The evolutionary ecology of symbiont-conferred resistance to parasitoids in aphids

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Abstract

Aphids may harbour a wide variety of facultative bacterial endosymbionts. These symbionts are transmitted maternally with high fidelity and they show horizontal transmission as well, albeit at rates too low to enable infectious spread. Such symbionts need to provide a net fitness benefit to their hosts to persist and spread. Several symbionts have achieved this by evolving the ability to protect their hosts against parasitoids. Reviewing empirical work and some models I explore the evolutionary ecology of symbiont-conferred resistance to parasitoids in order to understand how defensive symbiont frequencies are maintained at the intermediate levels observed in aphid populations. I further show that defensive symbionts alter the reciprocal selection between aphids and parasitoids by augmenting the heritable variation for resistance, by increasing the genetic specificity of the host-parasitoid interaction, and by inducing environment-dependent trade-offs. These effects are conducive to very dynamic, symbiont-mediated coevolution that is driven by frequency-dependent selection. Finally I argue that defensive symbionts represent a problem for biological control of pest aphids, and I propose to mitigate this problem by exploiting the parasitoids' demonstrated ability to rapidly evolve counteradaptations to symbiont-conferred resistance.

Keywords: Aphids, Biological Control, Coevolution, Genotype x genotype interaction, *Hamiltonella defensa*, Resistance, Parasitoid, Symbiosis

Aphid-parasitoid interactions

Due to their complex life-cycles and phenotypic plasticity and due to their importance as agricultural pests, aphids have long been a focus of attention in fundamental and applied entomology. As a numerically important food resource especially in temperature regions, aphids are exploited by a remarkable number and diversity of natural enemies. A particularly important group of their natural enemies are parasitoids (Schmidt et al., 2003). Primary parasitoids of aphids belong to two groups, the subfamily Aphidiinae (Hymenoptera: Ichneumonoidea: Braconidae) with more than 400 species worldwide (Starý, 1988), and the genus *Aphelinus* (Hymenoptera: Chalcidoidea: Aphelinidae) with some 80 species (Noyes, 2012). They play an important role for the natural control of aphid populations (Sigsgaard, 2002, Schmidt et al., 2003), and some species are also available commercially for augmentative control of pest aphids in confined spaces such as greenhouses (van Lenteren et al., 1997, Boivin et al., 2012). Here I focus mainly on the interaction between aphids and aphidiine parasitoids. The life-cycle of these solitary koinobiont parasitoids is relatively simple. Female wasps stab aphids for oviposition and normally inject a single egg. The parasitoid larva hatches soon after oviposition and develops through three larval instars inside the still active host, which they kill prior to pupation. For this the larva spins a cocoon either inside or below (parasitoids of the genus *Praon*) the dead host, in which metamorphosis takes place. This stage is called a 'mummy', from which the adult wasp emerges after metamorphosis.

Successful development of the parasitoid is always fatal to the host. Aphids have thus evolved a number of behavioral defences such as kicking or dropping off the plant to avoid parasitoid oviposition (Gross, 1993, Le Ralec et al., 2010), as well as physiological defences to prevent parasitoid development after oviposition (Griffiths, 1960, Li et al., 2002, Poirié &

Coustau, 2011). Behavioral resistance reduces parasitoid oviposition rate and physiological resistance is fatal to the parasitoid's egg or larva, thereby imposing selection for parasitoid counteradaptation. In the face of this intense reciprocal selection, natural populations of aphids tend to exhibit substantial genetic variation for resistance to parasitoids (e.g. Henter & Via, 1995, Ferrari et al., 2001, von Burg et al., 2008, Vorburger et al., 2009). Particularly impressive is the study by Henter & Via (1995), the first thorough quantification of genetic variation for aphid resistance to parasitoids. In total, 75 different clones of the pea aphid (*Acyrthosiphon pisum*) were exposed to the aphid parasitoid *Aphidius ervi* in a standard assay. Their rates of parasitism ranged from 0% mummification to over 80% mummification, that is from complete resistance to almost complete susceptibility. This enormous variation was the result of differences in physiological resistance, because parasitoids oviposited at equal rates in resistant and susceptible clones (Henter & Via, 1995). Still unknown at that time was that much of the variation for resistance to parasitoids in pea aphids is due to the presence or absence of defensive endosymbionts rather than to nuclear genetic variation among clones.

Defensive endosymbionts

Defensive endosymbionts entered the stage when Oliver et al. (2003) published the first paper showing that experimental infection with two species of facultative bacterial endosymbionts, later named *Hamiltonella defensa* and *Serratia symbiotica* (Moran et al., 2005a), increased the resistance of pea aphids to the parasitoid *A. ervi*. This finding triggered a burst of research with the result that today, only ten years later, there is a consensus that symbiont-conferred resistance to parasites and pathogens is an important and widespread phenomenon in aphids as well as in other insects (Duron & Hurst, 2013). As always, this

insight is perhaps less surprising in retrospect. It was well established that insects commonly harbour maternally transmitted bacteria (Buchner, 1965, Baumann et al., 2000), and the reproductive manipulations seen in the most abundant endosymbiont of insects, *Wolbachia*, illustrated that such symbionts can evolve means to promote their own transmission (Stouthamer et al., 1999, Werren et al., 2008). Once a symbiont has evolved vertical transmission, its persistence and spread become linked to host reproduction. The symbiont is therefore under selection to protect its host against natural enemies and/or other environmental challenges to ensure that the host survives at least until it reproduces. Models have indeed shown that host protection evolves readily in vertically transmitted parasites when they compete for the same host against horizontally transmitted parasites (Lively et al., 2005, Jones et al., 2007), particularly if the horizontally transmitted parasite strongly reduces host reproduction, as is the case for parasitoids (Jones et al., 2011). It is thus likely that many heritable defensive symbionts evolved from vertically transmitted pathogens.

Heritable symbionts affect a number of ecologically important traits in aphids, which is authoritatively reviewed in Oliver et al. (2010). Here I focus on protection against parasitoids, which has been demonstrated unambiguously for three facultative endosymbionts of aphids so far, namely *H. defensa*, *S. symbiotica* and for a particular strain of *Regiella insecticola* (Table 1). This list may well grow with additional investigations in the near future, as was the case for protection against the entomopathogenic fungus *Pandora neoaphidis*. Originally described for *R. insecticola* only (Scarborough et al., 2005), it was later found that several common endosymbionts of aphids increase resistance against this fungal pathogen (Łukasik et al., 2013b).

Mechanism of protection

A question of obvious interest is how defensive symbionts protect their hosts against parasitoids. The protective mechanism is best understood in the case of *H. defensa* in pea aphids. This bacterium is typically infected with temperate bacteriophages called APSEs (van der Wilk et al., 1999, Sandström et al., 2001), of which three types have been distinguished in *H. defensa* from pea aphids (APSE1-3). Each type encodes a different toxin gene (Moran et al., 2005b, Degnan & Moran, 2008), and it appears to be these toxins that kill the parasitoid egg or larva and thereby protect the aphid host. This was inferred when a spontaneous loss of APSE3 was observed from a strain of *H. defensa* in a laboratory-held clone of the pea aphid, which resulted in a loss of resistance in this clone (Oliver et al., 2009).

At about the same time a strongly protective strain of *R. insecticola*, a symbiont that generally does not increase resistance to parasitoids (Oliver et al., 2003, Vorburger et al., 2009), was discovered in the green peach aphid, *Myzus persicae* (Herzog et al., 2007, von Burg et al., 2008, Vorburger et al., 2010). The natural suspicion arose that the same phages might be responsible for the resistance conferred by this strain of *R. insecticola* (Vorburger et al., 2010), possibly acquired laterally from *H. defensa* in an aphid line harbouring a double infection. Infections with two or more facultative endosymbionts are frequently observed in aphids (Ferrari et al., 2004, Frantz et al., 2009, Nyabuga et al., 2010, Ferrari et al., 2012, Russell et al., 2013). However, sequencing of its genome revealed that phages were absent (Hansen et al., 2012). Instead, five categories of pathogenicity factors were discovered that were missing or inactivated in a non-protective strain of *R. insecticola*, making them likely candidates for a causal role in harming the wasps. Hence it appears that different endosymbionts have found mechanistically different solutions to the same evolutionary challenge. How protection against parasitoids by *S. symbiotica* functions mechanistically is still unclear. A possibility that should not be ruled out for all facultative symbionts of aphids is that indirect mechanisms via the host's immune system play an additional role in their

