

Hosts do not simply outsource pathogen resistance to protective symbionts

Running title: Resistance not outsourced to symbionts

Jan Hrček^{1*}, Benjamin J. Parker^{1*}, Ailsa H.C. McLean¹, Jean-Christophe
Simon², Ciara M. Mann¹, H. Charles J. Godfray¹

¹ Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS,
United Kingdom

² Institut de Génétique, Environnement et Protection des Plantes, UMR 1099 INRA,
Agrocampus Ouest, Université Rennes 1, Domaine de la Motte, 35653 Le Rheu Cedex 5,
France

* Joint first authors

Corresponding authors: janhrcek@gmail.com, benjamin.j.parker@gmail.com

Author contributions

All authors participated in study design, and prepared and approved the manuscript; JH, AHCM, CM and BJP carried out the experiments, JH & BJP the statistical analysis, HCJG the evolutionary modelling and J-CS provided molecular genetic reagents.

Acknowledgements

We thank Julia Ferrari for providing aphid lines, and Richard Humber at the USDA ARS Collection of Entomopathogenic Fungal Cultures for providing the strain of *Pandora*. The study was supported by NERC grant NE/K004972/1. JH was supported by J.E. Purkyně Fellowship from the Czech Academy of Sciences and BJP was supported by US NSF Fellowship DBI-1306387.

Data accessibility

Experimental data will be deposited to Dryad on acceptance.

Hosts do not simply outsource pathogen resistance to protective symbionts

Running title: Resistance not outsourced to symbionts

Jan Hrček^{1*}, Benjamin J. Parker^{1*}, Ailsa H.C. McLean¹, Jean-Christophe
Simon², Ciara M. Mann¹, H. Charles J. Godfray¹

¹ Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS,
United Kingdom

² Institut de Génétique, Environnement et Protection des Plantes, UMR 1099 INRA,
Agrocampus Ouest, Université Rennes 1, Domaine de la Motte, 35653 Le Rheu Cedex 5,
France

* Joint first authors

Corresponding authors: janhrcek@gmail.com, benjamin.j.parker@gmail.com

Author contributions

All authors participated in study design, and prepared and approved the manuscript; JH, AHCM, CM and BJP carried out the experiments, JH & BJP the statistical analysis, HCJG the evolutionary modelling and J-CS provided molecular genetic reagents.

Acknowledgements

We thank Julia Ferrari for providing aphid lines, and Richard Humber at the USDA ARS Collection of Entomopathogenic Fungal Cultures for providing the strain of *Pandora*. The study was supported by NERC grant NE/K004972/1. JH was supported by J.E. Purkyně Fellowship from the Czech Academy of Sciences and BJP was supported by US NSF Fellowship DBI-1306387.

Data accessibility

Experimental data will be deposited to Dryad on acceptance.

Hosts do not simply outsource pathogen resistance to protective symbionts

Abstract

Microbial symbionts commonly protect their hosts from natural enemies, but it is unclear how protective symbionts influence the evolution of host immunity to pathogens. One possibility is that ‘extrinsic’ protection provided by symbionts allows hosts to reduce investment in ‘intrinsic’ immunological resistance mechanisms. We tested this idea using pea aphids (*Acyrtosiphon pisum*) and their facultative bacterial symbionts that increase host resistance to the fungal pathogen *Pandora neoaphidis*. The pea aphid taxon is composed of multiple host plant associated populations called biotypes, which harbour characteristic communities of symbionts. We found that biotypes that more frequently carry protective symbionts have higher, rather than lower, levels of intrinsic resistance. Within a biotype there was no difference in intrinsic resistance between clones that did and did not carry a protective symbiont. The host plant on which an aphid feeds did not strongly influence intrinsic resistance. We describe a simple conceptual model of the interaction between intrinsic and extrinsic resistance and suggest that our results may be explained by selection favouring both the acquisition of protective symbionts and enhanced intrinsic resistance in habitats with high pathogen pressure. Such combined protection is potentially more robust than intrinsic resistance alone.

