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2   **A meta-analysis on the benefits and costs of hosting secondary endosymbionts in sap-sucking**

3   **insects**

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10   **Abstract**

11   Herbivorous insects host various bacteria that help them to feed, grow, and survive. Sap-sucking insects,

12   in particular, feed on a nutrient-poor resource and have evolved obligate symbioses with nutritional

13   bacteria for survival. Additionally, sap-sucking insects have formed facultative associations with bacterial

14   symbionts that are not essential for growth and survival but assumed to confer some benefits, such as

15   resistance to natural enemies. Several recent reviews have highlighted the importance of these

16   symbionts in understanding their hosts' biology, but currently there is a lack of a quantitative and

17   systematic analysis of the published evidences exploring whether the different endosymbionts are

18   actually beneficial or not. In this meta-analysis we explored the potential costs and benefits associated

19   with hosting facultative endosymbionts in sap-sucking insects. Our first result is that most of the empirical

20   experimental data information is limited to a few species of aphid and one species of whiteflies. Through

21   the meta-analysis we showed that hosting symbionts generally leads to costs through increased

22   development time, reduced longevity, and reduced fecundity, and benefits via increased resistance to

23   parasitic wasps in sap-sucking insects. However, the impact of these costs and benefits was strongly

24   insect and symbiont species dependent. Many of the insects studied are agricultural pests, and

25   understanding the impact of bacterial symbionts on their hosts across different environments can benefit

26   sustainable management of greenhouses and agricultural land.

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Introduction

Insect associations with mutualistic microbes are widespread, and it is nowadays widely accepted that these symbionts, and bacteria in particular, play key roles in the biology of their hosts (Brownlie & Johnson 2009; Feldhaar 2011). Thanks to important innovations in molecular techniques, the last two decades have provided deep insights into these diverse and often intricate interactions. The guts of most insects that feed on plant leaves, for instance, are colonised by complex communities of bacteria and fungi (Dillon & Dillon 2004). The role played by most of these microbes has yet to be understood, but evidence suggests that they are an important component of the hosts immune system, and that they often assist in the digestive process. Relative to leaf-feeding insects, the nutritional services provided by bacterial symbionts in sap-sucking insects are better understood (Moran, McCutcheon & Nakabachi 2008). This lifestyle has evolved multiple times among Hemiptera and includes most Sternorrhyncha (whiteflies, mealybugs, aphids and psyllids), many Auchenorrhyncha (planthoppers and leafhoppers) and most herbivorous Heteroptera (lygaeids, pentatomids, and coreids) (Dolling 1991).

Hemipterans are the most diverse group of hemimetabolous insects, with more than 100,000 species described (Stork 2018) and a large majority that have adopted sap-sucking life histories. Insects belonging to this guild feed on impoverished diets so that they all rely on mutualistic bacteria that live within or inside insect cells (they are termed endosymbionts) to synthesise essential nutrients that the insect cannot acquire directly from the diet (Douglas 1998). These primary, or obligate, symbionts are more like an organelle than an independent organism and are found in almost all aphids (they carry *Buchnera aphidicola*), psyllids (*Carsonella ruddii*), whiteflies (*Portiera aleyrodidarum*) and mealybugs (*Tremblaya princeps*), among others (Moran, McCutcheon & Nakabachi 2008). Such endosymbionts are required for insect survival and reproduction, transmitted from mother to offspring with high fidelity, and thought to be at the core of the diversification process of their insect hosts (Moran, McCutcheon & Nakabachi 2008). In addition to these obligate symbionts, sap-sucking insects have diverse associations with facultative (also, secondary) endosymbionts. Some of these symbionts are known to increase their hosts' tolerance to environmental stressors like heat shocks, or to protect them against natural enemies like fungal pathogens or parasitic wasps (Oliver, Smith & Russell 2014; Guo *et al.* 2017). Although these symbionts are not required for host survival, they are nowadays recognised as fundamental to understand the ecology of sap-sucking insects, including the niche they occupy, their distribution ranges and their interactions with other members in the community. Opposite to obligate symbionts, facultative

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59   endosymbionts of sap-sucking insects are often transmitted horizontally (Caspi-Fluger *et al.* 2012;

60   Ahmed *et al.* 2013; Chrostek *et al.* 2017) thus being mobile elements that can release herbivore

61   populations from their natural enemies, or allow them to colonise previously inhospitable habitats. The

62   role of facultative endosymbionts in host plant use, adaptation to abiotic conditions and interactions with

63   natural enemies has been studied for more than a decade, particularly in well-established model systems

64   like aphids and whiteflies (Moran, McCutcheon & Nakabachi 2008; Brownlie & Johnson 2009; Frago,

65   Dicke & Godfray 2012; Oliver, Smith & Russell 2014; Zchori-Fein, Lahav & Freilich 2014; Zytynska &

66   Weisser 2016; Guo *et al.* 2017; Vorburger 2018; Zytynska & Meyer 2019a).

67   A question that has yet to be resolved is why facultative endosymbionts are often found in only a fraction

68   of the individuals of a population, or why they are more abundant in some populations throughout the