124 protective effect (immune priming). There is indeed evidence that the presence of facultative
125 symbionts affects aphid cellular immunity (Schmitz et al., 2012).
126
127 **Determinants of infection frequencies**
128
129 The strongly increased resistance of aphids possessing heritable defensive symbionts
130 enables a rapid evolutionary response to selection by parasitoids. This is evidenced by
131 laboratory population experiments showing rapid increases in the frequency of infected
132 clones in the presence of parasitoids (Herzog et al., 2007, Oliver et al., 2008). However,
133 defensive symbionts do not go to fixation in natural populations of aphids. Most surveys
134 found them to occur at low to intermediate frequencies (e.g. Tsuchida et al., 2002, Oliver et
135 al., 2006, Frantz et al., 2009, Vorburger et al., 2009, Russell et al., 2013). Considering the
136 strong selective advantage of increased resistance to parasitoids, what is it that prevents the
137 fixation of such endosymbionts? Or more generally: what factors determine the frequency of
138 infection with defensive symbionts? The most important of these factors are illustrated in
139 Figure 1. In brief, it is the balance of selective benefits and costs conferred by the symbionts
140 as well as the balance between symbiont losses and gains that determine their frequency in a
141 population (Kwiatkowski & Vorburger, 2012). Below I try to summarize what is known
142 about the relevant factors, drawing mainly on results obtained from the pea aphid (*Ac. pisum*)
143 and the black bean aphid (*Aphis fabae*).
144
145 *Fidelity of vertical transmission*
146
147 How faithfully endosymbionts are inherited could have a strong influence on their
148 dynamics. In the case of aphids that reproduce by cyclical parthenogenesis, it is useful to

149 distinguish the vertical transmission during the parthenogenetic, viviparous generations over
150 the growth season, and the vertical transmission during the sexual generation via the
151 diapausing, overwintering eggs.
152 The vertical transmission under parthenogenetic reproduction appears to be virtually
153 perfect, at least under laboratory conditions. No transmission failures of *H. defensa*, for
154 example, were observed in the pea aphid (Darby & Douglas, 2003), and laboratory clones of
155 pea aphids as well as black bean aphids maintain their infections for many years and > 100
156 generations (Weldon et al., 2013; C. Vorburger, pers. obs.). The only reports of spontaneous
157 transmission failures I am aware of concern clones that were infected with two different
158 facultative endosymbionts, of which one was lost (Sandström et al., 2001, Moran & Dunbar,
159 2006). It is thus possible that superinfections are less stably transmitted than infections with a
160 single facultative endosymbiont. But how reliable is vertical transmission of single infections
161 during parthenogenesis under natural conditions? Unfortunately, there is no definitive answer
162 to this question yet, and this remains a serious gap in our understanding of symbiont
163 dynamics. There are only some indications in the literature that maternal inheritance of
164 facultative symbionts in the field may not be quite as faultless as under laboratory conditions.
165 One such observation is that symbiont-conferred resistance against parasitoids is reduced
166 under heat stress (Bensadia et al., 2006, Guay et al., 2009, Cayetano & Vorburger, 2013).
167 Combined with the fact that the bacterial endosymbiont *Wolbachia* can be eliminated in some
168 arthropods by exposure to high but naturally realistic temperatures (Van Opijnen &
169 Breeuwer, 1999, Kyei-Poku et al., 2003), this suggests that defensive endosymbionts of
170 aphids may be suppressed or even eliminated during bouts of high temperature (e.g. hot
171 summer days). For the moment this remains a speculation, though, as I am not aware of any
172 demonstrated cases of aphids being cured from facultative symbionts by exposure to heat. It
173 is interesting in this context that two symbionts providing resistance to parasitoids, namely *S.*

174 *symbiotica* and *H. defensa*, have also been shown to mitigate the negative effects of high
175 temperature on aphid life-history traits (Montllor et al., 2002, Russell & Moran, 2006).
176 A second hint comes from studies on pea aphids, showing that infected and uninfected
177 individuals of the same clone can be found in the field (Dion et al., 2011b). However, this
178 might just as well be a consequence of horizontal transmission (see below) rather than
179 vertical transmission failure and is thus no evidence for vertical transmission being less
180 reliable under natural conditions.
181 Maternal transmission via sexually produced eggs appears to occur very reliably as well.
182 In black bean aphids > 200 fundatrices hatched from eggs overwintered either in the
183 laboratory or under natural conditions outdoors were screened for infections with *R.*
184 *insecticola* or *H. defensa* present in their mothers. Just a single fundatrix was found to be
185 uninfected, suggesting that vertical transmission via eggs may at most be slightly less than
186 100% (C. Vorburger, G. Siegrist & N. Rhyner, unpublished data). In pea aphids, Moran &
187 Dunbar (2006) observed three losses of *H. defensa* in a total of 68 sexually produced lines
188 from mothers harbouring different facultative symbionts . This would imply a somewhat
189 higher rate of failed transmissions via the egg stage in pea aphids than in black bean aphids,
190 but further estimates are needed before any firm conclusions can be made.
191 Taking together the evidence currently available, aphid infections with facultative
192 endosymbionts are very stable across generations, both under asexual and under sexual
193 reproduction. Frequent loss of symbionts is thus unlikely to explain the intermediate levels of
194 infection in natural populations. However, there is a need for reliable estimates of vertical
195 transmission under natural conditions in the field. This preliminary conclusion may thus have
196 to be revised if such estimates become available and provide a different picture.
197
198 *Horizontal transmission of symbionts*

The rate of horizontal transmission is another important determinant of endosymbiont dynamics. Under very high rates of horizontal transmission, facultative symbionts could spread without providing any benefits to the host or even when harmful to the host (pathogens). But even very low rates of lateral transmission may be consequential, because they allow symbionts to jump ship and become associated with new host genotypes. This becomes important when genetic interactions between host and symbiont occur, such that particular combinations of host and symbiont genotypes are particularly fit or unfit, respectively (e.g. Simon et al., 2011, Vorburger & Gouskov, 2011).

That facultative endosymbionts of aphids are capable of horizontal transmission at least occasionally has been suggested by the incongruence of the symbionts' molecular phylogenies with those of their hosts. In *H. defensa*, *R. insecticola* and *S. symbiotica*, for example, closely related strains occur in distantly related aphids (Russell et al., 2003), implying the occurrence of lateral transfer even between species. There are a number of potential routes by which defensive symbionts of aphids could be transmitted horizontally between hosts and evidence is accumulating that several of these routes are indeed used by symbionts (Table 2). However, to assess their importance for the dynamics of defensive symbionts in host populations will require more than just a yes or no answer to whether symbionts can be transmitted via a particular route. It will require an understanding of the relative importance of different transmission routes and estimates of transmission rates via these routes, which will be more difficult to obtain.

One demonstrated route for horizontal transmission of facultative symbionts in aphids is by sex. When males from an infected clone mate with females from an uninfected clone, the symbionts may get transmitted in the males' ejaculate and passed on to the overwintering

224 eggs produced by the previously uninfected female. The fundatrices hatching from such eggs
225 the next spring will then start new lines carrying a heritable infection. This has been
226 demonstrated first in the pea aphid (Moran & Dunbar, 2006), and the observed rates of male-
227 to-female transfer were sometimes surprisingly high. In one experiment, all of 13 progeny
228 lines tested from a cross between an uninfected mother and an *R. insecticola*-infected father
229 had acquired the paternal symbiont, but other crosses yielded lower rates or even no
230 transmissions (Moran & Dunbar, 2006). A similar experiment in *Ap. fabae* detected only a
231 single acquired infection among 195 fundatrices from uninfected mothers mated to *R.*
232 *insecticola*-positive fathers and no acquired infections in 217 fundatrices from uninfected
233 mothers mated to *H. defensa*-positive fathers (C. Vorburger, G. Siegrist & N. Rhyner,
234 unpublished data). This corresponds to an estimated rate of transmission of only 0.2% across
235 the two symbionts. A tentative conclusion from this very limited set of observations would be
236 that sexual transmission of defensive endosymbionts is generally possible in aphids, but that
237 the efficacy of this route of horizontal transmission may vary considerably among species.
238 Importantly, this route is available only in cyclical parthenogens (several aphids are obligate
239 parthenogens), and only once per year during their sexual reproduction. It is not available
240 during the many asexual generations throughout the growth season, when selection by
241 parasitoids is particularly strong. Unless aphids hybridize frequently, this route is also more
242 likely to enable horizontal transmission within rather than between species.
243
244 These constraints apply to a lesser extent when facultative symbionts are conveyed by
245 vectors, a second demonstrated route of horizontal transmission in aphids. This route has long
246 suggested itself from the fact that most symbionts are readily transmittable by microinjection
247 of hemolymph from infected into uninfected aphids (Chen & Purcell, 1997), that is by a
248 human vector, but it was unclear what natural vectors could play this role. Prime suspects

were ectoparasitic mites (e.g. *Allothrombium* spp.), because they commonly attack aphids to feed on hemolymph, and because mites have been shown to laterally transmit endosymbionts of the genus *Spiroplasma* in *Drosophila* (Jaenike et al., 2007). However, no transmissions by mites were observed in 75 trials in which a symbiont-free black bean aphid was attacked by a mite that had fed before on black bean aphids harbouring either *H. defensa* or *R. insecticola* (Gehrer & Vorburger, 2012). This result does not rule out mites as potential vectors, but it implies that they may not be particularly effective. Other potential vectors are parasitoids. If a wasp sequentially stabs an infected and an uninfected aphid, it may transmit symbionts with its ovipositor. This transfer remains inconsequential if the recipient aphid is successfully parasitized and killed by the developing wasp. But if the recipient aphid resists the parasitoid, it may acquire a new, heritable infection with an endosymbiont. This was indeed observed in black bean aphids, in which *H. defensa* as well as *R. insecticola* were transmitted horizontally by the two parasitoid species *Lysiphlebus fabarum* and *Aphidius colemani* in a laboratory experiment (Gehrer & Vorburger, 2012). This occurred in 3.3% of all trials or in 8.6% of those trials in which the recipient aphid survived the parasitoid attack. These rates are not high, but considering the enormous population sizes of aphids and parasitoids in the field and the high frequency of their interactions, they nevertheless suggest that facultative endosymbionts enjoy substantial mobility in aphid populations. This route of horizontal transfer is available during the many clonal generations of the aphid life-cycle and it is likely to be particularly effective exactly when selecion by parasitoids is strongest. Ironically, by spreading defensive symbionts, parasitoids may contribute to 'vaccinating' their host population against themselves. Vectoring by parasitoids could also readily explain lateral transfer of defensive symbionts between species, because at least the more generalist parasitoid species have host ranges that may comprise several dozen aphid species (Starý, 2006). That other natural enemies of aphids, particularly predators, can occasionally act as

vectors for horizontal transmission of facultative symbionts is feasible but remains to be investigated.

