

Special issue: Symbionts in insect biology and pest control

Estimating costs of aphid resistance to parasitoids conferred by a protective strain of the bacterial endosymbiont *Regiella insecticola*

Anine R. Jamin^{1,2} & Christoph Vorburger^{1,2*}

¹Aquatic Ecology, Eawag, Überlandstrasse 133, PO Box 611, 8600 Dübendorf, Switzerland, and ²Institute of Integrative Biology, ETH Zürich, Universitätstrasse 16, 8092 Zürich, Switzerland

***Correspondence:** Christoph Vorburger, Aquatic Ecology, Eawag, Überlandstrasse 133, PO Box 611, 8600 Dübendorf, Switzerland. E-mail: christoph.vorburger@eawag.ch

Running title: *Costs of symbiont-conferred resistance*

Key words: *Acyrtosiphon pisum*, *Aphis fabae*, cost of resistance, defensive symbiosis, *Hamiltonella defensa*, lifespan, *Myzus persicae*, reproduction, Hemiptera, Aphididae, black bean aphid, pea aphid, green peach aphid

Accepted: 14 September 2018

Abstract

Heritable bacterial endosymbionts are common in aphids (Hemiptera: Aphididae), and they can influence ecologically important traits of their hosts. It is generally assumed that their persistence in a population is dependent on a balance between the costs and benefits they confer. A good example is *Hamiltonella defensa* Moran et al., a facultative symbiont that provides a benefit by strongly increasing aphid resistance to parasitoid wasps, but becomes costly to the host in the absence of parasitoids. *Regiella insecticola* Moran et al. is another common symbiont of aphids and generally does not influence resistance to parasitoids. In the green peach aphid, *Myzus persicae* (Sulzer), however, one strain (R5.15) was discovered that behaves like *H. defensa* in that it provides strong protection against parasitoid wasps. Here we compare R5.15-infected and uninfected lines of three *M. persicae* clones to test whether this protective symbiont is costly as well, i.e., whether it has any negative effects on aphid life-history traits. Furthermore, we transferred R5.15 to two other aphid species, the pea aphid, *Acyrtosiphon pisum* (Harris), and the black bean aphid, *Aphis fabae* Scopoli, where this strain is also protective against parasitoids and where we could compare its effects with those of additional, non-protective strains of *R. insecticola*. Negative effects of R5.15 on host survival and lifetime reproduction were limited and frequently non-significant, and these effects were comparable or in one case weaker than those of *R. insecticola* strains that are not protective against parasitoid wasps. Unless the benefit of protection is counteracted by detrimental effects on traits that were not considered in this study, *R. insecticola* strain R5.15 should have a high potential to spread in aphid populations.

Abbreviated abstract (2-3 sentences, max. 80 words)

Heritable bacterial endosymbionts are common in aphids (Hemiptera: Aphididae). Their spread and persistence in host populations will depend on the net effect of costs and benefits they confer. Here we show that the strong protection against parasitoids provided by an unusual strain of the facultative symbiont *Regiella insecticola* comes at limited costs to the host, suggesting that this strain has a high potential for spreading in aphid populations. [68 words]

Graphic for Table of Contents

TOC_graphic

1 Introduction

2 Insects are frequently infected with heritable bacterial endosymbionts (Baumann, 2005;
3 Duron et al., 2008; Feldhaar, 2011). Some insect-bacteria associations have evolved to the
4 point of mutual dependence, such that neither the host nor its symbiont are viable
5 independently (obligate endosymbionts; e.g., Akman et al., 2002; Tamas et al., 2002; Wu et
6 al., 2006), whereas other heritable endosymbionts are facultative, such that the host is also
7 viable in their absence (Moran et al., 2008; Ferrari & Vavre, 2011). Aphids (Hemiptera:
8 Aphididae) are an excellent model for the study of bacterial endosymbionts. Nearly all
9 species harbour the obligate endosymbiont *Buchnera aphidicola* Munson et al., which
10 supplies nutrients that are scarce in their phloem sap diet (Douglas, 1998), and aphids
11 commonly carry additional infections with facultative endosymbionts able to provide a
12 variety of ecological benefits (Oliver et al., 2010; Guo et al., 2017). Just like *B. aphidicola*,
13 facultative endosymbionts are transmitted vertically by exo-/endocytotic transport from
14 maternal cells (Koga et al., 2012; Michalik et al., 2014), i.e., they show transovarial
15 transmission. Of particular interest are so-called defensive symbionts, which have evolved
16 the ability to protect their hosts against natural enemies, thereby promoting their own
17 persistence (Oliver et al., 2014). The prime example is *Hamiltonella defensa* Moran et al.
18 (Moran et al., 2005b), which strongly increases aphid resistance to parasitoid wasps (Oliver et
19 al., 2003; Schmid et al., 2012; Asplen et al., 2014). Despite providing this strong benefit, *H.*
20 *defensa* is not fixed in aphid populations and tends to occur at intermediate frequencies in
21 natural aphid populations (e.g., Smith et al., 2015; Vorburger & Rouchet, 2016; Rock et al.,
22 2018). This suggests that the benefit of protection might be balanced by costs associated with
23 the possession of *H. defensa*, which is supported by empirical evidence. Pea aphids,
24 *Acyrtosiphon pisum* (Harris), as well as cowpea aphids, *Aphis craccivora* Koch, infected
25 with *H. defensa* are competitively inferior to uninfected aphids of the same clones (Oliver et
26 al., 2008; Dykstra et al., 2014). In the black bean aphid, *Aphis fabae* Scopoli, *H. defensa*
27 reduces lifespan and lifetime reproduction (Vorburger & Gouskov, 2011), suggesting that
28 there is a high cost for aphids of harbouring *H. defensa* in the absence of parasitoids
29 (Vorburger et al., 2013).