69   distribution range of a given species (Zytynska & Weisser 2016). One explanation is that in the absence

70   of the environmental stress or of natural enemy pressure, facultative symbionts can incur fitness costs

71   (Russell & Moran 2006; Vorburger & Gouskov 2011; Vorburger, Ganesanandamoorthy & Kwiatkowski

72   2013). Experimental work on the potential fitness costs and benefits of hosting bacterial symbionts

73   indicate that these effects are variable across symbiont species, host species, and also strains or

74   genotypes within these species (Russell & Moran 2006; Rouchet & Vorburger 2012). Variation in the

75   strength of costs and benefits across different environments can lead to the differences in infection rates

76   we see in natural systems (Zytynska & Weisser 2016; Zytynska & Venturino 2018). Here, we present

77   results from a meta-analysis study to understand the generality of the costs and benefits of hosting

78   symbionts across sap-sucking insects. The majority of work in this area has been on aphids and their

79   nine common facultative bacterial symbionts *Hamiltonella defensa*, *Regiella insecticola*, *Serratia*

80   *symbiotica*, *Rickettsia*, *Ricketsiella*, *Spiroplasma*, X-type (PAXS), *Arsenophonus*, and *Wolbachia*. A

81   number of recent reviews describe the roles these symbionts can have on their hosts, and therefore we

82   will not provide this detail here (Oliver, Smith & Russell 2014; Zytynska & Weisser 2016; Guo *et al.* 2017;

83   Vorburger 2018). Understanding the benefits different endosymbiont species confer to insect herbivores,

84   and the costs they impose is thus crucial to understand one of the most diverse communities of animals

85   in terrestrial ecosystems.

86   Several recent reviews have highlighted the importance of these symbionts in understanding their hosts'

87   biology, but currently there is a lack of a quantitative and systemic analysis of the published evidences

88   exploring whether the different endosymbionts are actually beneficial or not. Although most research on

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89   this topic has been limited to few model systems and symbiont species, we believe the amount of data

90   available is large enough to perform a meta-analysis. Such a systemic analysis will provide insights into

91   the role of these microbial symbionts in different sap-sucking insect groups and will identify knowledge

92   gaps at the level of both research questions and insect (or symbiont) taxa.

93   In this review we address the following questions:

94   1.   Symbionts are widespread in sap-sucking insects, but which groups of species have been

95   sufficiently experimentally examined for the effects of symbionts on the host?

96   2.   Many researchers make the assumption that symbionts must incur a cost to the host (otherwise

97   why don't all individuals co-host multiple beneficial symbionts?); are these costs general across

98   host and symbiont species?

99   3.   Benefits of hosting symbionts must outweigh any costs for associations to persist, but do we have

100   sufficient experimental data to show that symbionts confer general benefits across host and

101   symbiont species?

102   4.   The cost-benefit trade-off for hosting symbionts can be mediated by the immediate environment

103   (biotic and abiotic) depending on the benefit conferred. Sap-sucking insect communities are

104   dependent on their plant hosts, so to what extent can their host-plant species affect the costs and

105   benefits of hosting symbionts?

106

107   **Methods**

108   The majority of experimental work has used aphid systems, but there is growing popularity to study this

109   in whitefly. Literature and data pertaining to other phloem-feeders was searched for, but with little to no

110   papers relevant to our meta-analyses. We detail the individual search terms and methods below for each

111   group.

112   **Whitefly meta-analysis**

113   We searched for relevant literature using keyword searches in Web of Science finding papers published

114   until the end of March 2018. We used the terms: whitefl* OR bemisia* OR siphoninus* OR Trialeurodes

115   OR Aleurodicus OR Aleuronudus OR Dialeurodicus OR Metaleurodicus OR Palaealeurodicus OR

116   Paraleyrodes AND symbio*. Resulting in 220 potential papers. To be included in the meta-analysis,

117   papers had to satisfy the following inclusion criteria: (1) data on at least one whitefly species; (2) an

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118   experimental test of symbiont effects on traits, either experimentally cured, a comparison of field-

119   collected infected and uninfected whitefly, or introgression of symbiont via crossing; (3) any of the

120   following types of variables tested: any behaviour, growth, fecundity, survival, or parasitism related

121   variable; and, (4) data on means, an estimation of variation and sample size. For the whitefly data, two

122   studies were eliminated because the antibiotic treatment also eliminated the primary symbiont. The final

123   dataset consisted of 9 papers (from 1993-2017, see Appendix 1 for list of included papers), and the only

124   variables of interest with sufficient data were growth rate (development time, growth rate and longevity),

125   fecundity, adult mass, and survival. To calculate overall symbiont effect on growth the variables

126   development time, growth rate and longevity were pooled together. For growth rate and longevity, larger

127   is better for the insect, but the opposite is true for development time (based on the slow-growth-high-

128   mortality hypothesis). In the overall test for growth, y_i was thus multiplied by -1.