A third and potentially important route for lateral transfer of facultative symbionts is by ingestion. This possibility suggests itself from the fact that it is possible to infect aphids by feeding them an artificial diet mixed with symbiont cells (Darby & Douglas, 2003). It is also known that *H. defensa* cells are contained in the honeydew as well as in cornicle secretions of pea aphids (Darby & Douglas, 2003). Taken together these findings make it appear feasible that horizontal transmission of facultative symbionts by oral acquisition could occur in aphid colonies comprising infected and uninfected individuals. However, to my knowledge we still lack a clear demonstration of this process actually occurring in laboratory or natural populations, despite some suggestive observations (e.g. Oliver et al., 2008). Noteworthy in this context is a recent report that *Rickettsia* symbionts of whiteflies, distant relatives of aphids, can be transmitted between hosts via the plant phloem (Caspi-Fluger et al., 2012). Whether this is possible in aphids as well remains to be investigated.

Overall it is clear that defensive endosymbionts of aphids are capable of horizontal transmission. This can occur via different routes such as sexual transfer, vectoring by parasitoids and possibly ingestion (Table 2), of which tranfer via vectors may be particularly important. Some additional routes probably remain to be detected. The rates at which horizontal transmissions occur in the field are difficult to judge with the limited information available so far. Considering that the observed rates are mostly low even in laboratory trials specifically staged to readily allow and detect lateral transmission events, it is likely that natural rates are too low to enable infectious spread. Further considering that harbouring defensive symbionts tends to be associated with costs to the host (see below), it appears that a

299 significant benefit in terms of protection against parasitoids is required for their persistence in
300 aphid populations.
301
302 *Strength of protection*
303
304 By all standards, the protection against parasitoids provided by defensive symbionts of
305 aphids can be strong. The very first demonstration of symbiont-conferred resistance to *A. ervi*
306 in pea aphids reported a 41.5% reduction of parasitism by *H. defensa* and a 22.5% reduction
307 by *S. symbiotica* (Oliver et al., 2003). The first study on protection by *H. defensa* in *A. fabae*,
308 using naturally infected or uninfected clones from the field, found almost complete resistance
309 of infected clones against the aphid parasitoid *L. fabarum* (Vorburger et al., 2009; Fig. 2).
310 The defensive strain of *R. insecticola* originally found in *Myzus persicae* reduced parasitism
311 by *A. colemani* in three different clones of *M. persicae* by 100% and induced near-complete
312 resistance in a clone of *Ap. fabae* (Vorburger et al., 2010). The same strain of *R. insecticola*
313 also reduces parasitism by *A. ervi* in pea aphids by between 48% and 11%, depending on
314 aphid genetic background (Hansen et al., 2012). Clearly, possessing defensive symbionts can
315 provide a significant selective advantage in the presence of parasitoids (Herzog et al., 2007,
316 Oliver et al., 2008). However, more refined studies showed that there is significant variation
317 at multiple levels. For example, different isolates of *H. defensa* provide different degrees of
318 protection against *A. ervi* in pea aphids (Oliver et al., 2005), and this could later be linked to
319 the presence of different variants of the APSE phage in *H. defensa* (Degnan & Moran, 2008,
320 Oliver et al., 2009, Weldon et al., 2013). Among-strain variation in the strength of protection
321 is also observed in *H. defensa* present in *Ap. fabae* (Schmid et al., 2012, Cayetano &
322 Vorburger, 2013). A recent study by Łukasik et al. (2013a) even suggests that protection
323 against parasitoids may not be a universal attribute of *H. defensa*, since the strains found in

the grain aphid *Sitobion avenae* did not provide significant protection against the parasitoids *A. ervi* and *Ephedrus plagiator*, even though these strains did contain APSE (Łukasik et al., 2013a).

The strength of protection provided by *H. defensa* (and presumably other defensive symbionts as well) also depends on the parasitoid an aphid is confronted with. This is particularly evident in the *Ap. fabae*/*L. fabarum* system, in which the occurrence of thelytokous (asexual) lines in the parasitoid allows a detailed analysis of how resistant different aphid clones with or without particular isolates of *H. defensa* are against different genotypes of the parasitoid. Such experiments have shown that different genotypes of *L. fabarum* vary in their ability to overcome the resistance conferred by *H. defensa* (Rouchet & Vorburger, 2012, Schmid et al., 2012, Cayetano & Vorburger, 2013). This variation is so pronounced as to make the same isolates of *H. defensa* appear defensive or non-defensive depending on which parasitoid genotype their host is attacked by (e.g. Cayetano & Vorburger, 2013). More specifically, particular isolates of *H. defensa* may protect strongly against some but not or only weakly against other parasitoid genotypes, and *vice versa* (Schmid et al., 2012, Cayetano & Vorburger, 2013). This specificity of symbiont-conferred resistance results in significant aphid line-by-parasitoid line interactions on rates of parasitism (Rouchet & Vorburger, 2012), which are due to genotype-by-genotype interactions between the parasitoids and the symbionts defending their hosts and are therefore not observed in the absence of defensive symbionts (Sandrock et al., 2010). If these interactions are related to the presence of different toxin-encoding phages in the different strains of the symbiont remains to be tested. Even though genetic specificity is pervasive in this aphid symbiont-parasitoid interaction, it does not drown variation in mean effects, such that some isolates of *H. defensa* remain significantly more protective when averaged across many

parasitoid genotypes and some parastoids remain more infective on symbiont-protected hosts (L. Cayetano & C. Vorburger, unpublished data).

Genetic variation for infectivity is of course also present in sexual species of aphid parasitoids, such as *A. ervi* (Henter, 1995), and an experimental evolution study by Dion et al. (2011a) has shown that this species also harbours genetic variation for the ability to overcome the resistance conferred by *H. defensa*. Within only four generations of selection, parasitoids improved their ability to parasitize pea aphids harbouring *H. defensa* to the point that they were no longer protected compared to aphids without the symbiont (Dion et al., 2011a).

To summarize this part, there is ample evidence from laboratory investigations that protection against parasitoids by defensive symbionts can be very strong and is thus likely to provide a significant selective advantage for infected clones in the presence of parasitoids, although this remains to be corroborated in the field (but see Brady & White, 2013, Oliver et al., 2013). It is important to remember, though, that there is among-strain variation in the level of protection provided by defensive symbionts, and that this protection may also be conditional on the genotypes of the attacking parasitoids. These are the ingredients for very dynamic, symbiont-mediated coevolution that may contribute to the maintenance of symbiont diversity (Kwiatkowski et al., 2012).

Costs of harbouring symbionts

Given that defensive symbionts are inherited with near-perfect fidelity and provide aphids with strong protection against an important group of natural enemies, it appears necessary to postulate costs of harbouring these symbionts to explain why they do not go to fixation in aphid populations (Kwiatkowski & Vorburger, 2012). The first clear evidence for such costs came from population cage experiments with pea aphids, showing that in the absence of

parasitoids, the frequency of infection with *H. defensa* declined over time (Oliver et al., 2008). It remained unclear, however, why infected aphids were competitively inferior. A life-table experiment on black bean aphids later showed that experimental infections with *H. defensa* shortened aphid lifespan (Fig. 3), which translated into significant reductions in lifetime reproduction (Vorburger & Gouskov, 2011), a result that was corroborated by similar observations in pea aphids (Simon et al., 2011). Interestingly, the magnitude of this longevity cost was influenced by significant genotype-by-genotype interactions between host and symbiont (Vorburger & Gouskov, 2011), such that the negative effect on lifespan was very strong for some combinations of host and symbiont genotypes, but moderate for others. It is therefore questionable if comparisons of experimentally infected aphids with their uninfected clone mates provide a fair representation of the costs in natural populations, because particularly unfit combinations may not persist in the field to begin with. Similar comparisons with naturally infected clones that were cured from their infections could clarify this. Mechanistically, the curtailed lifespan of aphids harbouring *H. defensa* may simply be a consequence of the metabolic demands imposed by the presence of a large bacterial population in the host, it may reflect costs of immune activation in the presence of symbionts, or it may be due to 'collateral damage' to the host resulting from the symbiont's production of toxins. It will be interesting to address these possibilities experimentally.