30 Another abundant facultative symbiont of aphids is *Regiella insecticola* Moran et al.
31 (Moran et al., 2005b), which occurs in a wide variety of aphid species (Henry et al., 2015).
32 The phenotypic effects of this endosymbiont have mainly been investigated in pea aphids,
33 where it consistently provides protection against the fungal pathogen *Pandora neoaphidis*

(Remaudiere & Hennebert) Humber (Entomophthoraceae) (Scarborough et al., 2005; Łukasik et al., 2013; Parker et al., 2017), but where there is no clear evidence for protection against parasitoid wasps (Oliver et al., 2003; Ferrari et al., 2004; Nyabuga et al., 2010). In the green peach aphid, *Myzus persicae* (Sulzer), on the other hand, a strain of *R. insecticola* has been discovered that strongly increases resistance to parasitoid wasps (Herzog et al., 2007; von Burg et al., 2008; Vorburger et al., 2010). This strain, referred to as R5.15, could be transferred to black bean aphids and pea aphids by microinjection, and it retained its capacity to protect against parasitoids in the new hosts (Vorburger et al., 2010; Hansen et al., 2012). In both of these species, *R. insecticola* also occurs naturally, but no parasitoid-protective strains have yet been reported (Oliver et al., 2003, 2014; Vorburger et al., 2009). Although it 'behaves' virtually like *H. defensa*, the protection provided by *R. insecticola* strain R5.15 appears to rely on different mechanisms. In *H. defensa*, the resistance conferred against parasitoids is contingent on the presence of a toxin-encoding bacteriophage called APSE in the symbiont's genome (Oliver et al., 2009), but this bacteriophage is not present in the genome of *R. insecticola* strain R5.15 (Hansen et al., 2012).

Only a few studies have addressed potential costs of infection with *R. insecticola*. The limited data available suggest that strains protecting against fungal pathogens in pea aphids tend to reduce host survival in the absence of the pathogen (Parker et al., 2017; McLean et al., 2018). Unfortunately, similar data are not available for the strain protecting against parasitoids. We have therefore estimated the effects of *R. insecticola* R5.15 on survival and other components of fitness in six aphid clones belonging to three species and, in the species where this was possible, we compared these effects with those imposed by other strains of *R. insecticola* that are not protective against parasitoids. Our experiment revealed only moderate costs of infection with R5.15 under laboratory conditions, and these costs – if significant at all – were comparable or in one case even weaker than those imposed by other strains of *R. insecticola*.

Materials and methods

Aphid lines

The 15 aphid lines used in the experiment (Table 1) included *M. persicae* clone 5.15, which was collected in 2003 in Bacchus Marsh, Australia, and in which *R. insecticola* strain R5.15 was originally discovered (Herzog et al., 2007; von Burg et al., 2008). We further used a line of the same clone from which *R. insecticola* was eliminated with antibiotics, and five lines of

previously uninfected aphid clones from three species (two *M. persicae*, one *A. fabae*, two *Ac. pisum*) that had been infected artificially with R5.15 by microinjection, as well as their uninfected counterparts for comparison. These five clones had been checked previously to not carry any other facultative endosymbionts (von Burg et al., 2008; Vorburger et al., 2009; Hansen et al., 2012). Finally, for the *A. fabae* and *Ac. pisum* clones, we also included one line each that was artificially infected with another, non-protective strain of *R. insecticola*, obtained from the same species as the recipient clone. No such lines were available for the *M. persicae* clones. Facultative endosymbionts appear to be generally rare in this species (Henry et al., 2015), and we are not aware of any other phenotypically characterized strain of *R. insecticola* from *M. persicae*.

Most of the aphid lines used here have been used in previous studies (Vorburger et al., 2010; Hansen et al., 2012), and in all cases the manipulations of the facultative symbionts (antibiotic curing or transfection by microinjection) took place at least 150 generations prior to this experiment. Since then they have stably maintained their infection status in laboratory culture, and the protective phenotype of *R. insecticola* strain R5.15 remained stable as well, as evidenced in a recent experiment (Hertäg & Vorburger, 2018). Nevertheless, the presence or absence of *R. insecticola* in each line was re-confirmed prior to the experiment using diagnostic PCR with primers and cycling conditions as described in Ferrari et al. (2012).

Experimental procedures

We measured four life-history traits in all aphid lines, including development time (time from birth to adult ecdysis), adult mass, lifespan, and lifetime reproduction. The experiment was set up in a randomized complete block design with 10 replicates per line, such that one replicate of each line was placed on randomly assigned positions within each of 10 plastic trays (blocks). Aphids grew in an E-36L plant growth chamber (Percival Scientific Perry, IA, USA) at 17.5 °C and L16:D8 photoperiod, on seedlings of their respective host plants, i.e., radish, *Raphanus raphanistrum* ssp. *sativus* (L.) Domin (Brassicaceae), for *M. persicae* and broad bean, *Vicia faba* L. (Fabaceae), for *A. fabae* and *Ac. pisum*. Plants were grown in 0.07-l plastic pots in commercial seed raising mixture (H1 substrate; Tref, Moerdijk, The Netherlands) and covered with cages made from clear plastic cylinders and fine gauze to prevent aphid escape. To avoid that environmental maternal effects carried over from the laboratory stock culture could influence our measurements, we first reared all replicates for one generation before measuring the life-history traits in the second generation. To start the test generation, five adult aphids from the first generation were transferred to a fresh plant.