129   **Aphid meta-analysis**

130   We searched for relevant literature using keyword searches in Web of Science finding papers published

131   until the end of 2018. We used the terms: ("aphid*" AND ("Hamiltonella" OR "Regiella" OR "Serratia" OR

132   "Rickettsia" OR "Rickettsiella" OR "Sprioplasma" OR "Arsenophonus" OR "Wolbachia" OR "X-type" OR

133   "PAXS")). This resulted in the extraction of 459 papers. To be included in the meta-analysis, papers had

134   to satisfy the following inclusion criteria: (1) data on at least one aphid species (Hemiptera: Aphididae);

135   (2) an experimental test of symbiont effects on aphids traits, either experimentally cured (Experimental)

136   or a comparison of field-collected infected and uninfected aphids (Natural); (3) any of the following types

137   of variables tested: aphid behaviour, growth, fecundity, survival, or parasitism-related; and, (4) data on

138   means, an estimation of variation and sample size. This resulted in 75 potential papers, but we then

139   included an additional inclusion criterion to include only traits with independent data points across at

140   least three independent studies, to reduce bias from single-lab results. Further, only data where aphids

141   hosted single symbionts were able to be used, as there was not sufficient data on multiple infections (10

142   studies, 37 data points [35 from *A. pisum* aphids] across 8 different symbiont combinations). In our final

143   57 papers (1997-2018; Figure 1a, see Appendix 2 for list of included papers) we were able to analyse the

144   effect of bacterial symbionts on: Development (age at first reproduction), Lifespan (longevity), Mass

145   (fresh weight), Fecundity (number of offspring), and Parasitism (proportion of aphids parasitized).

146   To account for differences between studies that artificially cured/inoculated aphid lines, and those

147   comparing naturally-collected infected to naturally uninfected aphid lines, we pooled data within studies

148   across aphid genotypes and symbiont strains (i.e. removing effects of within-species genetic variation);

149   this was necessary since no cross-comparison of common aphid or symbiont genetic lines was possible.

150   Data was still separated within a study for aphid species, symbiont species, and host-plant (experimental

151   plant, and plant of collection where possible).

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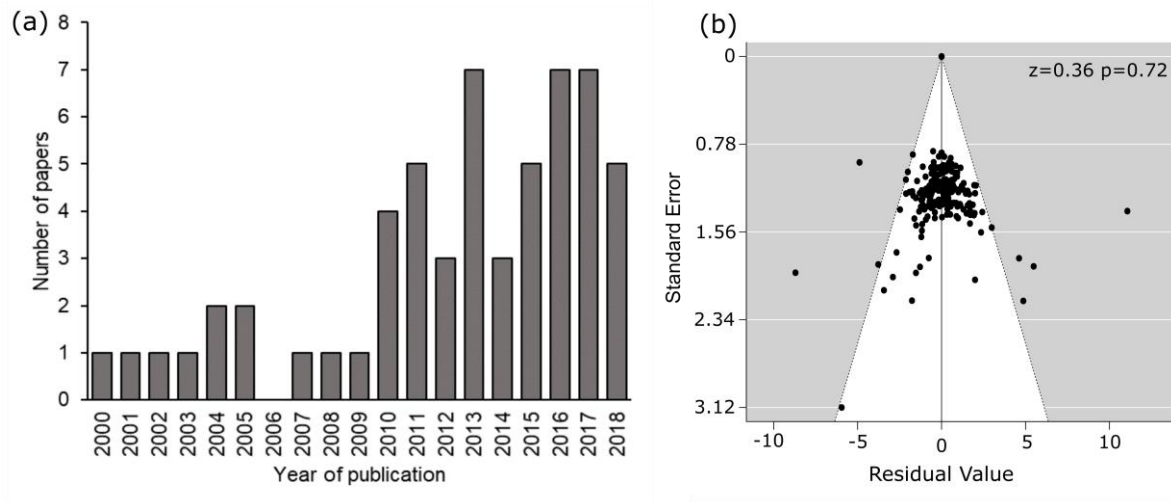
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154   **Figure 1. Summary of aphid meta-analysis data** (a) Number of aphid symbiont papers across year of
155   publication for the 57 papers used in the meta-analysis, and (b) funnel plot showing no strong publication
156   bias across these papers
157

158   The meta-analysis was conducted in R v3.5.1 (R Core Team 2019) in RStudio v1.1.463 (RStudio Team

159   2018) using the package metafor (Viechtbauer 2010). The standardised mean difference was used with

160   unbiased estimates of the sampling variances (SMDH, giving Hedges' g). This measure was used since

161   it gives a direct effect size comparison of the treated (infected with a symbiont) to untreated (no symbiont

162   control) data. We used a meta-analytic linear mixed effects model (rma) to test the effect of hosting

163   symbionts on the different aphid traits. 'Study' was included as random effect to account for multiple data

164   point across aphid and symbiont species within individual studies. Publication bias was assessed by

165   testing the funnel-plot asymmetry (Figure 1b for overall data, Figure S1 for data subsets). Data were also

166   subset into those where aphid lines had been directly compared through experimental curing/infecting

167   (Experimental) or a comparison of field-collected infected and uninfected aphids (Natural), and analyses

168   run as above on each separate dataset. The mean effect size and 95% confidence intervals are

169   presented; the mean effect size was considered significantly different from 0 if its 95% CI did not include

170   zero, and level of significant given from model outputs.