94

95 **Key words**

96 ecological immunology, resistance, robustness, symbiosis

97

98 **Introduction**

99 Eukaryotes frequently benefit from symbiotic associations with bacteria, viruses, fungi
100 and other microorganisms (McFall-Ngai et al. 2013). Symbionts provide nutrients absent
101 in their hosts' diets or confer resistance against pathogens and other natural enemies
102 (Haine 2008; Brownlie and Johnson 2009). Because associations with beneficial
103 microbes are common, it is not possible to understand the ecology and evolution of hosts
104 without considering their symbionts. Protective microbes are also of applied importance:
105 the presence of a symbiont can affect the ability of a vector to transmit human, animal or
106 plant diseases (Gottlieb et al. 2010; Sasser et al. 2013; Caragata et al. 2016), and
107 symbiotic microbiomes can inhibit the colonisation of the gut or other organs by
108 pathogens (Koch and Schmid-Hempel 2011; Zipperer et al. 2016). To address both
109 fundamental and applied questions about symbiosis we need to better understand how
110 hosts and symbionts coevolve to create compound phenotypes (Heath and Stinchcombe
111 2014).

The evolution of symbiosis has been most closely studied in associations where the symbiont provides key nutrients for its host (Akman et al. 2002; Douglas 2014). A common observation is that metabolic pathways evolve to become shared between the host and symbiont so that what was originally a facultative association becomes obligate (Bennett and Moran 2015). Similar processes might operate in protective partnerships. If a host population becomes more resistant to a natural enemy due to an acquired microorganism, the host might be selected to reduce investment in intrinsic resistance, effectively outsourcing this function to the protective symbiont (Rolff and Siva-Jothy 2003; Altincicek et al. 2008; Hurst and Darby 2009; Boughton et al. 2011; Parker et al. 2011; Jaenike 2012; King and Bonsall 2017). One possibility is that because intrinsic resistance mechanisms are costly to maintain (Kraaijeveld and Godfray 1997; Barribeau et al. 2014), symbionts may be able to provide this function at a lower net cost to host fitness. For example, Martinez *et al.* (2016) recently showed by experimental evolution that selection in *Drosophila melanogaster* for an allele which increases resistance to the *Drosophila C* virus was reduced in the presence of the protective endosymbiotic bacterium *Wolbachia*. However, whether this pattern applies in natural populations remains unknown.

Under what conditions would we expect to see a negative correlation between investment in intrinsic defence and the presence of protective symbionts in a host population? The amount a host invests in defence against a pathogen (or other natural enemy) depends on the likelihood of infection, the effectiveness of resistance, and the constitutive costs of investment that are paid regardless of whether infection occurs. Figure 1 describes a

stylised model showing how these three factors interact to determine optimum investment, and two paths through which this may be affected by the presence of a protective symbiont. The simplest way that a symbiont may affect selection on host resistance is by decreasing the rate of infection experienced by the host. If this occurs, we would expect hosts to respond by reducing investment in intrinsic resistance. Were we to sample a range of hosts that had adapted to symbionts offering differing degrees of protection, we would expect to see a negative correlation between intrinsic and extrinsic (symbiont-based) resistance. Alternatively, hosts may be selected to acquire symbionts only in environments where pathogen incidence is high. The optimum level of investment in intrinsic resistance may then be reduced in the presence of symbionts, but could still be higher than in low incidence (no symbiont) environments. If we were to sample hosts across a spectrum of environments that co-varied in pathogen prevalence and symbiont frequency, we might find a positive correlation between intrinsic and extrinsic resistance (more complicated interactions are possible, which we return to in the Discussion). The aim of our study is to test which of these alternative relationships between intrinsic and extrinsic resistance is operating using empirical data from a natural system.

The pea aphid (*Acyrtosiphon pisum*) is an important model for endosymbiont research. In addition to its primary obligate nutritional symbiont (*Buchnera aphidicola*), pea aphids can host at least seven species of secondary, facultative endosymbionts from three major bacterial lineages. Pea aphid secondary symbionts have been shown to provide a variety of fitness benefits for their host (Oliver et al. 2010; McLean et al. 2016), including protection against fungal pathogens and parasitoids (Oliver et al. 2003; Scarborough et al.