Because facultative endosymbionts can influence several ecologically relevant traits of their aphid hosts (Oliver et al., 2010), their presence may also impose ecological costs or trade-offs in addition to the physiological cost just described. There are a number of intriguing observations suggesting that this is indeed the case. For example, Dion et al. (2011b) reported that pea aphids enjoying increased resistance to parasitoids from harbouring *H. defensa* reduced costly behavioural defences such as dropping off the host plant. This might entail an increased susceptibility to other natural enemies, particularly predators. An

ecological cost might also arise if defensive symbionts influence the aphids' interaction with other mutualists like ants, which is an understudied question in my opinion (but see Erickson et al., 2012). Many aphid species are facultatively or obligately tended by ants, which provide protection against natural enemies in exchange for honeydew. If the possession of defensive symbionts somehow impaired the aphids' attractiveness to ants, this might reduce attendance and hence protection.

Another important observation in this context is the link between host plants and the facultative symbionts of associated aphid populations. A number of aphid species have diversified into host-associated lineages that are variably referred to as host races or subspecies, e.g. the pea aphid (Via, 1991, Peccoud et al., 2009), the black bean aphid (Müller, 1982), or the cowpea aphid, *A. craccivora* (Coeur d'Acier et al., 2007). In these cases, the frequencies of infection with different facultative symbionts vary significantly among populations from different host plants (e.g. Ferrari et al., 2012, Rouchet, 2012, Brady & White, 2013, Russell et al., 2013). If host-associated populations are reproductively isolated from each other, this may simply reflect historical legacies or drift effects, but there are indications that facultative symbionts influence the fitness on particular host plants. For example, infection with *R. insecticola* can improve pea aphid performance on clover (Tsuchida et al., 2004), although the generality of this effect is still debated (Leonardo, 2004, Ferrari et al., 2007, McLean et al., 2011). Of particular relevance here is that the frequency of infection with *H. defensa* in European pea aphids can range from very low (< 5%) on some plants such as peas (*Pisum sativum*) to rather high (~ 70%) on other plants such as alfalfa (*Medicago sativa*) (Ferrari et al., 2012). This is at least suggestive of this defensive symbiont also playing a functional role in host plant use and specialization (Ferrari & Vavre, 2011). An increase in the frequency of *H. defensa* in response to selection by parasitoids may thus potentially compromise performance on particular host plants. This hypothetical example

serves to illustrate the more general point that if defensive symbionts influence more than one host trait simultaneously, as appears to be the case (Oliver et al., 2010), the increased resistance to parasitoids they provide will be associated with other phenotypic effects that may be maladaptive in a given environment and thus equate to an ecological cost of symbiont-conferred resistance.

All of the known or hypothesized costs of symbiont-conferred resistance I mentioned above are constitutive or standing costs in the jargon of ecological immunology (Kraaijeveld et al., 2002), also referred to as evolutionary costs (Schmid-Hempel, 2003). Such costs arise from having the ability to resist parasitoids, here from possessing defensive symbionts. They are also incurred in the absence of parasitoids and can therefore select against protection when the risk of parasitism is low. Distinct are the induced costs of actually using a defence when attacked (Kraaijeveld et al., 2002, Schmid-Hempel, 2003). They can also influence the evolution of resistance in that they reduce the benefits of increased resistance if successfully resisting a parasitoid entails a strong reduction in the aphids' residual reproductive value (Vorburger et al., 2008). It is currently unclear whether the defence provided by symbionts in aphids is persistent or to some extent turned on when an aphid is attacked, which will be an important question to address. Induced costs could feasibly occur if symbionts increased their density or the release of toxins when their host was attacked, and a mathematical model has shown that induced costs of symbiont-conferred resistance could facilitate the coexistence of protected and unprotected hosts (Kwiatkowski & Vorburger, 2012), which is what we typically observe in aphids. However, a first test with black bean aphids did not provide any evidence for induced costs of resistance conferred by *H. defensa* (Vorburger et al., 2013). On the contrary, aphids harbouring a strongly protective isolate of *H. defensa* enjoyed an increase in longevity and lifetime reproduction after resisting one parasitoid attack compared to control individuals that were not attacked. This was a surprising observation and it is yet

448 unclear whether it can be generalized to other aphid species or symbiont strains, so the
449 question if there are any induced costs of symbiont-conferred resistance remains open.
450 Nevertheless, at least for the best-studied defensive symbiont of aphids, *H. defensa*,
451 evidence is accumulating that its possession is associated with costs to the host. These
452 include physiological costs affecting aphid life-history traits, as well as other phenotypic
453 effects that – depending on the aphids' environment – could feasibly translate into a selective
454 disadvantage. Such costs not only help to explain why defensive symbionts do not go to
455 fixation in natural populations of aphids, they could also help to explain the large variation in
456 symbiont complements, e.g. among host-associated populations, because the context-
457 dependence of the potentially manifold ecological costs implies that the net benefit or cost of
458 harbouring a particular defensive symbiont will be environment-specific.
459
460 **Defensive symbionts modify host-parasitoid coevolution**
461
462 The strong reciprocal selection between aphids and their parasitoids is expected to result in
463 intense and dynamic coevolution. It is thus fair to ask if and how host-parasitoid coevolution
464 is influenced by the presence of defensive symbionts in the host. The present understanding
465 of antagonistic coevolution between hosts and parasites has benefitted greatly from the
466 development of genetic models that are based on so-called interaction loci (reviewed in
467 Salathé et al., 2008). Such models assume that host resistance and parasite infectivity are
468 genetically determined traits and that the probability of infection is determined by the
469 combination of host and parasite genotypes according to some interaction model like the
470 gene-for-gene model (GFG, Flor, 1971) or the matching alleles model (MA, Frank, 1993). It
471 was later shown that these models can be regarded as the endpoints of a continuum from high
472 (MA) to low (GFG) specficity of the host-parasite interaction (Agrawal & Lively, 2002). One

473 important lesson from these models was that if the host-parasite interaction shows strong
474 genetic specificity and/or if increased resistance or infectivity come at a cost, host-parasite
475 systems will exhibit negative frequency-dependence. This is a powerful mechanism to
476 maintain genetic variation, because in both antagonists, rare genotypes will be favoured and
477 common genotypes disfavoured by selection (e.g. Judson, 1995, Howard & Lively, 2002).
478 More generally, these models have shown that there are three main determinants of the
479 dynamics of host-parasite coevolution: (i) the genetic variation available to selection, (ii) the
480 degree of genetic specificity in the interaction, and (iii) the costs or trade-offs associated with
481 increased resistance and infectivity, respectively. This list immediately implies that defensive
482 symbionts do indeed have the potential to influence the coevolutionary dynamics, because
483 they have an impact on all three of these attributes.
484 (i) Defensive symbionts clearly increase the heritable variation for resistance to parasitoids
485 in host populations. Although aphid populations do exhibit significant clonal variation for
486 resistance to parasitoids in the absence of symbionts (von Burg et al., 2008, Sandrock et al.,
487 2010), this variation is of smaller magnitude than the differences between uninfected clones
488 and clones harbouring a defensive symbiont (Oliver et al., 2005, von Burg et al., 2008,
489 Vorburger et al., 2009).
490 (ii) Defensive symbionts increase the genetic specificity of the host-parasitoid interactions.
491 This is demonstrated most clearly in the *Ap. fabae*/*L. fabarum* system. When the aphids are
492 uninfected with *H. defensa*, there are no significant interactions between aphid clones and
493 parasitoid lines on the observed rates of parasitism (Sandrock et al., 2010). Hence, there is no
494 evidence for genotype-specificity in their interaction. When the aphids do harbour *H.*
495 *defensa*, on the other hand, strong host clone-by-parasitoid line interactions are observed
496 (Rouchet & Vorburger, 2012), and this specificity has been demonstrated to result from a

497 genotype-by-genotype interaction beween the parasitoids and the defensive symbionts of
498 their hosts (Schmid et al., 2012).
499 (iii) Defensive symbionts induce costs of resistance. The added line of defence provided by
500 *H. defensa* (and possibly other defensive symbionts) comes at its own physiological costs to
501 the aphid host and potential trade-offs with other ecologically relevant traits that are
502 concurrently influenced by the symbiont (see above).
503 Taken together, these findings imply that defensive symbionts alter the reciprocal selection
504 between aphids and parasitoids, supplying the ingredients for very dynamic coevolution
505 driven by frequency-dependence. This notion is supported by a recent model that
506 incorporates defensive symbionts in host-parasite interactions (Kwiatkowski et al., 2012).
507 Particularly if the interaction between parasites and defensive symbionts is more specific than
508 that between parasitoids and the host's own defences (the situation observed in the *Ap.*
509 *fabae*/*L. fabarum* system), the symbionts can drive coevolutionary genetic cycling, also
510 referred to as 'Red Queen dynamics' (Woolhouse et al., 2002). If sufficient horizontal
511 transmission is allowed for, symbionts may even take over the coevolutionary interaction
512 with the parasite, at the expense of genetic variation in the host population (Kwiatkowski et
513 al., 2012). The ever-increasing ability to genotype parasitoids, hosts and their symbionts with
514 high throughput and resolution might soon allow the observation of such dynamics in real
515 time. It will also be interesting to study the parasitoids' counteradaptations to the presence of
516 defensive symbionts in their hosts. Parasitoids can be expected to show plastic behavioural
517 responses to the presence of symbionts as well as to evolve adaptations that reduce the
518 suscpetibility of their eggs or larvae to the symbiont-conferred defence mechanisms. An
519 experiment by Oliver et al. (2012) indicates that the parasitoid *A. ervi* is able to detect the
520 presence of *H. defensa* in pea aphids and responds by laying two or more eggs in infected
521 aphids to increase the chance of successful parasitism despite the defensive symbiont. A very