171   Further, we subset the data by aphid trait (i.e. one model for each trait) and explored differences across

172   aphid or symbiont species within these using meta-analytic linear mixed effects models by including

173   'aphid species' and 'symbiont species' as moderators (equivalent to main effects in standard linear

174   models). The interaction term was considered but in no case was there sufficient data for this to be a

175   meaningful term to include. We used model comparisons to estimate the effect of symbiont species and

176   aphid species using a LRT (likelihood ratio test) giving Chi-square and associated p-values. While the

177   overall effects of different aphid and symbionts species were analysed and relevant results presented in

178   the main text, for visual representation we present results from additional analyses that separated the

179   aphid species data into two categories: (a) *Acyrthosiphon pisum* aphid data (the commonly-used model

180   pea aphid species) and, (b) all other aphids (often representing less than half of the total data points).

181   Only aphid-symbiont combinations with at least two data points are presented in the figures.

182   **Results**

183   ***Whitefly traits***

184   The included studies are limited to a single species of whitefly (*B. tabaci*) and include some of its

185   biotypes (Q, B, among others) and three symbionts: *Hamiltonella*, *Wolbachia* and *Rickettsia*. Tests are

186   performed on different host plants and symbionts usually removed with antibiotics or via introgression

187   (Asiimwe, Kelly & Hunter 2014). Across all symbiont species, there was a cost of carrying symbionts in

188   *B. tabaci* through reduced fecundity (number of offspring). The following traits were not significantly

189   affected by symbiont carrying: growth (development time (days), growth rate, longevity (days)), adult

190   mass (body length (mm)) and survival (from egg to adult) (Figure 2a).

191   ***Aphid traits***

192   Our meta-analysis was based on data from 57 papers (2000-2018; Figure 1a, see Appendix 2 for list of

193   included papers) yielding 233 data points from 11 aphid species: *Acyrthosiphon pisum* (n=123), *Aphis*

194   *fabae* (n=32), *Sitobion avenae* (n=32), *Aphis craccivora* (n=13), *Acyrthosiphon kondoi* (n=12),

195   *Macrosiphum euphorbiae* (n=5), *Megoura crassicauda* (n=5), *Aphis glycines* (n=4), *Myzus persicae*

196   (n=3), *Rhopalosiphum padi* (n=3), *Obtusicauda frigidae* (n=1). We compared aphid traits of aphids

197   infected with a symbiont to uninfected aphids (data were pooled within a study across aphid genotypes

198   and symbiont strains, but separated by aphid species, symbiont species, and host-plant). The data were

199   robust against publication bias as measured through funnel-plot asymmetry (z = 0.363, p = 0.717; Figure

200   1b).

7

201   The number of data points within our final dataset for the different response variables were: Age at first

202   reproduction (N=27: Experimental n=26, Natural n=1; from 14 papers), Longevity (N=23: Experimental

203   n=20, Natural n=3; from 12 papers), Mass (N=26: Experimental n=18, Natural n=8; from 15 papers),

204   Fecundity (N=104: Experimental n=87, Natural n=17; from 40 papers), Parasitism (N=53: Experimental

205   n=36, Natural n=17; from 28 papers).
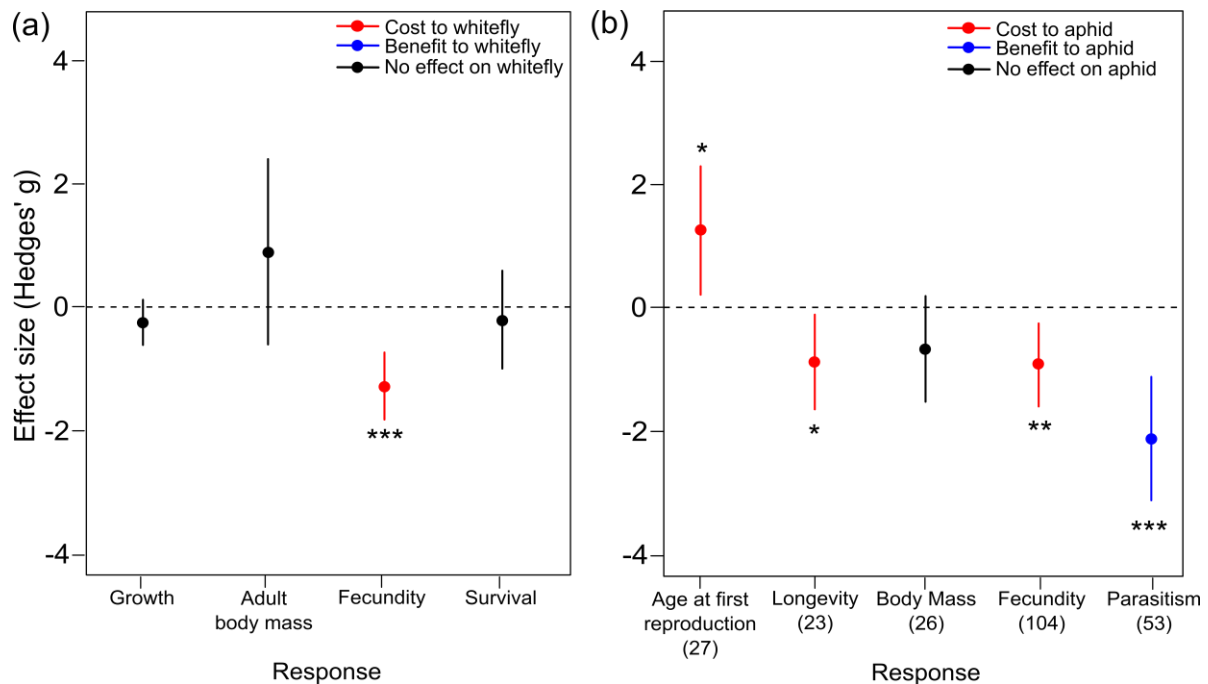
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207   **Figure 2. Overall effect of endosymbionts** on (a) whitefly *Bemisia tabaci* across four different B. tabaci