2005; Łukasik et al. 2013), and possibly also improved performance on particular host plants (Tsuchida et al. 2004; McLean et al. 2011). Most studies investigating the benefits of symbiosis have been conducted in the laboratory, but two recent experiments have demonstrated protection against natural enemies in the field (Hrček et al. 2016; Rothacher et al. 2016). The pea aphid taxon is made up of multiple ‘biotypes’: populations that are to varying degrees adapted to different host plant species within the family Fabaceae. There are patterns in the frequencies of the seven secondary symbionts found in the different biotypes that are consistent across multiple continents (Peccoud et al. 2009; Ferrari et al. 2012; Henry et al. 2013; Russell et al. 2013), but there can also be considerable variation over the course of a season (Smith et al. 2015).

The pea aphid endosymbiont *Regiella insecticola* confers protection against the fungal pathogen *Pandora neoaphidis* (Scarborough et al. 2005), and at least one other specialist fungal pathogen of aphids (Parker et al. 2013). *Regiella* is found in many pea aphid biotypes but is particularly common in pea aphids adapted to *Trifolium* (Ferrari et al. 2012; Henry et al. 2013). At least some isolates of four other symbionts (*Spiroplasma*, *Rickettsia*, *Rickettsiella* and X-type) also provide protection against fungal pathogens (Łukasik et al. 2013; Heyworth and Ferrari 2015). Biotypes adapted to *Medicago sativa* and *Trifolium* have the highest net frequencies of protective symbionts, while the biotype adapted to *Ononis* has the lowest (Fig. 2). It is not yet known whether variation in the frequency of protective symbionts across the nine biotypes for which there are data reflects host-plant specific risks of infection, or has arisen through non-adaptive historical or idiosyncratic processes. The mechanism of the protective symbiosis against fungal

pathogens is currently unknown, but seems to be largely independent of intrinsic resistance. Thus the two types of resistance tend to reinforce each other with the symbionts improving overall resistance in hosts with low as well as high intrinsic resistance (Parker et al. 2017).

Here we explore the relationship between intrinsic and extrinsic resistance across biotypes (host-associated populations) of pea aphid. In Experiment 1 we ask whether aphid biotypes that frequently carry protective symbionts have relatively low or high intrinsic resistance. Separating genetic (aphid biotype) and environmental (host plant) effects on resistance is challenging because aphids from a given biotype are naturally specialised on certain host plants (Ferrari et al. 2012). However, cultivated *Vicia faba* appears to be a universal host that is acceptable to all biotypes (Ferrari et al. 2012). We thus measure intrinsic resistance for each clone on both *V. faba* and its natural host plant. In Experiment 2 we examine the relationship between intrinsic and extrinsic resistance in a broader set of aphid genotypes from a single biotype.

Material and Methods

Experiment 1

We obtained five aphid clonal lines from each of four different pea aphid biotypes (for clone details see Supporting Information, Table S1). The biotypes differ in the frequency

with which they harbour symbionts that are protective against *Pandora* (Fig. 2). The lines were collected in the south of the United Kingdom with one exception from Germany. We used a standard set of seven microsatellite loci to confirm that each line represented a unique genotype and that each belonged to the aphid biotype corresponding to the plant from which it was collected (Peccoud et al. 2009). Asexually reproducing lines were kept on *Vicia faba* plants at 14°C and a 16h:8h light:dark regime. All lines were screened for the secondary symbionts known from pea aphids using diagnostic PCR (Henry et al. 2013) and the original microbes they harboured are reported in Table S1. Almost all of the lines initially carried some secondary symbionts, which is to be expected given previous observations that 85% of aphids from these biotypes in this geographical area carry at least one symbiont (Henry et al. 2013). Secondary endosymbionts were removed from all 20 lines by antibiotic treatment as described by McLean et al. (2011). Symbiont removal with antibiotics was carried out at least 8 generations prior to the experiments to minimize trans-generational effects on the performance of the aphids (Koga et al. 2003). The absence of secondary symbionts was reconfirmed by diagnostic PCR (Henry et al. 2013) before the start of the experiments.