522 recent study by Łukasik et al. (2013a) also found that the parasitoids *A. ervi* and *E. plagiator*
523 are able to distinguish infected from uninfected grain aphids (*S. avenae*), yet there the
524 response was reduced attacks on aphids possessing *H. defensa*. Clearly, much is still to be
525 learnt about how parasitoids cope with symbiont-conferred resistance and it will be exciting
526 to find out how increased infectivity on protected aphids, which can evolve surprisingly
527 quickly (Dion et al., 2011a, Rouchet, 2012), is achieved mechanistically.
528
529 **Implications for biological control**
530
531 Several species of aphidiine parasitoids are available commercially and employed for
532 biological control of pest aphids in greenhouses. Even though this has so far been
533 demonstrated at a smaller scale of population cages only (Herzog et al., 2007, Oliver et al.,
534 2008), it is reasonable to assume that such biocontrol releases impose strong selection for
535 clones that are protected by defensive symbionts. Similar to the notorious evolution of
536 insecticide resistance (Foster et al., 2007), the application of parasitoids for aphid control may
537 lead to increased levels of symbiont-conferred resistance in pest aphids and thus compromise
538 the efficiency of biocontrol. Although it is reassuring that harbouring defensive symbionts is
539 associated with costs to the aphid host (see above), the same is true for many forms of
540 insecticide resistance (Foster et al., 2000), yet this could not prevent the evolution of high
541 levels of resistance in intensively treated areas (e.g. Foster et al., 1998).
542 Could the problem be avoided by foresighted breeding of parasitoids for biocontrol
543 releases? After all, parasitoid populations harbour genetic variation for the ability to
544 overcome symbiont-conferred resistance (Rouchet & Vorburger, 2012, Schmid et al., 2012),
545 and they can be selected for higher infectivity on protected aphids (Dion et al., 2011a,
546 Rouchet, 2012). This could be achieved if producers of parasitoids for biocontrol bred their

stocks on hosts with defensive symbionts. However, since this is likely to make mass rearing more difficult and less yielding, at least initially, it may not be a commercially viable solution. Alternatively, users could rely on a high evolvability of released parasitoids, such that an increase in the frequency of infection with defensive symbionts is tracked by counteradaptations in the parasitoids (coevolution). The speed at which parasitoids evolved higher infectivity on symbiont-protected hosts in the study by Dion et al. (2011a) gives reason for optimism that this might be at least partially successful. It would, however, require a conscious effort to maintain the necessary genetic variation in the parastoids bred for biocontrol rather than relying on a few easy-to-breed stocks. This could be achieved by founding populations from many sources and maintaining them at high effective population size, by repeatedly introgressing new genetic material, or by keeping different stocks separately and mixing them prior to greenhouse releases. While it is still too early to sketch optimal solutions, I think there is now sufficient evidence to recognize defensive symbionts of aphids as a likely problem for biological control, albeit one that may be mitigated by clever breeding of biocontrol agents.

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References

Agrawal, A. and Lively, C.M. (2002) Infection genetics: gene-for-gene versus matching-alleles models and all points in between. *Evolutionary Ecology Research,* **4**, 79-90.

573 Baumann, P., Moran, N.A. and Baumann, L. (2000) Bacteriocyte-associated endosymbionts of
574 insects. *The Prokaryotes.* (ed. M. Dworkins), pp. 1-55. Springer, New York.
575 Bensadia, F., Boudreault, S., Guay, J.F., Michaud, D. and Cloutier, C. (2006) Aphid clonal resistance
576 to a parasitoid fails under heat stress. *Journal of Insect Physiology,* **52**, 146-157.
577 Boivin, G., Hance, T. and Brodeur, J. (2012) Aphid parasitoids in biological control. *Canadian*
578 *Journal of Plant Science,* **92**, 1-12.
579 Brady, C.M. and White, J.A. (2013) Cowpea aphid (*Aphis craccivora*) associated with different host
580 plants has different facultative endosymbionts. *Ecological Entomology,* **38**, 433-437.
581 Buchner, P. (1965) *Endosymbiosis of Animals with Plant Microorganisms,* Interscience, New York.
582 Caspi-Fluger, A., Inbar, M., Mozes-Daube, N., Katzir, N., Portnoy, V., Belausov, E., Hunter, M.S.
583 and Zchori-Fein, E. (2012) Horizontal transmission of the insect symbiont *Rickettsia* is plant-
584 mediated. *Proceedings of the Royal Society B-Biological Sciences,* **279**, 1791-1796.
585 Cayetano, L. and Vorburger, C. (2013) Genotype-by-genotype specificity remains robust to average
586 temperature variation in an aphid/endosymbiont/parasitoid system. *Journal of Evolutionary*
587 *Biology,* **26**, 1603-1610.
588 Chen, D.Q. and Purcell, A.H. (1997) Occurrence and transmission of facultative endosymbionts in
589 aphids. *Current Microbiology,* **34**, 220-225.
590 Coeur D'acier, A.C., Jousselin, E., Martin, J.F. and Rasplus, J.Y. (2007) Phylogeny of the genus *Aphis*
591 Linnaeus, 1758 (Homoptera : Aphididae) inferred from mitochondrial DNA sequences. *Molecular*
592 *Phylogenetics and Evolution,* **42**, 598-611.
593 Darby, A.C. and Douglas, A.E. (2003) Elucidation of the transmission patterns of an insect-borne
594 bacterium. *Applied and Environmental Microbiology,* **69**, 4403-4407.
595 Degnan, P.H. and Moran, N.A. (2008) Diverse phage-encoded toxins in a protective insect
596 endosymbiont. *Applied and Environmental Microbiology,* **74**, 6782-6791.
597 Dion, E., Zele, F., Simon, J.C. and Outreman, Y. (2011a) Rapid evolution of parasitoids when faced
598 with the symbiont-mediated resistance of their hosts. *Journal of Evolutionary Biology,* **24**, 741-
599 750.
600 Dion, E., Polin, S.E., Simon, J.C. and Outreman, Y. (2011b) Symbiont infection affects aphid
601 defensive behaviours. *Biology Letters,* **7**, 743-746.
602 Duron, O. and Hurst, G.D.D. (2013) Arthropods and inherited bacteria: from counting the symbionts
603 to understanding how symbionts count. *Bmc Biology,* **11**.
604 Erickson, D.M., Wood, E.A., Oliver, K.M., Billick, I. and Abbot, P. (2012) The effect of ants on the
605 population dynamics of a protective symbiont of aphids, *Hamiltonella defensa*. *Annals of the*
606 *Entomological Society of America,* **105**, 447-453.
607 Ferrari, J. and Vavre, F. (2011) Bacterial symbionts in insects or the story of communities affecting
608 communities. *Philosophical Transactions of the Royal Society B-Biological Sciences,* **366**, 1389-
609 1400.