208   life history traits, and (b) aphids (Hemiptera: Aphididae) across five different variables, including four

209   related to aphid life history traits and the last related to resistance to parasitism (proportion of aphids

210   parasitized).

211   * P<0.05, ** P<0.01, *** P<0.001

212

213   **Effects across all aphids and symbionts**

214   Across all aphid and symbiont species, there was a cost to aphids through increased development time

215   (age at first reproduction), reduced longevity (days), reduced fecundity (number of offspring) (Figure 2b).

216   However, there was also a strong benefit to aphids of hosting endosymbionts that conferred resistance

217   against attacks by parasitic wasps (reduced proportion of aphids with a symbiont are parasitized; Figure

218   2b). The measures for age at first reproduction, longevity, and parasitism, were relatively consistent

219   across studies and therefore we are able to present mean values for these effects. We found that age at

220   first reproduction for the aphids was increased from 8.75±1.21 days (uninfected controls) to 9.06±1.21

221   days when hosting a symbiont, and longevity was decreased by five days when hosting a symbiont

222   (control: 30.31±5.02 days, symbiont: 24.33±4.95 days). The proportion of aphids parasitized reduced

223   from 0.54±0.25 (uninfected controls) to 0.36±0.29 when hosting a symbiont. The measures of body mass

224   and fecundity varied across studies, thereby reducing our ability to provide reliable mean values for these

225   traits.

226   **Experimental lines versus naturally-collected lines of aphid**

227   When the data were compared between those aphid lines that had been experimentally infected/cured

228   and those that were collected from the field as infected or uninfected, we see that there is substantial

229   variation in the results (Figure S2). The unequal distribution of data points (higher number of data points

230   for experimental lines compared to natural lines) needs to be noted here and results interpreted with this

231   potential strong bias in mind. The results indicate that the effects are stronger and less variable within

232   experimental studies, with a lack of overall significant results and greater range of data for 'natural' aphid

233   lines collected from the field. In some cases, e.g. fecundity, the data shows a potential for a change in

234   the direction of the result; however, further exploration of the data suggests that this may be influenced

235   by a few individual data points owing to the lack of data across multiple species, symbionts, and

236   laboratories.

237   **Effects within aphid traits by symbiont and aphid species**

238   The age at first reproduction (days) in the aphid was increased by symbionts for all aphid species, but

239   the magnitude varied across aphid species (X^2=18.54, df=6, P<0.001), and the effect varied across the

240   different symbiont species (X^2=12.07, df=7, P=0.007). *Hamitonella defensa* symbionts did not increase

241   aphid age at first reproduction, while *Rickettsia* sp. increased only for *A. pisum* aphids and *Serratia*

242   *symbiotica* symbionts for all aphids (Figure 3a).

243   Overall, aphid fecundity was reduced for aphids hosting a symbiont (Figure 2b) but the effect size was

244   dependent on the aphid species (X^2=13.79, df=9, P=0.003) and, in part, the symbiont (X^2=12.44, df=7,

245   P=0.088). Both *H. defensa* and *Regiella insecticola* reduced the fecundity of *A. pisum* aphids but there

246   was no evidence for this to affect other aphid species (Figure 3b). *Rickettsia*, *S. symbiotica*, and

247   *Arsenophonus sp.* had no effect on aphid fecundity, while the *X-type* symbiont decreased *A. pisum* aphid

248   fecundity (not studied in other aphids) (Figure 3b).

9

249 Symbiont-conferred resistance to parasitic wasps was variable across symbiont (X^2=18.57, df=7,

250 P=0.002) and aphid species (X^2=3.73, df=7, P=0.053). *Regiella insecticola* symbionts reduced the

251 proportion of aphids parasitized for all aphids studied, whereas *H. defensa* only reduced this for *A. pisum*

252 aphids, no other symbionts showed a significant effect (Figure 3c).