We assessed the intrinsic resistance of all 20 symbiont-free aphid lines against *Pandora neoaphidis* on both their natural host plant and on *Vicia faba*. We reared aphids for the experiment at low densities (10-20 per pot containing several young plants) to avoid crowding (which increases the frequency of winged morphs) and we used only unwinged individuals in assays. To synchronize aphid genotypes, which differ in their development rates, we kept some at 14°C and others at 20°C during their early instars. The more

slowly developing genotypes kept at 20°C were all five *Lotus* genotypes, and some *Trifolium* (C63, C317) and *Ononis* (C101) genotypes (for genotype codes see Table S1). All of the aphids were kept in the same room at 20°C in the final four days before the experiment to minimize the effects of temperature, moisture and growth rate. All biotypes were tested on their native host plant and on *V. faba*. We reared aphids from each host plant treatment for two generations (the parental and experimental generations) on the appropriate plant species to incorporate any maternal effects into our response variable. We used six- to eight-week old plants of the natural host plant species and three week old *Vicia faba* plants throughout the experiment (natural host plants grow more slowly than the cultivated *V. faba*, which meant that all plants were of similar size and developmental stage when used).

A strain of the fungal pathogen *P. neoaphidis* (strain ARSEF 2588; collected in Lansing, New York in 1988) was obtained from the USDA Agricultural Research Service's Collection of Entomopathogenic Fungal Cultures. Fungal virulence is known to attenuate under laboratory conditions, and thus we maintained our fungal stocks by passaging through *A. pisum* every 2 weeks (using a symbiont-free aphid clone that is hybrid of multiple aphid biotypes).

The experiment was performed in a single temporal block to help standardize the pathogen dose (we have previously observed temporal variation in *Pandora* infectivity and spore output per *Pandora* cadaver). Before use in the experiments, fungal cadavers were hydrated by placing them on tap-water agar in Petri dishes for twelve hours

overnight. 30-50 ten-day-old wingless adult aphids were used in the experiment for each combination of genotype (20 genotypes), host plant (natural vs. *V. faba*) and treatment (fungal infection vs. control). Aphids were assigned to the fungal infection and control treatments at random. Fungal infection followed standard protocol (Hajek et al. 2012; Parker et al. 2014); briefly, aphids were subjected to a two hour-long ‘spore shower’ from two fungal cadavers in an infection chamber painted with Fluon® (so that aphids could not climb the walls of the chamber). The hydrated cadavers were rotated among randomly ordered infection chambers so that each cadaver was present for the same time above each chamber in order to ensure equal spore dose and risk of infection in all treatments. This protocol allowed us to control for a variety of variables, including fungal cadaver age, the dose with which the cadaver itself was initially infected, quality of the aphids used to make the cadaver, and the conditions under which the cadaver was stored. Aphids assigned to the control treatment were placed for two hours in Petri dishes of similar size to the infection chambers and in all other ways were treated identically to those in the fungal treatment.

High humidity is essential for successful *Pandora* infection, but also difficult to standardize on whole plants due to differences in transpiration rates between plant species. After the infection, we therefore placed aphids in groups of four in Petri dishes on a plant leaf whose stalk was inserted in agar made with 2% tap water. Each dish was individually wrapped in parafilm and kept at 20°C for 48 hours. In a pilot study, we found this resulted in >95% humidity in all dishes independent of plant species. The aphids were then moved to new dishes with fresh plant material and kept at 20°C for 72

hours, and then transferred again to new dishes and kept for a further 48 hours. The experiment was blinded by assigning a random code to each dish so that data were collected without knowledge of the treatment. We recorded successful infection frequencies (the presence of sporulating cadavers) and survival daily from day two to day seven.