After 4 h, the adults and all but one of their newly born offspring were removed. After 7 days we began to check these focal individuals daily to record the date of their final moult (adult ecdysis). The newly moulted adults were weighed to the nearest microgram on an MX5 microbalance (Metther Toledo, Greifensee, Switzerland) and then returned to their plants. Once the aphids were 2 weeks old we began moving them to new plants at weekly intervals and counting all offspring on the old plants. Additionally, we checked survival at 2-day intervals until all aphids had died. In this way we obtained data on the approximate lifespan of each individual as well as the total number of offspring it produced during its lifetime.

Statistical analysis

We analysed the survival data with a Cox proportional hazards regression using the Survival package in R v.3.4.3 (R Core Team, 2017). First we focused just on the uninfected aphids and the aphids infected with *R. insecticola* strain R5.15, testing for the effect of aphid clone, R5.15 infection, as well as their interaction. We did this for all clones and for the three *M. persicae* clones only, as R5.15 was originally discovered in this species. For the three clones with a third line carrying an additional, non-protective strain of *R. insecticola* (Table 1), we also ran separate models for each clone to test for variation among the three lines. Development time, adult mass, and lifetime reproduction were analysed with ANOVA, including the same comparisons as for survival. Individuals that had died before they were adult had to be excluded from these analyses. We used type III SS to account for the resulting imbalance and because we were interested in an effect of R5.15 over and above potential interactions with aphid clone, even if the latter were non-significant. The block variance was pooled into the residual because block did not have a significant effect on any of the analysed traits.

Results

Aphid survival

Survival differed among clones, both when all aphids were considered (Cox regression: LR $\chi^2 = 23.059$, d.f. = 5, $P < 0.001$), and when the analysis was restricted to *M. persicae* (LR $\chi^2 = 9.310$, d.f. = 2, $P = 0.010$). The survivorship curves of the uninfected lines and the lines infected with *R. insecticola* strain R5.15 indicate a weak negative effect of the symbiont (Figure 1), which was significant overall ($\chi^2 = 5.229$, d.f. = 1, $P = 0.022$), but not when only *M. persicae* is considered ($\chi^2 = 2.137$, d.f. = 1, $P = 0.14$). Just in *M. persicae* clone 7.09 it

1 looked like R5.15 reduced survival to some extent (Figure 1C), and this individual difference
2 was indeed significant (coefficient \pm SE = 1.103 ± 0.527 , $z = 2.094$, $P = 0.036$). There were
3 no significant clone*R5.15 interactions (all clones: $\chi^2 = 5.229$, d.f. = 5, $P = 0.70$; *M. persicae*:
4 $\chi^2 = 3.088$, d.f. = 2, $P = 0.21$).

5 Of the clones for which we also had lines with other strains of *R. insecticola*, *A. fabae*
6 clone A06-405 stood out for showing variation among the three lines ($\chi^2 = 10.231$, d.f. = 2, P
7 = 0.006; Figure 1D), which was mainly due to the non-protective strain R49 strongly
8 reducing aphid survival compared to the uninfected line ($z = 3.116$, $P = 0.002$), whereas
9 R5.15 had no significant effect in this particular clone ($z = 0.701$, $P = 0.48$). For the two *Ac.*
10 *pisum* clones, variation among lines was not significant (LSR1: $\chi^2 = 0.902$, d.f. = 2, $P = 0.64$;
11 5A: $\chi^2 = 3.425$, d.f. = 2, $P = 0.18$; Figures 1E, F).

13 Aphid life-history traits

14 Development time of aphids was not influenced by infection with R5.15, neither across all
15 clones ($F_{1,88} = 0.105$, $P = 0.75$), nor across the three clones of *M. persicae* ($F_{1,44} = 0.034$, $P =$
16 0.86; Figure 2A). Development time did not vary among the aphid clones ($F_{5,88} = 0.847$, $P =$
17 0.52), nor was there a significant clone*R5.15 interaction ($F_{5,88} = 1.881$, $P = 0.11$). For the
18 three clones with additional lines carrying a non-protective strain of *R. insecticola*,
19 development time did not differ among lines either (*A. fabae* A06-405: $F_{2,18} = 2.624$, $P =$
20 0.10; *Ac. pisum* LSR1: $F_{2,24} = 0.158$, $P = 0.86$; *Ac. pisum* 5A: $F_{2,21} = 2.561$, $P = 0.10$).

21 Unsurprisingly for clones belonging to three different species, among-clone variation in
22 adult mass was large ($F_{5,88} = 64.945$, $P < 0.001$), with pea aphids being about 4× heavier than
23 the other two species (Figure 2B), but also among the three *M. persicae* clones the mass
24 differences were nearly significant ($F_{2,44} = 3.141$, $P = 0.053$). Infection with R5.15, on the
25 other hand, did not affect adult mass, neither across all clones ($F_{1,88} = 0.340$, $P = 0.56$), nor in
26 *M. persicae* only ($F_{1,44} = 0.171$, $P = 0.68$). The clone*R5.15 interaction was not significant in
27 either case (all clones: $F_{5,88} = 0.822$, $P = 0.54$; *M. persicae* clones: $F_{2,44} = 2.097$, $P = 0.14$).
28 Adult mass varied in none of the clones with three lines (*A. fabae* A06-405: $F_{2,18} = 0.321$, $P =$
29 0.73; *Ac. pisum* LSR1: $F_{2,24} = 1.479$, $P = 0.25$; *Ac. pisum* 5A: $F_{2,21} = 0.274$, $P = 0.76$).

