of*
 456 *Entomology*, 51, 187-208.

457 van Veen, F.J.F., van Holland, P.D. & Godfray, H.C.J. (2005) Stable coexistence in insect
 458 communities due to density- and trait-mediated indirect effects. *Ecology*, 86, 3182-3189.

459 Vorburger, C. (2017) Symbiont-conferred resistance to parasitoids in aphids – challenges for
 460 biological control. *Biological Control*, In press (available online:
 461 <http://dx.doi.org/10.1016/j.biocontrol.2017.1002.1004>).

462 Vorburger, C., Gehrer, L. & Rodriguez, P. (2010) A strain of the bacterial symbiont *Regiella*
 463 *insecticola* protects aphids against parasitoids. *Biology Letters*, 6, 109-111.

464 Vorburger, C. & Gouskov, A. (2011) Only helpful when required: A longevity cost of
 465 harbouring defensive symbionts. *Journal of Evolutionary Biology*, 24, 1611-1617.

466 Vorburger, C. & Rouchet, R. (2016) Are aphid parasitoids locally adapted to the prevalence
 467 of defensive symbionts in their hosts? *BMC Evolutionary Biology*, 16, 271.

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

471 White, J.F. & Torres, M.S. (2009) Defensive Mutualism in Microbial Symbiosis. CRC Press,
472 Boca Raton FL.

473 Zytynska, S.E. & Weisser, W.W. (2016) The natural occurrence of secondary bacterial
474 symbionts in aphids. *Ecological Entomology*, 41, 13-26.

475

476

Figure caption

Fig. 1. Population dynamics in experimental communities

Plots illustrating the temporal trajectories of population density estimates from caged communities of two aphid species (*Aphis fabae* and *Myzus persicae*) with and without the defensive endosymbiont *Regiella insecticola* (red circles) either in the absence (A-D) or in the presence of parasitoids (E-H). Plot labels A-H correspond to treatments detailed in Table 1. For treatments with parasitoids, the temporal dynamics of parasitism (mummy formation) are illustrated as well. Values depict means of six replicate cages \pm 1 SE. Note that the y-axis is on a logarithmic scale.

Table 1. Results of linear mixed models testing for the effects of *Regiella insecticola* infection in each of the competing aphid species as well as time (day of count) on (A) the population densities of *Aphis fabae* and *Myzus persicae* (individuals per cm plant stem length) in the absence of parasitoids, (B) the population densities of *A. fabae* and *M. persicae* in the presence of parasitoids, and (C) the densities of *A. fabae* and *M. persicae* mummies (successfully parasitized aphids) in the presence of parasitoids. Densities were cube root-transformed before analysis. *P* values of fixed effects are based on *F* tests with Satterthwaite’s approximation carried out with the lmerTest library in R (Kuznetsova, Brockhoff & Christensen 2015). Cage was included as a random effect in all models.

Effect	ndf, ddf	A: Parasitoids absent: Aphids			B: Parasitoids present: Aphids			C: Parasitoids present: Mummies		
		MS	<i>F</i>	<i>P</i>	MS	<i>F</i>	<i>P</i>	MS	<i>F</i>	<i>P</i>
<i>Aphis fabae</i>										
<i>Regiella</i> in <i>A. fabae</i> (RegA)	1, 20	4.839	6.186	0.022	1.368	2.984	0.100	1.349	7.644	0.012
<i>Regiella</i> in <i>M. persicae</i> (RegM)	1, 20	4.009	5.124	0.035	1.316	2.870	0.106	0.107	0.061	0.808
Time (day)	16, 320	31.994	40.897	< 0.001	7.038	15.347	< 0.001	2.111	11.961	< 0.001
RegA × RegM	1, 20	9.009	11.516	0.003	2.337	5.097	0.035	0.059	0.336	0.569
RegA × Time	16, 320	0.958	1.225	0.247	2.985	6.508	< 0.001	0.972	5.509	< 0.001
RegM × Time	16, 320	0.589	0.753	0.738	1.712	3.733	< 0.001	0.117	0.665	0.828
RegA × RegM × Time	16, 320	0.846	1.082	0.371	1.028	2.242	0.004	0.037	0.209	0.999
<i>Myzus persicae</i>										
<i>Regiella</i> in <i>A. fabae</i> (RegA)	1, 20	10.885	12.939	0.002	0.341	1.099	0.307	0.000	0.001	0.983
<i>Regiella</i> in <i>M. persicae</i> (RegM)	1, 20	1.786	2.123	0.161	0.762	2.457	0.133	2.219	29.842	< 0.001
Time (day)	16, 320	21.563	25.513	< 0.001	3.527	11.373	< 0.001	1.371	18.443	< 0.001
RegA × RegM	1, 20	4.992	5.934	0.024	0.358	1.155	0.295	0.021	0.280	0.603
RegA × Time	16, 320	2.423	2.880	< 0.001	1.487	4.797	< 0.001	0.042	0.561	0.912
RegM × Time	16, 320	0.611	0.727	0.766	2.422	7.811	< 0.001	0.802	10.786	< 0.001
RegA × RegM × Time	16, 320	0.748	0.889	0.582	0.752	2.424	0.002	0.022	0.292	0.997

Figure 1