Extrinsic resistance across biotypes

We used data on the natural frequency of symbiont carriage from Henry *et al.* (2013) to quantify extrinsic (symbiont conferred) resistance across aphid biotypes. These data come from aphids collected in the UK, France and Germany, with 50-159 (mean of 84) genotypes per biotype screened for seven pea aphid secondary symbionts. We categorised aphids as carrying a protective symbiont if they carried at least one of the five symbionts that have been shown to protect against *Pandora*: *Regiella*, *Spiroplasma*, *Rickettsia*, *Rickettsiella* and X-type (Scarborough *et al.* 2005; Łukasik *et al.* 2013; Heyworth and Ferrari 2015). We calculated an average frequency of protective symbionts for each aphid biotype (Fig. 2). Because not all strains of these symbionts protect against *Pandora*, the heterogeneity being most pronounced in *Spiroplasma* and X-type (Łukasik *et al.* 2013; Heyworth and Ferrari 2015; Parker *et al.* 2017), we conducted a robustness analysis based on three conservative scenarios. In these we assumed, i) all *Spiroplasma* are non-protective (Fig. S1A), ii) all X-type are non-protective (Fig. S1B) or iii) both *Spiroplasma* and X-type are non-protective except for co-infections of the two which have been found to be protective (Heyworth and Ferrari 2015) (Fig. S1C). These

scenarios are conservative because we know that at least some of the *Spiroplasma* and X-type strains are protective.

Experiment 2

In the second experiment, we tested whether intrinsic susceptibility is associated with the carriage of a protective symbiont within a single biotype. We took ten aphid clonal lines collected on *Medicago sativa* (including five used in the first experiment) and as before confirmed that they were genetically distinct and belonged to the *Medicago sativa* biotype (see Table S1). We also included one genotype (C132) from the *Lotus pedunculatus* biotype which in Experiment 1 showed high intrinsic susceptibility in order to have a vulnerable control in the experiment. We removed any secondary symbionts by antibiotics using the protocols described above—six of the lines had a potentially protective secondary symbiont, and four were either symbiont-free upon collection or had a non-protective symbiont species. We assessed aphid intrinsic susceptibility to *Pandora* on *Vicia faba* as detailed above.

Data analysis

We first explored the data using a quasibinomial GLM before conducting further analyses using a binomial GLMM (packages lme4 and car in the R environment (Fox and Weisberg 2011; Bates et al. 2015; R Core Team 2015)). Our response variable was sporulation, measured as the proportion of aphids that became a sporulating cadaver by day 7 of the experiment from all aphids alive on day 2. Sporulation provides

unambiguous evidence that the aphid succumbed to *Pandora* and hence is the best measure of intrinsic susceptibility (the inverse of intrinsic resistance) in this experimental system. Further, aphids become infected with fungal pathogens when exposed to spores produced by infected conspecifics, and aphids that do not produce spores do not transmit the infection. Sporulation is likely to be a more relevant measure of intrinsic susceptibility than survival for the disease dynamics of this system. We removed aphids that died before day 2 from the analysis (159 out of 1719 fungal treatment aphids) because mortality at this early stage cannot be attributed to the fungus (Hajek et al. 2012). Raw data from both experiments will be deposited in Dryad. The first experiment was analysed at the biotype level. Explanatory variables were biotype-level frequency of symbionts protective against *Pandora* for each pea aphid biotype (Fig. 2) and host plant treatment (natural host plant versus *V. faba*). Further, we included survival of control aphids (Figure S2) as an explanatory variable in some models in order to account for variation that could have resulted from differential survival. Aphid genotype was treated as a random factor. The second experiment was analysed at the clone level, and the binary explanatory variable was whether or not each clone carried at least one protective symbiont when collected in the field.

For both experiments, we repeated the analysis using survival rather than sporulation as the dependent variable.

We then repeated the sporulation analyses in a Bayesian framework using the package MCMCglmm (Hadfield 2010), which allowed us to include a measure of relatedness in

the analysis. We excluded data from aphids on *Ononis* due to a probable experimental artefact discussed below. To obtain a measure of relatedness, we scored seven standard pea aphid microsatellite loci from each line according to published protocols (Peccoud et al. 2009). We then computed genetic distances between the lines used in the experiment using a genotype sharing index derived from the microsatellite data based on the methods in Blouin *et al.* (1996) implemented in the package Demerelate (Kraemer and Gerlach 2013). The index matrix was inverted to obtain a distance matrix using functions in the R package MASS (Venables and Ripley 2002), and the latter was incorporated into the MCMCglmm analysis using the “ginverse” parameter. We included aphid genotype as a random factor and used uninformative priors. We report DIC (Deviance Information Criterion) and P-value (pMCMC; for comparison with previous models) for models with analogous structure to the frequentist models without genetic distance described above.