30 Lifetime reproduction varied among all six aphid clones ($F_{5,88} = 3.321$, $P = 0.009$) – with
31 the two *Ac. pisum* clones being most fecund on average (Figure 2C) –, but not among the
32 three clones of *M. persicae* separately ($F_{2,44} = 2.453$, $P = 0.098$). Compared to uninfected
33 aphids in all clones except *Ac. pisum* clone LSR1, aphids infected with R5.15 produced
34 slightly fewer offspring over their lifetime, but this effect was neither significant across all

clones ($F_{1,88} = 2.009$, $P = 0.16$), nor in *M. persicae* only ($F_{1,44} = 1.770$, $P = 0.19$) (Figure 2C). The clone*R5.15 interactions were non-significant as well (all clones: $F_{5,88} = 0.404$, $P = 0.85$; *M. persicae* clones: $F_{2,44} = 0.298$, $P = 0.74$). In the two *Ac. pisum* clones for which two *R. insecticola*-infected lines were available, the lines did not differ (LSR1: $F_{2,24} = 1.599$, $P = 0.22$; 5A: $F_{2,21} = 0.193$, $P = 0.83$). Also in *A. fabae* clone A06-405 the three lines did not vary ($F_{2,18} = 2.370$, $P = 0.12$), but *R. insecticola* strain R49 seemed to reduce lifetime reproduction more strongly than the protective strain R5.15 (Figure 2), and the individual difference between uninfected vs. R49-infected lines was marginally significant (estimate \pm SE = -23.4 ± 10.81 , $t = -2.169$, $P = 0.044$), presumably as a consequence of the lower average lifespan of this line (Figure 1D).

Aphid wing polyphenism

An unexpected observation was that in one aphid clone, LSR1 of *Ac. pisum*, a substantial proportion of the focal individuals we reared developed into winged adults (alates) despite the low-density rearing conditions. The occurrence of alates was unequal among the three lines of this clone (Fisher's exact test: $P = 0.017$), with the uninfected line developing mostly into alates (7 of 8) and the R5.15-infected line producing mostly unwinged (= apterous) individuals (8 of 10). The line infected with the non-protective strain of *R. insecticola* did not show any clear tendency (5 alate, 4 apterous). For three of the 30 aphids from clone LSR1 the morph is unknown because they died before reaching the adult stage.

It is generally the case in aphids that alates are less fecund than apterous individuals (Walters & Dixon, 1983; Zhang et al., 2009), but this was not the case for clone LSR1 here (morph effect: $F_{1,21} = 0.327$, $P = 0.57$; line*morph interaction: $F_{2,21} = 0.493$, $P = 0.62$). The result that R5.15 does not reduce lifetime reproduction across all clones remained therefore unchanged when all alates were omitted from the analysis ($F_{1,77} = 2.785$, $P = 0.099$).

Discussion

Consistent with two previous studies (Parker et al., 2017; McLean et al., 2018), aphids infected with *R. insecticola* tended to have slightly lower survival and lifetime reproduction than uninfected aphids in the absence of natural enemies, but the overall effect of the protective strain R5.15 was only significant for survival. In the two *Ac. pisum* clones, infection with R5.15 was similarly benign to infection with other, 'native' strains of *R. insecticola* that are not protective against parasitoids, and in our single *A. fabae* clone, R5.15

1 was even less harmful than another *A. fabae*-derived strain. In *M. persicae*, the species in
2 which R5.15 was originally discovered, a comparison with other native strains was not
3 possible, unfortunately. It is worth noting that in Parker et al.'s (2017) multi-strain
4 comparison, *R. insecticola* R5.15 was included as well, and also there it had virtually no
5 effect on pea aphid survival in the absence of natural enemies (Figure S3 in Parker et al.,
6 2017).

7 These observations from R5.15 stand in contrast to those from multiple strains of *H.*
8 *defensa*, which also provide strong protection against parasitoids. In *A. fabae*, these protective
9 strains of *H. defensa* clearly do reduce survival in the absence of parasitoids, which translates
10 into a lower lifetime reproduction (Vorburger & Gouskov, 2011). Those effects were highly
11 significant despite a somewhat lower replication than in the present study (8 vs. 10
12 individuals per aphid clone/symbiont combination). Furthermore, in *A. craccivora* as well as
13 *Ac. pisum*, *H. defensa*-infected aphids suffer from a reduced competitive ability (Oliver et al.,
14 2008; Dykstra et al., 2014). Although there is some evidence that infection with *R. insecticola*
15 strain R5.15 can represent a weak liability in a competitive situation as well (Hertäg &
16 Vorburger, 2018), the effect appears again very moderate compared to the strong effects
17 imposed by *H. defensa*.

18 At this point we can only speculate about the explanation of this difference. One
19 possibility is a difference in symbiont densities, because detrimental effects of endosymbionts
20 on insect fitness are in some cases related with their densities in the host (Weldon et al.,
21 2013; Martinez et al., 2015). *Hamiltonella defensa* does indeed grow to high densities that we
22 estimated to be in the range of 50 million symbiont genomes per young adult black bean
23 aphid (Schmid et al., 2012). Unfortunately, we do not have such estimates for the *R.*
24 *insecticola* strains used here, but a study by Chandler et al. (2008) on black bean aphids
25 found that titers of *H. defensa* and *R. insecticola* were comparable, as is their localization
26 within the host. Both occur intracellularly in secondary bacteriocytes that are interspersed
27 with primary bacteriocytes (containing *B. aphidicola*) and in sheath cells adjacent to
28 bacteriocytes, and both are also found free-living in the hemolymph at high densities (Moran
29 et al., 2005b).