253 The influence of aphid or symbiont species was not presented for the aphid traits of body mass or

254 longevity due to strong effects of publication bias within these traits (Figure S2), potentially leading to

255 biased conclusions; however, analysis indicated little variation in the effect of symbiont hosting across

256 the different aphid and symbiont species tested.
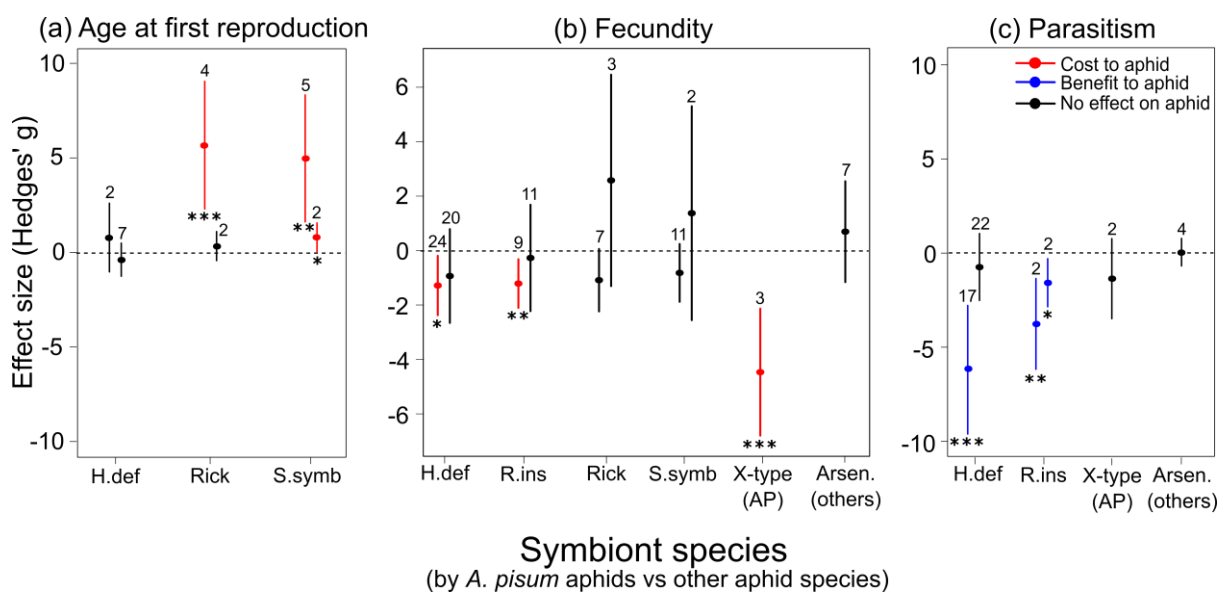
Figure 3. Variation in effect size of hosting symbionts for *A. pisum* pea aphids compared to other
aphid species on (a) aphid age at first reproduction (days) where a positive effect indicates a
lengthened development time when hosting the symbiont; (b) fecundity, where a negative effect indicates
fewer offspring produced when hosting the symbiont; and, (c) parasitism, where a negative effect
indicates a reduced proportion of aphids were parasitized by a parasitic wasp when hosting the
symbiont. The clustered lines show results from *A. pisum* pea-aphids (left lines, AP) compared to all
other aphid species combined (right lines, others). Data points are the mean effect size with 95%
confidence intervals, numbers above the lines indicates the number of data points in the meta-analysis.

Discussion

268 In this meta-analysis we have explored the benefits and potential costs associated with carrying

269 facultative endosymbionts in phloem-feeding insects. This is the first study that explores this question

270 quantitatively across phloem-feeding Hemiptera [i.e. whiteflies, mealybugs, aphids, psyllids,

271 planthoppers, leafhoppers, lygaeids, pentatomids and coreids (Dolling 1991)] and Thysanoptera. Our first

272 highlight is that, although these two Orders comprise almost 110,000 described species (Stork 2018),

273 most of them sap-suckers, information on the costs and benefits of facultative symbiont infection is

274   limited to aphids and whiteflies. Within these two groups, we found a strong bias towards a few well-

275   studied species, such as the model pea aphid (*A. pisum),* the black-bean aphid (*Aphis fabae*), the cereal

276   aphid (*Sitobion avenae*), and the whitefly *Bemisia tabaci*. This bias towards a few model species is likely

277   due to their importance as agricultural pest species and long history of ecological studies involving these

278   species. However, there are many other agriculturally-important sap-feeders, particularly aphid species,

279   that have been little used in symbiont studies. Other than lack of research effort, one reason for this bias

280   might be due to the difficulty of artificially removing symbionts in some insect species. In aphids using

281   antibiotics to "cure" them from secondary symbionts is simple and well-documented, albeit time-

282   consuming (Simon *et al.* 2007); however, this technique does not work for the potato aphid *Macrosiphum*

283   *euphorbiae* because the antibiotic treatment eliminates both facultative and the obligatory *Buchnera*

284   symbionts resulting in aphid death (Hackett, Karley & Bennett 2013). One work around, as done by the

285   authors working on *M. euphorbiae*, is by testing symbiont effects in various field-collected genotypes with

286   and without the bacterium (termed 'Natural' aphid lines in this meta-analysis), or creating infected lines

287   via introgression (as done in whiteflies, e.g. (Asiimwe, Kelly & Hunter 2014)).