610 Ferrari, J., Scarborough, C.L. and Godfray, H.C.J. (2007) Genetic variation in the effect of a
611 facultative symbiont on host-plant use by pea aphids. *Oecologia,* **153**, 323-329.
612 Ferrari, J., Müller, C.B., Kraaijeveld, A.R. and Godfray, H.C.J. (2001) Clonal variation and
613 covariation in aphid resistance to parasitoids and a pathogen. *Evolution,* **55**, 1805-1814.
614 Ferrari, J., West, J.A., Via, S. and Godfray, H.C.J. (2012) Population genetic structure and secondary
615 symbionts in host-associated populations of the pea aphid complex. *Evolution,* **66**, 375-390.
616 Ferrari, J., Darby, A.C., Daniell, T.J., Godfray, H.C.J. and Douglas, A.E. (2004) Linking the bacterial
617 community in pea aphids with host-plant use and natural enemy resistance. *Ecological*
618 *Entomology,* **29**, 60-65.
619 Flor, H.H. (1971) Current status of the gene-for-gene concept. *Annual Review of Phytopathology,* **9**,
620 275-296.
621 Foster, S.P., Denholm, I. and Devonshire, A.L. (2000) The ups and downs of insecticide resistance in
622 peach-potato aphids (Myzus persicae) in the UK. *Crop Protection,* **19**, 873-879.
623 Foster, S.P., Devine, G. and Devonshire, A.L. (2007) Insecticide resistance. *Aphids as Crop Pests.*
624 eds. H. Van Emden & R. Harringtons), pp. 261-285. CAB International, Wallingford, UK.
625 Foster, S.P., Denholm, I., Harling, Z.K., Moores, G.D. and Devonshire, A.L. (1998) Intensification of
626 insecticide resistance in UK field populations of the peach-potato aphid, *Myzus persicae*
627 (Hemiptera : Aphididae) in 1996. *Bulletin of Entomological Research,* **88**, 127-130.
628 Frank, S.A. (1993) Specificity versus detectable polymorphism in host-parasite genetics. *Proceedings*
629 *of the Royal Society B-Biological Sciences,* **254**, 191-197.
630 Frantz, A., Calcagno, V., Mieuzet, L., Plantegenest, M. and Simon, J.C. (2009) Complex trait
631 differentiation between host-populations of the pea aphid *Acyrthosiphon pisum* (Harris):
632 implications for the evolution of ecological specialisation. *Biological Journal of the Linnean*
633 *Society,* **97**, 718-727.
634 Gehrer, L. and Vorburger, C. (2012) Parasitoids as vectors of facultative bacterial endosymbionts in
635 aphids. *Biology Letters,* **8**, 613-615.
636 Griffiths, D.C. (1960) Immunity of aphids to insect parasites. *Nature,* **187**, 346-346.
637 Gross, P. (1993) Insect behavioral and morphological defenses against parasitoids. *Annual Review of*
638 *Entomology,* **38**, 251-273.
639 Guay, J.F., Boudreault, S., Michaud, D. and Cloutier, C. (2009) Impact of environmental stress on
640 aphid clonal resistance to parasitoids: Role of *Hamiltonella defensa* bacterial symbiosis in
641 association with a new facultative symbiont of the pea aphid. *Journal of Insect Physiology,* **55**,
642 919-926.
643 Hansen, A.K., Vorburger, C. and Moran, N.A. (2012) Genomic basis of endosymbiont-conferred
644 protection against an insect parasitoid. *Genome Research,* **22**, 106-114.
645 Henter, H.J. (1995) The potential for coevolution in a host-parasitoid system. II. Genetic variation
646 within a population of wasps in the ability to parasitize an aphid host. *Evolution,* **49**, 439-445.

Henter, H.J. and Via, S. (1995) The potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. *Evolution,* **49**, 427-438.

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

Howard, R.S. and Lively, C.M. (2002) The Ratchet and the Red Queen: the maintenance of sex in parasites. *Journal of Evolutionary Biology,* **15**, 648-656.

Jaenike, J., Polak, M., Fiskin, A., Helou, M. and Minhas, M. (2007) Interspecific transmission of endosymbiotic *Spiroplasma* by mites. *Biology Letters,* **3**, 23-25.

Jones, E.O., White, A. and Boots, M. (2007) Interference and the persistence of vertically transmitted parasites. *Journal of Theoretical Biology,* **246**, 10-17.

Jones, E.O., White, A. and Boots, M. (2011) The evolution of host protection by vertically transmitted parasites. *Proceedings of the Royal Society B-Biological Sciences,* **278**, 863-870.

Judson, O.P. (1995) Preserving genes - a model of the maintenance of genetic variation in a metapopulation under frequency-dependent selection. *Genetical Research,* **65**, 175-191.

Kraaijeveld, A.R., Ferrari, J. and Godfray, H.C.J. (2002) Costs of resistance in insect-parasite and insect-parasitoid interactions. *Parasitology,* **125**, S71-S82.

Kwiatkowski, M. and Vorburger, C. (2012) Modeling the ecology of symbiont-mediated protection against parasites. *The American Naturalist,* **179**, 595-605.

Kwiatkowski, M., Engelstadter, J. and Vorburger, C. (2012) On genetic specificity in symbiont-mediated host-parasite coevolution. *PLoS computational biology,* **8**, e1002633.

Kyei-Poku, G.K., Floate, K.D., Benkel, B. and Goettel, M.S. (2003) Elimination of *Wolbachia* from *Urolepis rufipes* (Hymenoptera : Pteromalidae) with heat and antibiotic treatments: Implications for host reproduction. *Biocontrol Science and Technology,* **13**, 341-354.

Le Ralec, A., Anselme, C., Outreman, Y., Poirié, M., Van Baaren, J., Le Lann, C. and Van Alphen, J.J.M. (2010) Evolutionary ecology of the interactions between aphids and their parasitoids. *Comptes Rendus Biologies,* **333**, 554-565.

Leonardo, T.E. (2004) Removal of a specialization-associated symbiont does not affect aphid fitness. *Ecology Letters,* **7**, 461-468.

Li, S., Falabella, P., Giannantonio, S., Fanti, P., Battaglia, D., Digilio, M.C., Volkl, W., Sloggett, J.J., Weisser, W. and Pennacchio, F. (2002) Pea aphid clonal resistance to the endophagous parasitoid *Aphidius ervi*. *Journal of Insect Physiology,* **48**, 971-980.

Lively, C.M., Clay, K., Wade, M.J. and Fuqua, C. (2005) Competitive co-existence of vertically and horizontally transmitted parasites. *Evolutionary Ecology Research,* **7**, 1183-1190.

Łukasik, P., Dawid, M.A., Ferrari, J. and Godfray, H.C.J. (2013a) The diversity and fitness effects of infection with facultative endosymbionts in the grain aphid, *Sitobion avenae*. *Oecologia*, 985-906.

Łukasik, P., Van Asch, M., Guo, H.F., Ferrari, J. and Godfray, H.C.J. (2013b) Unrelated facultative endosymbionts protect aphids against a fungal pathogen. *Ecology Letters,* **16**, 214-218.

Mclean, A.H.C., Van Asch, M., Ferrari, J. and Godfray, H.C.J. (2011) Effects of bacterial secondary symbionts on host plant use in pea aphids. *Proceedings of the Royal Society B-Biological Sciences,* **278**, 760-766.

Montllor, C.B., Maxmen, A. and Purcell, A.H. (2002) Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. *Ecological Entomology,* **27**, 189-195.

Moran, N.A. and Dunbar, H.E. (2006) Sexual acquisition of beneficial symbionts in aphids. *Proceedings of the National Academy of Sciences of the United States of America,* **103**, 12803-12806.

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

Moran, N.A., Degnan, P.H., Santos, S.R., Dunbar, H.E. and Ochman, H. (2005b) The players in a mutualistic symbiosis: Insects, bacteria, viruses, and virulence genes. *Proceedings of the National Academy of Sciences of the United States of America,* **102**, 16919-16926.

Müller, F.P. (1982) Das Problem *Aphis fabae*. *Zeitschrift für angewandte Entomologie,* **94**, 432-446.

Noyes, J.S. (2012) Universal Chalcidoidea Database. World Wide Web Electronic Publication. http://www.nhm.ac.uk/chalcidoids.

Nyabuga, F.N., Outreman, Y., Simon, J.C., Heckel, D.G. and Weisser, W.W. (2010) Effects of pea aphid secondary endosymbionts on aphid resistance and development of the aphid parasitoid *Aphidius ervi*: a correlative study. *Entomologia Experimentalis Et Applicata,* **136**, 243-253.

Oliver, K.M., Moran, N.A. and Hunter, M.S. (2005) Variation in resistance to parasitism in aphids is due to symbionts not host genotype. *Proceedings of the National Academy of Sciences of the United States of America,* **102**, 12795-12800.

Oliver, K.M., Moran, N.A. and Hunter, M.S. (2006) Costs and benefits of a superinfection of facultative symbionts in aphids. *Proceedings of the Royal Society B-Biological Sciences,* **273**, 1273-1280.

Oliver, K.M., Smith, A.H. and Russell, J.A. (2013) Defensive symbiosis in the real world - advancing ecological studies of heritable, protective bacteria in aphids and beyond. *Functional Ecology*, in press.

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

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

Oliver, K.M., Degnan, P.H., Hunter, M.S. and Moran, N.A. (2009) Bacteriophages encode factors required for protection in a symbiotic mutualism. *Science,* **325**, 992-994.