30 More relevant may be that the protection against parasitoids conferred by *H. defensa*
31 appears to have a different mechanistic basis than that conferred by R5.15. It is possible that
32 the APSE-encoded factors, presumably toxins (Moran et al., 2005a), that have been shown to
33 be causally involved in *H. defensa*-mediated disruption of parasitoid development (Brandt et
34 al., 2017), will also cause some 'collateral damage' to the host. This APSE phage is not

1 present in the genome of *R. insecticola* strain R5.15 (Hansen et al., 2012). Unfortunately, the
2 mechanistic basis of R5.15-mediated protection against parasitoids is not known, although
3 genome comparisons between R5.15 and a non-protective strain provided some candidate
4 effectors (Hansen et al., 2012). It will be interesting to eventually work out how R5.15
5 achieves such an effective elimination of another insect inside its insect host's body with
6 relatively little harm to the host itself.

7 It is important to acknowledge that a lack of strong negative effects on host life-history
8 traits is not tantamount to a lack of symbiont-induced costs to the hosts. First of all, using 1-3
9 clones per species cannot be representative of these aphid species as a whole, and with only
10 10 infected and 10 uninfected individuals tested per clone, the power to detect more subtle
11 effects that could still be biologically relevant was certainly very limited. Secondly, costs of
12 infection with a defensive symbiont may also be ecological in nature. In pea aphids, for
13 example, infection with *H. defensa* leads to reduced defensive behaviour (Dion et al., 2011),
14 making infected aphids more susceptible to predation (Polin et al., 2014). Other studies
15 detected effects of heritable endosymbionts on dietary breadth. The most convincing example
16 comes from *A. craccivora*, in which the endosymbiont *Arsenophonus* improves fitness on one
17 host plant, black locust, but reduces fitness on an another plant, alfalfa (Wagner et al., 2015).
18 There is also evidence for a strain of *R. insecticola* altering the feeding niche of pea aphids
19 (Tsuchida et al., 2004). Depending on the local availability of different host plants, a change
20 in the ability to exploit particular plants may well represent an ecological cost of symbiont
21 infection. In the context of ecological costs it is also interesting that *R. insecticola* appeared
22 to suppress the formation of winged morphs in pea aphid clone LSR1. This finding is based
23 on a very limited sample size here, and additional tests would be required to verify its
24 robustness. Nevertheless, reduced wing induction has been observed before in *R. insecticola*-
25 infected pea aphids (Leonardo & Mondor, 2006), although there aphids were exposed to
26 crowded conditions. The production of a winged dispersal morph is indeed a response to
27 crowding or to chemical cues signalling a high risk of predation or parasitism (Weisser et al.,
28 1999; Müller et al., 2001; Sloggett & Weisser, 2002; Kunert et al., 2005). Thus, it is at least
29 feasible that a reduced ability to respond to such challenges by dispersal could be detrimental
30 to the fitness of an aphid clone. In our experiment, however, aphids were neither crowded nor
31 did they experience the presence of natural enemies. Hence, the lower proportion of winged
32 adults in the R5.15-infected line of clone LSR1 should not be assumed to be maladaptive,
33 especially considering that all other clones produced virtually no alates under the benign
34 conditions of our experiment.

1 In summary, our experiment shows that in comparison to the protection by *H. defensa*, the
2 strong protection against parasitoids conferred by *R. insecticola* strain R5.15 does not come
3 at equally strong and obvious costs to the host in terms of survival and reproductive
4 performance in the absence of parasitoids. Further research is required to assess whether
5 R5.15 affects other traits of its aphid hosts which might entail ecological costs of protection.
6

7 **Acknowledgements**

8 We thank N. Moran for kindly providing the pea aphid lines used in this study, and P.
9 Rodriguez for the skilful maintenance of insect cultures. The drawings of aphids in the
10 graphical abstract are by C. Hertäg. This study was supported by the Swiss National Science
11 Foundation (Sinergia grant nr. CRSII3_154396 to CV). Data are available at Dryad Digital
12 Repository: <https://doi.org/10.5061/dryad.m464p56>
13 .
14

15 **References**

- 16 Akman L, Yamashita A, Watanabe H, Oshima K, Shiba T et al. (2002) Genome sequence of
17 the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nature*
18 *Genetics* 32: 402-407.
- 19 Asplen MK, Bano N, Brady CM, Desneux N, Hopper KR et al. (2014) Specialisation of
20 bacterial endosymbionts that protect aphids from parasitoids. *Ecological Entomology* 39:
21 736-739.
- 22 Baumann P (2005) Biology of bacteriocyte-associated endosymbionts of plant sap-sucking
23 insects. *Annual Review of Microbiology* 59: 155-189.
- 24 Brandt JW, Chevignon G, Oliver KM & Strand MR (2017) Culture of an aphid heritable
25 symbiont demonstrates its direct role in defence against parasitoids. *Proceedings of the*
26 *Royal Society B* 284: 20171925.
- 27 von Burg S, Ferrari J, Müller CB & Vorburger C (2008) Genetic variation and covariation of
28 susceptibility to parasitoids in the aphid *Myzus persicae* – no evidence for trade-offs.
29 *Proceedings of the Royal Society B* 275: 1089-1094.
- 30 Chandler SM, Wilkinson TL & Douglas AE (2008) Impact of plant nutrients on the
31 relationship between a herbivorous insect and its symbiotic bacteria. *Proceedings of the*
32 *Royal Society B* 275: 565-570.
- 33 Dion E, Polin SE, Simon JC & Outreman Y (2011) Symbiont infection affects aphid

defensive behaviours. *Biology Letters* 7: 743-746.

Douglas AE (1998) Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annual Review of Entomology* 43: 17-37.

Duron O, Bouchon D, Boutin S, Bellamy L, Zhou LQ et al. (2008) The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biology* 6: 27.

Dykstra HR, Weldon SR, Martinez AJ, White JA, Hopper KR et al. (2014) Factors limiting the spread of the protective symbiont *Hamiltonella defensa* in *Aphis craccivora* aphids. *Applied and Environmental Microbiology* 80: 5818-5827.

Feldhaar H (2011) Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecological Entomology* 36: 533-543.