288   We find support for an overall fitness cost of hosting bacterial symbionts in the aphid and whitefly species

289   studied; yet, the impact of these is strongly insect and symbiont species dependent. The general costs to

290   the aphid occur through increased time until the first reproduction, reduced fecundity and reduced

291   longevity. Thus, symbionts decrease aphid fitness by delaying development, reducing lifespan, and

292   reducing offspring production during this time. Hosts that carry costly symbionts but that do not confer

293   any benefit are expected to be lost in populations via purifying selection. We could show that there are

294   general benefits of symbionts through decreased parasitism rates, such that certain symbionts protect

295   certain aphid species from attack by specialist parasitic wasps (first shown by Oliver *et al.* 2003).

296   Although other benefits, such as resistance to entomopathogenic fungi or heat stress have been

297   highlighted in reviews on aphid symbiont effects (Oliver, Smith & Russell 2014; Zytynska & Weisser

298   2016; Guo *et al.* 2017; Vorburger 2018), these traits lack sufficient data across multiple aphid species

299   and symbionts to be included in a meta-analysis. In whiteflies, facultative symbionts were found to be

300   costly only through reduced fecundity, but as far as we are aware any benefits such as symbiont-

301   mediated resistance to natural enemies have never been tested in this insect group. Despite this, costly

302   symbionts like *Hamiltonella* are highly prevalent in whiteflies (Gueguen *et al.* 2010; Zchori-Fein, Lahav &

303   Freilich 2014), which suggests that the benefits associated to these bacteria are yet to be discovered.

304 The species-specific costs and benefits we identified in this meta-analysis have the potential to

305 contribute to the variation in symbiont-hosting frequencies observed in the field within and among

306 populations (Zytynska & Weisser 2016). All the experiments included in this meta-analysis compared

307 infected with uninfected aphid lines, and in the field both infected and uninfected aphids coexist

308 (Zytynska & Weisser 2016). The reduced fitness of aphids hosting a symbiont means they will be

309 outcompeted by the uninfected aphids when there is no benefit (e.g. through resistance), confirming the

310 'only helpful when required' statement of Vorburger and Gouskov (2011). Yet, the magnitude of the costs

311 and benefits will determine the impact on individual populations.

312 In this meta-analysis, we compared experiments that either directly assessed symbiont effects using

313 lines of aphid that had been artificially cured or infected, or compared lines of naturally-infected to

314 naturally-uninfected aphids collected to the field. We found that this separation resulted in strong

315 differences in the effects. A greater amount of variation with reduced effects on the aphid were seen for

316 those aphid lines that were collected from the field. Since this data comparison was potentially highly

317 biased, with small sample sizes for the naturally-collected aphids, we must interpret this carefully.

318 However, it may suggest that 'successful' aphid-symbiont combinations incur fewer costs, but perhaps

319 also reduced benefits. Or, this increased variation in effect size might indicate it is strongly dependent on

320 the particular combination of aphid (species, genotype), symbiont (species, strain), and surrounding

321 challenges (e.g. microclimate, host-plant, parasitism rate). Perhaps important benefits can only be

322 appreciated under more natural conditions. A field study in the US, for example, revealed that the

323 prevalence of the defensive symbiont *H. defensa* in *A.* pisum aphids increased throughout the season in

324 response to increased densities of parasitic wasps (Smith *et al.* 2015). This correlation, however, was

325 only significant in one of the two sites studied, a result that may reflect that parasitic wasps are not the

326 only natural enemies dictating the fate of symbiont-carrying insects. In addition, most studies have been

327 done on a restricted set of host plants, while only a small proportion of sap-sucking insects are

328 monophagous (most feed on more than one plant species). Data were collected on 'experimental host-

329 plant' and 'host-plant of aphid collection' but was insufficient for inclusion in the meta-analysis, indicating

330 the need for more empirical studies in this area.

331 Bringing the plant layer into account will certainly change our understanding of the cost-benefit balance

332 of symbiont infection in phloem feeders, with work suggesting the surrounding plant community can have

333 strong impacts on aphid endosymbiont communities (Zytynska *et al.* 2016; Zytynska & Meyer 2019a).

334 Moreover, in the aphids *A. pisum* and *C. cedri,* the facultative symbiont *S. symbiotica* can assist the

335 obligatory symbiont at the nutritional level, potentially enabling host-feeding on a wider selection of plants

336 (Koga, Tsuchida & Fukatsu 2003; Lamelas *et al.* 2011). In whiteflies (Su *et al.* 2015) and aphids (Frago

337 *et al.* 2017) recent studies also revealed that symbionts are able to help their hosts circumvent plant

338 defences that are triggered upon insect attack. Altogether, this means that symbionts could have a much

339 wider impact on aphid populations than is currently empirically tested. Thus, to better understand the

340 balance between costs and benefits of symbiont infection, and ultimately their prevalence in natural

341 populations, a wider community perspective is necessary. A recent review discusses the importance of

342 the immediate surrounding biotic community (plant diversity, natural enemy diversity) in combination with

343 the abiotic environment on mediating aphid-symbiont interactions (Zytynska & Meyer 2019a).