720 Oliver, K.M., Degnan, P.H., Burke, G.R. and Moran, N.A. (2010) Facultative symbionts in aphids and
721 the horizontal transfer of ecologically important traits. *Annual Review of Entomology,* **55**, 247-266.
722 Oliver, K.M., Noge, K., Huang, E.M., Campos, J.M., Becerra, J.X. and Hunter, M.S. (2012) Parasitic
723 wasp responses to symbiont-based defense in aphids. *BMC Biology,* **10**, 11.
724 Peccoud, J., Ollivier, A., Plantegenest, M. and Simon, J.C. (2009) A continuum of genetic divergence
725 from sympatric host races to species in the pea aphid complex. *Proceedings of the National*
726 *Academy of Sciences of the United States of America,* **106**, 7495-7500.
727 Poirié, M. and Coustau, C. (2011) The evolutionary ecology of aphids' immunity. *Isj-Invertebrate*
728 *Survival Journal,* **8**, 247-255.
729 Rouchet, R. (2012) Host-parasitoid coevolution: The role of parasitoid adaptation to endosymbiont-
730 mediated defence in aphids. PhD thesis. p. 102. ETH Zürich, Zürich, Switzerland.
731 Rouchet, R. and Vorburger, C. (2012) Strong specificity in the interaction between parasitoids and
732 symbiont-protected hosts. *Journal of Evolutionary Biology,* **25**, 2369-2375.
733 Russell, J.A. and Moran, N.A. (2006) Costs and benefits of symbiont infection in aphids: variation
734 among symbionts and across temperatures. *Proceedings of the Royal Society B-Biological*
735 *Sciences,* **273**, 603-610.
736 Russell, J.A., Latorre, A., Sabater-Muñoz, B., Moya, A. and Moran, N.A. (2003) Side-stepping
737 secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea.
738 *Molecular Ecology,* **12**, 1061-1075.
739 Russell, J.A., Weldon, S., Smith, A.H., Kim, K.L., Hu, Y., Lukasik, P., Doll, S., Anastopoulos, I.,
740 Novin, M. and Oliver, K.M. (2013) Uncovering symbiont-driven genetic diversity across North
741 American pea aphids. *Molecular Ecology,* **22**, 2045-2059.
742 Salathé, M., Kouyos, R.D. and Bonhoeffer, S. (2008) The state of affairs in the kingdom of the Red
743 Queen. *Trends in Ecology & Evolution,* **23**, 439-445.
744 Sandrock, C., Gouskov, A. and Vorburger, C. (2010) Ample genetic variation but no evidence for
745 genotype specificity in an all-parthenogenetic host-parasitoid interaction. *Journal of Evolutionary*
746 *Biology,* **23**, 578-585.
747 Sandström, J.P., Russell, J.A., White, J.P. and Moran, N.A. (2001) Independent origins and horizontal
748 transfer of bacterial symbionts of aphids. *Molecular Ecology,* **10**, 217-228.
749 Scarborough, C.L., Ferrari, J. and Godfray, H.C.J. (2005) Aphid protected from pathogen by
750 endosymbiont. *Science,* **310**, 1781-1781.
751 Schmid-Hempel, P. (2003) Variation in immune defence as a question of evolutionary ecology.
752 *Proceedings of the Royal Society of London Series B-Biological Sciences,* **270**, 357-366.
753 Schmid, M., Sieber, R., Zimmermann, Y.S. and Vorburger, C. (2012) Development, specificity and
754 sublethal effects of symbiont-conferred resistance to parasitoids in aphids. *Functional Ecology,* **26**,
755 207-215.

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

Schmitz, A., Anselme, C., Ravallec, M., Rebuf, C., Simon, J.C., Gatti, J.L. and Poirié, M. (2012) The cellular immune response of the pea aphid to foreign intrusion and symbiotic challenge. *PLoS One,* **7**, e42114.

Sigsgaard, L. (2002) A survey of aphids and aphid parasitoids in cereal fields in Denmark, and the parasitoids' role in biological control. *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie,* **126**, 101-107.

Simon, J.C., Boutin, S., Tsuchida, T., Koga, R., Le Gallic, J.F., Frantz, A., Outreman, Y. and Fukatsu, T. (2011) Facultative symbiont infections affect aphid reproduction. *PLoS One,* **6**, e21831.

Starý, P. (1988) Aphidiidae. *Aphids: Their Biology, Natural Enemies, and Control. Vol. 2B.* eds. A.K. Minks & P. Harrewijns), pp. 171-184. Elsevier, Amsterdam.

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

Stouthamer, R., Breeuwer, J.a.J. and Hurst, G.D.D. (1999) *Wolbachia pipientis*: Microbial manipulator of arthropod reproduction. *Annual Review of Microbiology,* **53**, 71-102.

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

Tsuchida, T., Koga, R., Shibao, H., Matsumoto, T. and Fukatsu, T. (2002) Diversity and geographic distribution of secondary endosymbiotic bacteria in natural populations of the pea aphid, *Acyrthosiphon pisum*. *Molecular Ecology,* **11**, 2123-2135.

Van Der Wilk, F., Dullemans, A.M., Verbeek, M. and Van Den Heuvel, J.F.J.M. (1999) Isolation and characterization of APSE-1, a bacteriophage infecting the secondary endosymbiont of *Acyrthosiphon pisum*. *Virology,* **262**, 104-113.

Van Lenteren, J.C., Drost, Y.C., Van Roermund, H.J.W. and Posthuma-Doodeman, C.J.a.M. (1997) Aphelinid parasitoids as sustainable biological control agents in greenhouses. *Journal of Applied Entomology,* **121**, 473-485.

Van Opijnen, T. and Breeuwer, J.a.J. (1999) High temperatures eliminate *Wolbachia*, a cytoplasmic incompatibility inducing endosymbiont, from the two-spotted spider mite. *Experimental and Applied Acarology,* **23**, 871-881.

Via, S. (1991) Specialized host plant performance of pea aphid clones is not altered by experience. *Ecology,* **72**, 1420-1427.

Von Burg, S., Ferrari, J., Müller, C.B. and Vorburger, C. (2008) Genetic variation and covariation of susceptibility to parasitoids in the aphid *Myzus persicae* – no evidence for trade-offs. *Proceedings of the Royal Society B-Biological Sciences,* **275**, 1089-1094.

792 Vorburger, C. and Gouskov, A. (2011) Only helpful when required: A longevity cost of harbouring
793 defensive symbionts. *Journal of Evolutionary Biology,* **24**, 1611-1617.
794 Vorburger, C., Gouskov, A. and Von Burg, S. (2008) Genetic covariation between effectiveness and
795 cost of defence in aphids. *Biology Letters,* **4**, 674-676.
796 Vorburger, C., Gehrer, L. and Rodriguez, P. (2010) A strain of the bacterial symbiont *Regiella*
797 *insecticola* protects aphids against parasitoids. *Biology Letters,* **6**, 109-111.
798 Vorburger, C., Ganesanandamoorthy, P. and Kwiatkowski, M. (2013) Comparing constitutive and
799 induced costs of symbiont-conferred resistance to parasitoids in aphids. *Ecology and Evolution,* **3**,
800 706-713.
801 Vorburger, C., Sandrock, C., Gouskov, A., Castañeda, L.E. and Ferrari, J. (2009) Genotypic variation
802 and the role of defensive endosymbionts in an all-parthenogenetic host-parasitoid interaction.
803 *Evolution,* **63**, 1439-1450.
804 Weldon, S.R., Strand, M.R. and Oliver, K.M. (2013) Phage loss and the breakdown of a defensive
805 symbiosis in aphids. *Proceedings of the Royal Society B-Biological Sciences,* **280**.
806 Werren, J.H., Baldo, L. and Clark, M.E. (2008) *Wolbachia*: master manipulators of invertebrate
807 biology. *Nature Reviews Microbiology,* **6**, 741-751.
808 Woolhouse, M.E.J., Webster, J.P., Domingo, E., Charlesworth, B. and Levin, B.R. (2002) Biological
809 and biomedical implications of the co-evolution of pathogens and their hosts. *Nature Genetics,* **32**,
810 569-577.
811
812

Figure captions

Fig. 1. Main factors affecting the frequency of infection with defensive symbionts (S) in aphid populations either positively (green arrows) or negatively (red arrows).

Fig. 2. Protection against parasitoids by a defensive symbiont. Average percentage of successful parasitism by the aphid parasitoid *Lysiphlebus fabarum* in clones of the black bean aphid (*Aphis fabae*) that did or did not harbour natural infections with the defensive endosymbiont *Hamiltonella defensa*. Modified from Vorburger et al. (2009).

Fig. 3. *Hamiltonella defensa* reduces aphid lifespan in the absence of parasitoids. Survivorship curves of black bean aphids (*Aphis fabae*) without any facultative symbiont (their natural state) and of aphids harbouring experimental infections with *H. defensa*. The figure summarizes the results over two different genetic backgrounds (clones) and six different isolates of *H. defensa*. Modified from Vorburger & Gouskov (2011).

Table 1. Facultative bacterial endosymbionts of aphids shown to increase host resistance to parasitoids by comparing parasitism between infected and uninfected sublines of the same aphid clones.

Symbiont	Aphid host	Protection against	References
Hamiltonella defensa	*Acyrthosiphon pisum*	*Aphidius ervi*	Oliver et al. (2003; 2005)
	Aphis fabae	*Lysiphlebus fabarum*	Schmid et al. (2012)
Serratia symbiotica	*Acyrthosiphon pisum*	*Aphidius ervi*	Oliver et al. (2003)
*Regiella insecticola**	*Myzus persicae*	*Aphidius colemani*	Vorburger et al. (2010)
	Aphis fabae	*Aphidius colemani*	Vorburger et al. (2010)
	Acyrthosiphon pisum	*Aphidius ervi*	Hansen et al. (2012)

* so far only one strain was found to be protective

Table 2. Routes of horizontal transmission of facultative bacterial symbionts in aphids.