Ferrari J, Darby AC, Daniell TJ, Godfray HCJ & Douglas AE (2004) Linking the bacterial community in pea aphids with host-plant use and natural enemy resistance. *Ecological Entomology* 29: 60-65.

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

Ferrari J, West JA, Via S & Godfray HCJ (2012) Population genetic structure and secondary symbionts in host-associated populations of the pea aphid complex. *Evolution* 66: 375-390.

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

Hansen AK, Vorburger C & Moran NA (2012) Genomic basis of endosymbiont-conferred protection against an insect parasitoid. *Genome Research* 22: 106-114.

Henry LM, Maiden MCJ, Ferrari J & Godfray HCJ (2015) Insect life history and the evolution of bacterial mutualism. *Ecology Letters* 18: 516-525.

Hertäg C & Vorburger C (2018) Defensive symbionts mediate species coexistence in phytophagous insects. *Functional Ecology* 32: 1057-1064.

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

Koga R, Meng X-Y, Tsuchida T & Fukatsu T (2012) Cellular mechanism for selective vertical transmission of an obligate insect symbiont at the bacteriocyte-embryo interface. *Proceedings of the National Academy of Sciences of the USA* 109: E1230-E1237.

Kunert G, Otto S, Röse USR, Gershenzon J & Weisser WW (2005) Alarm pheromone

mediates production of winged dispersal morphs in aphids. *Ecology Letters* 8: 596-603.

Leonardo TE & Mondor EB (2006) Symbiont modifies host life-history traits that affect gene flow. *Proceedings of the Royal Society B* 273: 1079-1084.

Łukasik P, van Asch M, Guo HF, Ferrari J & Godfray HCJ (2013) Unrelated facultative endosymbionts protect aphids against a fungal pathogen. *Ecology Letters* 16: 214-218.

Martinez J, Ok S, Smith S, Snoeck K, Day JP & Jiggins FM (2015) Should symbionts be nice or selfish? Antiviral effects of *Wolbachia* are costly but reproductive parasitism is not. *PLoS Pathogens* 11: e1005021.

McLean AHC, Parker BJ, Hreck J, Kavanagh JC, Wellham PAD & Godfray HCJ (2018) Consequences of symbiont co-infections for insect host phenotypes. *Journal of Animal Ecology* 87: 478-488.

Michalik A, Szklarzewicz T, Jankowska W & Wieczorek K (2014) Endosymbiotic microorganisms of aphids (Hemiptera: Sternorrhyncha: Aphidoidea): ultrastructure, distribution and transovarial transmission. *European Journal of Entomology* 111: 91-104.

Moran NA, Degnan PH, Santos SR, Dunbar HE & Ochman H (2005a) The players in a mutualistic symbiosis: insects, bacteria, viruses, and virulence genes. *Proceedings of the National Academy of Sciences of the USA* 102: 16919-16926.

Moran NA, McCutcheon JP & Nakabachi A (2008) Genomics and evolution of heritable bacterial symbionts. *Annual Review of Genetics* 42: 165-190.

Moran NA, Russell JA, Koga R & Fukatsu T (2005b) Evolutionary relationships of three new species of *Enterobacteriaceae* living as symbionts of aphids and other insects. *Applied and Environmental Microbiology* 71: 3302-3310.

Müller CB, Williams IS & Hardie J (2001) The role of nutrition, crowding and interspecific interactions in the development of winged aphids. *Ecological Entomology* 26: 330-340.

Nyabuga FN, Loxdale HD, Heckel DG & Weisser WW (2010) Spatial population dynamics of a specialist aphid parasitoid, *Lysiphlebus hirticornis* Mackauer (Hymenoptera: Braconidae: Aphidiinae): evidence for philopatry and restricted dispersal. *Heredity* 105: 433-442.

Oliver KM, Campos J, Moran NA & Hunter MS (2008) Population dynamics of defensive symbionts in aphids. *Proceedings of the Royal Society B* 275: 293-299.

Oliver KM, Degnan PH, Burke GR & Moran NA (2010) Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. *Annual Review of Entomology* 55: 247-266.

Oliver KM, Degnan PH, Hunter MS & Moran NA (2009) Bacteriophages encode factors

required for protection in a symbiotic mutualism. *Science* 325: 992-994.

Oliver KM, Russell JA, Moran NA & Hunter MS (2003) Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences of the USA* 100: 1803-1807.

Oliver KM, Smith AH & Russell JA (2014) Defensive symbiosis in the real world - advancing ecological studies of heritable, protective bacteria in aphids and beyond. *Functional Ecology* 28: 341-355.

Parker BJ, Hrčák J, McLean AHC & Godfray HCJ (2017) Genotype specificity among hosts, pathogens, and beneficial microbes influences the strength of symbiont-mediated protection. *Evolution* 71: 1222-1231.

Polin S, Simon JC & Outreman Y (2014) An ecological cost associated with protective symbionts of aphids. *Ecology and Evolution* 4: 826-830.

R Core Team (2017) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rock DI, Smith AH, Joffe J, Albertus A, Wong N et al. (2018) Context-dependent vertical transmission shapes strong endosymbiont community structure in the pea aphid, *Acyrtosiphon pisum*. *Molecular Ecology* 27: 2039-2056.

Scarborough CL, Ferrari J & Godfray HCJ (2005) Aphid protected from pathogen by endosymbiont. *Science* 310: 1781-1781.

Schmid M, Sieber R, Zimmermann YS & Vorburger C (2012) Development, specificity and sublethal effects of symbiont-conferred resistance to parasitoids in aphids. *Functional Ecology* 26: 207-215.