344 Complex community interactions also occur among symbionts inside their hosts, with potential

345 implications for the benefits that these bacteria provide (Ferrari & Vavre 2011). Our meta-analysis is

346 based only on insects with single symbiont infections since we lacked sufficient data on the role of

347 multiple symbiont infections. In the field it is estimated that aphids, for instance, host 0-4 symbionts per

348 individual (Ferrari *et al.* 2012; Russell *et al.* 2013; Smith *et al.* 2015; Zytynska *et al.* 2016), and that

349 multiple infections are particularly common in some genus like *Macrosiphum* (Henry et al. 2015). More

350 importantly, where symbiont co-infections occur strong fitness costs are often observed (Oliver, Moran &

351 Hunter 2006; Guay *et al.* 2009; Leclair *et al.* 2017; McLean *et al.* 2018). It would be very interesting to

352 test this across different aphid species, asking whether the proportion of multiple infections correlates

353 negatively with the fitness costs they impose. To better understand how multiple infections arise, more

354 work on how facultative symbionts are horizontally transferred within populations of the same species or

355 among species is also needed. While aphid symbionts are predominantly vertically transmitted from

356 mother to offspring, there is also evidence of horizontal transfer of symbionts among aphids during

357 sexual reproduction (Moran & Dunbar 2006), by parasitoids when ovipositing eggs into aphids (Gehrer &

358 Vorburger 2012), or even through infected honeydew (Darby & Douglas 2003). Based on a few

359 laboratory studies, aphid hosts impose little constrains to symbiont acquisition even if the host already

360 carries a facultative symbiont, demonstrated by successful microinjecting of different symbiont species

361 and strains into pea aphids (Leclair *et al.* 2017; McLean *et al.* 2018). A recent paper used mathematical

362 modelling to further show the importance of horizontal transmission of symbionts among aphids, focusing

363 on the potential of parasitoid wasps to transmit protective symbionts among aphids (Zytynska &

364   Venturino 2018). A low rate of horizontal transmission led to coexistence of uninfected aphids, infected

365   aphids, and parasitoid wasps, with the percentage of infected aphids ranging from 30-70% which is in

366   agreement with infection rates observed in field surveys (Zytynska & Weisser 2016; Zytynska &

367   Venturino 2018).

368

369   Many of these symbionts protect agricultural insect pests from their natural enemies, which is

370   counteractive to the aims of biological control programs (Vorburger 2018). Understanding how different

371   insect species interact with the common symbionts species, how symbionts are transmitted between

372   insect individuals, and the likely costs and benefits, all help to devise management programs to reduce

373   the impact of these symbionts. In a closed greenhouse system, the spread of a protective symbiont can

374   hinder biological control efforts, yet strategies to increase natural enemy diversity could mitigate this

375   impact (Vorburger 2018). In the field, if natural enemy density is low, as might occur in a monoculture

376   crop field, then insects with no symbionts (higher fitness) would outcompete insects with symbionts

377   (Zytynska & Meyer 2019b). Coupled with pesticide resistance evolution (e.g. seen in cereal aphids in

378   Europe; Malloch *et al.* (2016)), this can lead to increased risks of pest outbreaks.

379

380   **Conclusion**

381   Current molecular methods allow us to study the intricate ways insects establish mutualistic symbioses

382   with microbial partners. We used meta-analysis techniques to show the general costs (through increased

383   development time, reduced longevity and reduced fecundity) and benefits (increases resistance to

384   parasitic wasps) of hosting bacterial symbionts in sap-sucking insects. Current data is strongly biased

385   towards a few species of aphid and whitefly, and that there is a large variation of effects among insect as

386   well as symbiont species. Thus, the results cannot reliably be extrapolated to other phloem-feeding taxa,

387   and not even to other aphid or whitefly species. The impact of cost-benefit trade-offs in natural systems

388   are still to be uncovered, but an appreciation of the diversity of potential outcomes due to the species or

389   genetics of the insect/symbiont and the surrounding environment (plant diversity, natural enemy diversity,

390   microclimate) will benefit the design of future studies. While many of the studied insect species are

391   agricultural pests, studies in which the phenotypic consequences of facultative symbiont infection are

392   measured in non-model species are urgently needed. For agricultural systems, the spread of protective

393   symbionts in sap-sucking insects can hinder biological control efforts while reduced densities of natural

394   enemies might select for uninfected aphids with higher reproductive fitness. In both cases, this can lead

395   to pest outbreaks.

396

397   **Data availability**

398   All papers used in the meta-analysis are detailed in the appendices. Data will be made readily available

399   through contact with the corresponding author until final publication of the paper when data will be made

400   publically available.

401

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408

409   **References**

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