Route/mechanism	Aphid host	Symbiont	References
Sexual transfer (male to female)	*Acyrthosiphon pisum*	*Hamiltonella defensa* *Regiella insecticola*	Moran & Dunbar (2006)
Transfer by parasitoids (*L. fabarum* & *A. colemani*)	*Aphis fabae*	*Hamiltonella defensa* *Regiella insecticola*	Gehrer & Vorburger (2012)
Ingestion*	*Acyrthosiphon pisum* *Aphis fabae*	*Hamiltonella defensa*	Darby & Douglas (2003)

* so far only demonstrated for symbionts added to artificial diet

Fig. 1

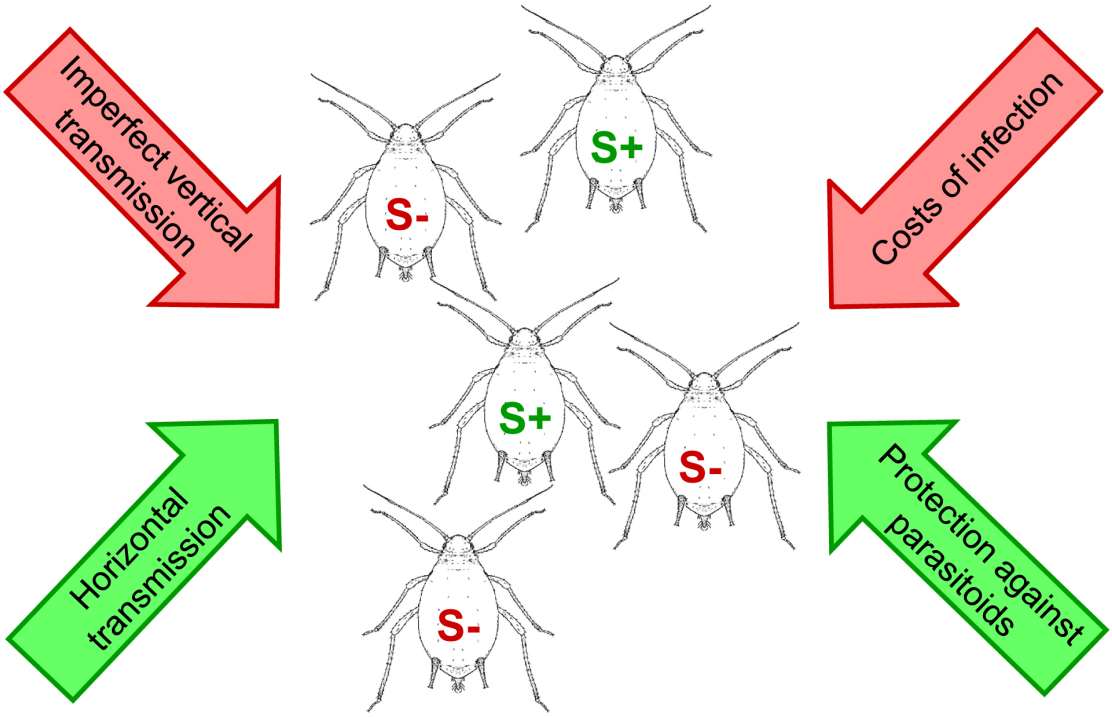

Imperfect vertical transmission
Costs of infection
Horizontal transmission
Protection against parasitoids
S-
S+
S+
S-
S-

Fig. 2

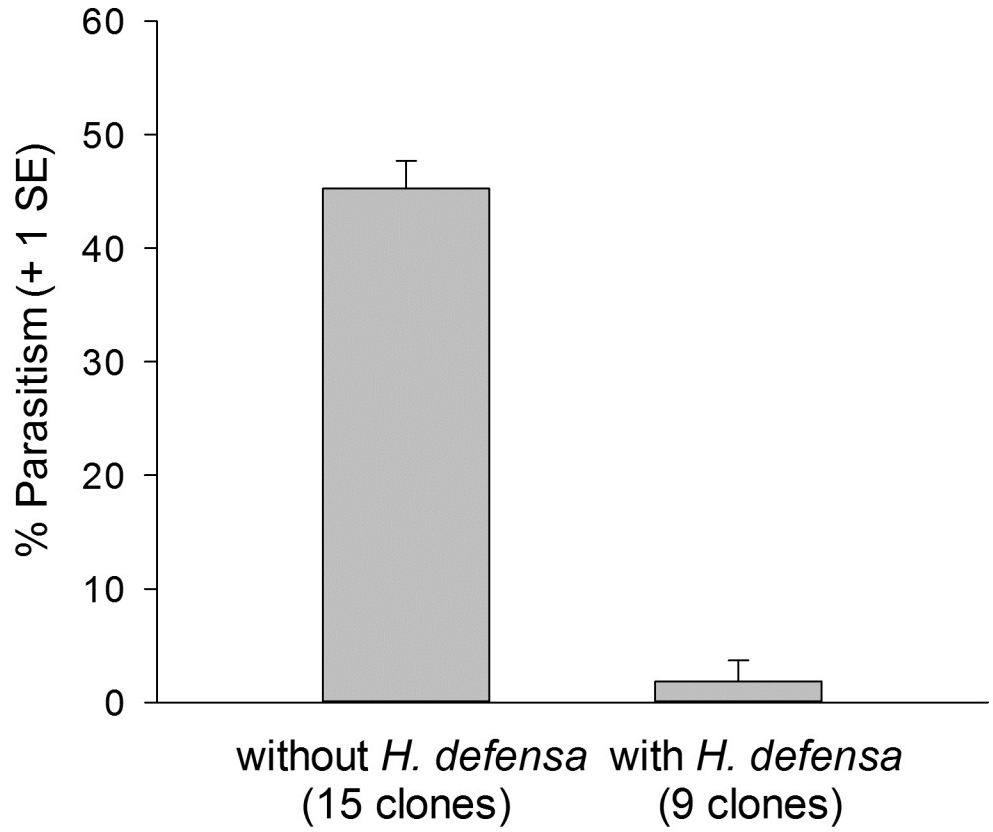
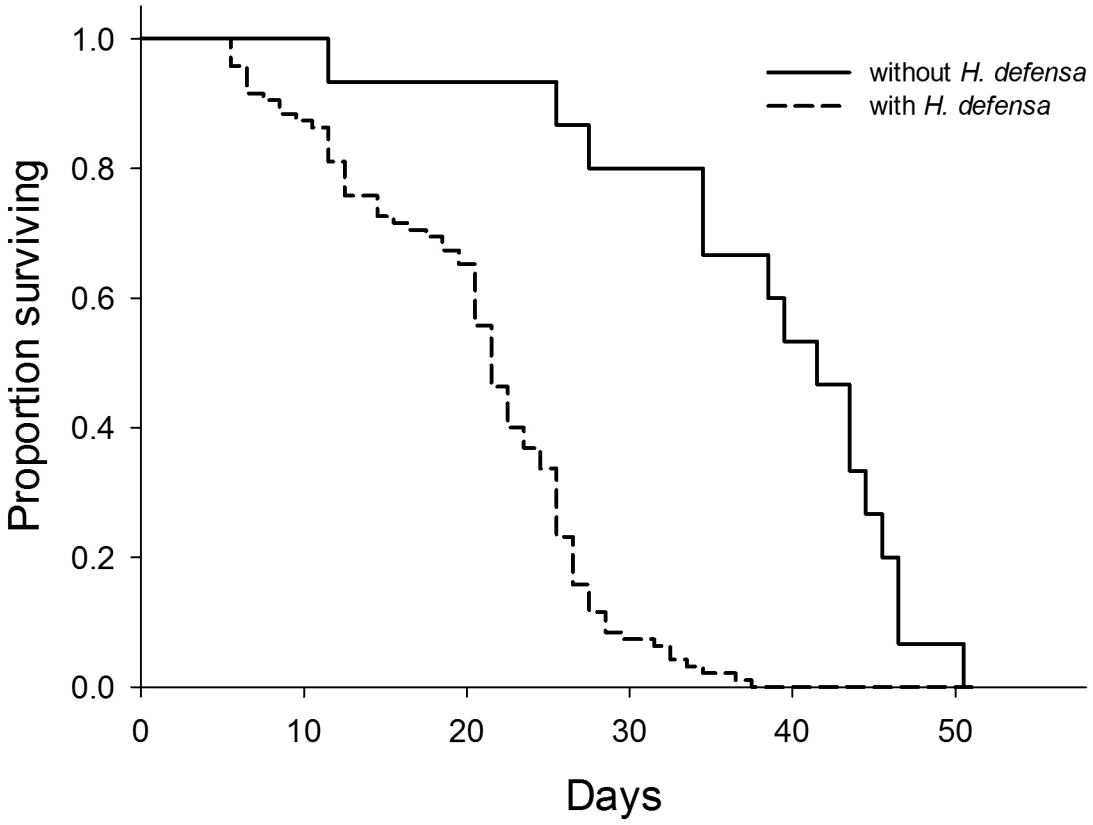

60
50
40
30
20
10
0
% Parasitism (+ 1 SE)
without H. defensa
(15 clones)
with H. defensa
(9 clones)

Fig. 3


1.0
0.8
0.6
0.4
0.2
0.0
Proportion surviving
0
10
20
30
40
50
Days
without H. defensa
with H. defensa