Sloggett JJ & Weisser WW (2002) Parasitoids induce production of the dispersal morph of the pea aphid, *Acyrtosiphon pisum*. *Oikos* 98: 323-333.

Smith AH, Lukasik P, O'Connor MP, Lee A, Mayo G et al. (2015) Patterns, causes and consequences of defensive microbiome dynamics across multiple scales. *Molecular Ecology* 24: 1135-1149.

Tamas I, Klasson L, Canback B, Naslund AK, Eriksson AS et al. (2002) 50 million years of genomic stasis in endosymbiotic bacteria. *Science* 296: 2376-2379.

Tsuchida T, Koga R & Fukatsu T (2004) Host plant specialization governed by facultative symbiont. *Science* 303: 1989-1989.

Vorburger C, Ganesanandamoorthy P & Kwiatkowski M (2013) Comparing constitutive and induced costs of symbiont-conferred resistance to parasitoids in aphids. *Ecology and Evolution* 3: 706-713.

- 1 Vorburger C, Gehrler L & Rodriguez P (2010) A strain of the bacterial symbiont *Regiella*
2 *insecticola* protects aphids against parasitoids. *Biology Letters* 6: 109-111.
- 3 Vorburger C & Gouskov A (2011) Only helpful when required: a longevity cost of
4 harbouring defensive symbionts. *Journal of Evolutionary Biology* 24: 1611-1617.
- 5 Vorburger C & Rouchet R (2016) Are aphid parasitoids locally adapted to the prevalence of
6 defensive symbionts in their hosts? *BMC Evolutionary Biology* 16: 271.
- 7 Vorburger C, Sandrock C, Gouskov A, Castañeda LE & Ferrari J (2009) Genotypic variation
8 and the role of defensive endosymbionts in an all-parthenogenetic host-parasitoid
9 interaction. *Evolution* 63: 1439-1450.
- 10 Wagner SM, Martinez AJ, Ruan YM, Kim KL, Lenhart PA et al. (2015) Facultative
11 endosymbionts mediate dietary breadth in a polyphagous herbivore. *Functional Ecology*
12 29: 1402-1410.
- 13 Walters KFA & Dixon AFG (1983) Migratory urge and reproductive investment in aphids -
14 variation within clones. *Oecologia* 58: 70-75.
- 15 Weisser WW, Braendle C & Minoretti N (1999) Predator-induced morphological shift in the
16 pea aphid. *Proceedings of the Royal Society B* 266: 1175-1181.
- 17 Weldon SR, Strand MR & Oliver KM (2013) Phage loss and the breakdown of a defensive
18 symbiosis in aphids. *Proceedings of the Royal Society B* 280: 20122103.
- 19 Wu D, Daugherty SC, Van Aken SE, Pai GH, Watkins KL et al. (2006) Metabolic
20 complementarity and genomics of the dual bacterial symbiosis of sharpshooters. *PLoS*
21 *Biology* 4: 1079-1092.
- 22 Zhang Y, Wu KM, Wyckhuys KAG & Heimpel GE (2009) Trade-offs between flight and
23 fecundity in the soybean aphid (Hemiptera: Aphididae). *Journal of Economic*
24 *Entomology* 102: 133-138.
- 25
26
27

Figure captions

Figure 1 Survivorship curves of lines of six clones of three aphid species, that were uninfected with *Regiella insecticola*, infected with a protective strain (R5.15) of *R. insecticola*, or infected with strains of *R. insecticola* that are not protective against parasitoids.

Figure 2 Mean (+ SE) (A) development time (days), (B) adult mass (mg), and (C) lifetime reproduction (total no. offspring) of six clones of three aphid species, that were uninfected with *Regiella insecticola*, infected with a protective strain (R5.15) of *R. insecticola*, or infected with strains of *R. insecticola* that are not protective against parasitoids. The asterisk indicates a significant difference between an uninfected and a *R. insecticola*-infected line of the same clone ($t = -2.169$, $P < 0.05$).

1 **Table 1** Summary of aphid lines used in this study, indicating all combinations of genetic
2 backgrounds (six aphid clones belonging to three species) and *Regiella insecticola* infections
3 available for measurements of aphid life-history traits

Species	Clone	<i>Regiella insecticola</i> infection		
		None	Strain R5.15	Other strains
<i>Myzus persicae</i>	5.15	5.15 ^{R-}	5.15	
	5.03	5.03	5.03 ^{R5.15}	
	7.09	7.09	7.09 ^{R5.15}	
<i>Aphis fabae</i>	A06-405	A06-405	A06-405 ^{R5.15}	A06-405 ^{R49}
<i>Acyrthosiphon pisum</i>	LSR1	LSR1	LSR1 ^{R5.15}	LSR1-Ri
	5A	5A	5A ^{R5.15}	5A-U

4

Figure 1

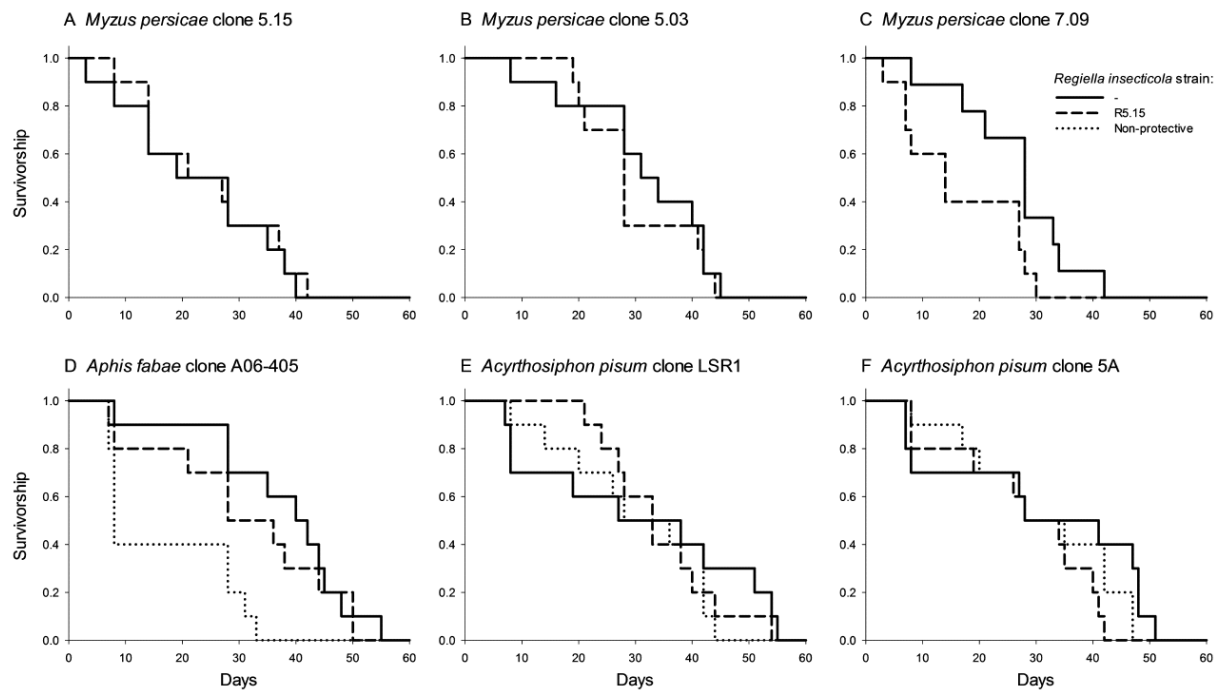


Figure 2

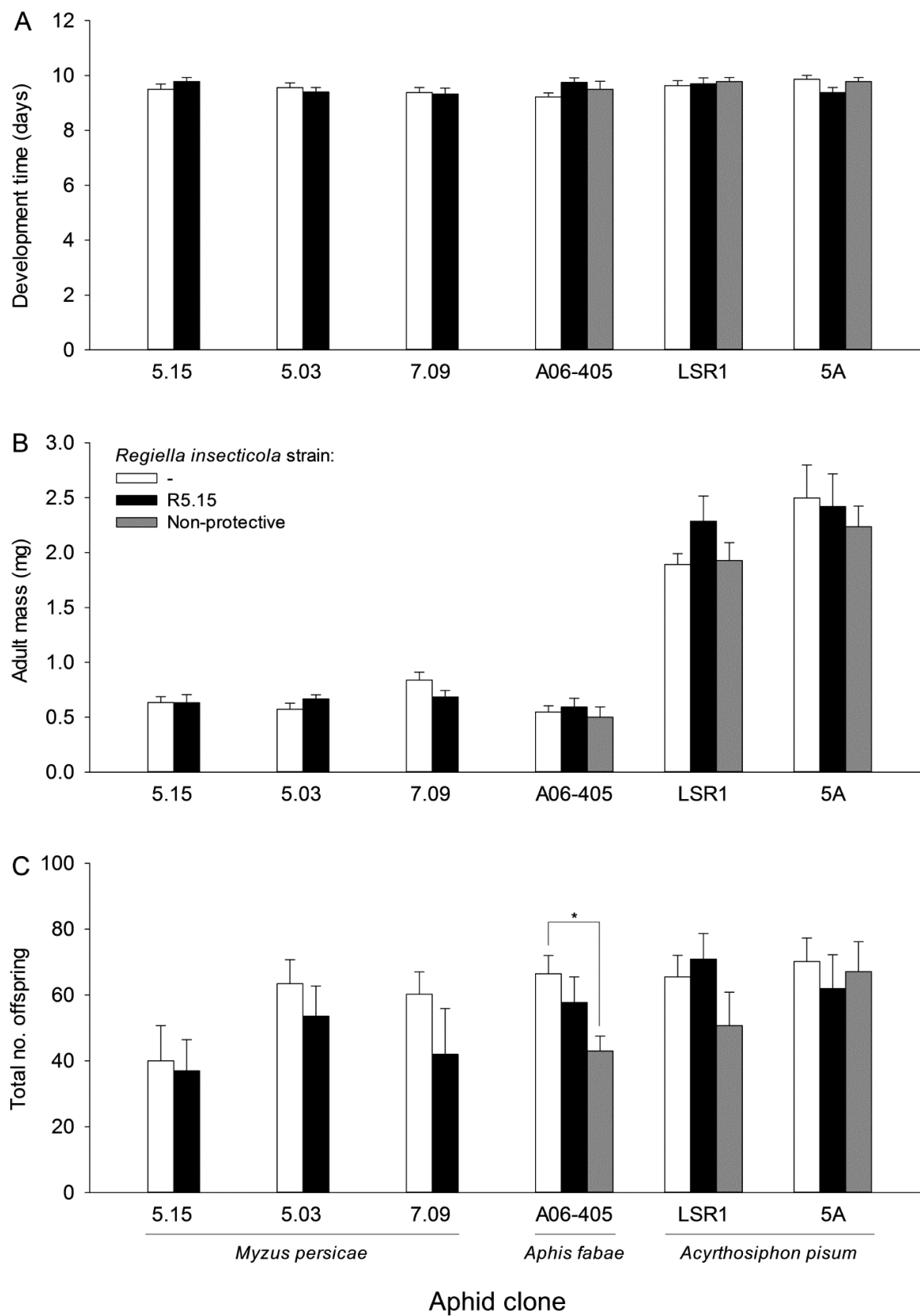


Table of Contents Figure