Results

Experiment 1

We assessed the intrinsic susceptibility (the probability of forming a sporulating cadaver after being exposed to the spore shower) of 20 clones of pea aphid from four biotypes against the fungal pathogen *Pandora* on each clone’s natural host plant and on the universal host plant, *Vicia faba*. Overall, 46.3% of aphids survived the experiment, 28.7% sporulated and 25.0% died without sporulation. There was wide variation in relative

susceptibility across clones, much of which was explained by aphid biotype (Fig. 3A, $F_{3,39} = 4.633$, $P = 0.008$), but not host plant ($F_{3,39} = 1.079$, $P = 0.306$).

We asked whether there was a correlation between the experimentally measured intrinsic susceptibility to *Pandora* and the frequency of protective symbionts across different biotypes (obtained from the survey in Henry *et al.* (2013) and shown in Fig. 2). We found a significant negative correlation between intrinsic susceptibility and extrinsic (symbiont-mediated) resistance (Figs. 3A and 3B, Wald $\chi_1 = 6.439$, $P = 0.011$). We then explored the effect of host plant (the biotype's natural host species versus *V. faba*) and found a strong effect (Fig. 3A, Wald $\chi_1 = 18.873$, $P < 0.001$). However, this result was solely due to the low sporulation rate of a single biotype (*Ononis*) on its natural host plant, and we have reason to suspect this is an experimental artefact. *Ononis* plants are small shrubs and our technique of letting aphids feed on cuttings with stems inserted in agar in Petri dishes appeared to be less suitable for this plant compared to the other herbaceous species, resulting in high mortality relative to the universal host plant from causes other than the fungal pathogen and therefore a low apparent sporulation rate. We tested this suspicion statistically and found that *Ononis* aphids differed significantly from the other three biotypes in relative survival of control aphids on *Vicia faba* vs. their natural host plant (Fig. S2). There was significant effect of the interaction between host plant and aphid biotype on control survival (Wald $\chi_3 = 20.762$, $P < 0.001$), and multiple comparisons showed significant differences between *Ononis* and *Trifolium* ($P < 0.001$), *Medicago* ($P = 0.019$) and *Lotus* ($P = 0.036$), but no significant differences among the other three biotypes. The overall effect of host plant on aphid resistance disappears when the *Ononis*

natural host plant data are removed from the analysis (Wald $\chi_1 = 0.100$, $P = 0.752$), while the effect of frequency of symbiont carriage persists (Wald $\chi_1 = 12.899$, $P < 0.001$). Accepting this as an artefact, our data suggests that host plant does not influence aphid resistance to *Pandora*, which means that the effect of biotype is due to differences between aphids and not to the effects of feeding on different host plants.

To assess the robustness of these conclusions we conducted a series of further analyses. First, we included control survival as an explanatory variable in the model. In line with the tests reported above, control survival had a much stronger effect with the full dataset (Wald $\chi_1 = 42.562$, $P < 0.001$) compared to the reduced dataset that excluded the *Ononis* natural host plant data (Wald $\chi_1 = 6.005$, $P = 0.014$). In the latter case, the effect of frequency of symbiont carriage was again significant (Wald $\chi_1 = 9.1145$, $P = 0.003$), while host plant was not (Wald $\chi_1 = 1.962$, $P = 0.161$). Second, we repeated the analysis (without the *Ononis* native host plant data but with control survival) making three different conservative assumptions about the protective effects of *Spiroplasma* and X-type symbionts (see Methods). The results were unaffected by these alternative assumptions (control survival and the frequency of symbiont carriage always had a statistically significant effect while the effect of host plant remained non-significant). Last, we repeated our analyses reported above with survival (instead of sporulation) as the response variable. The tests were conducted with control survival as explanatory variable on i) full dataset, ii) dataset without *Ononis* native host plant data, and iii-v) with three different conservative assumptions about symbiont protective effects (see Methods). In all tests there was a significant negative correlation between aphid survival due to

388 fungal infection and extrinsic (symbiont-mediated) resistance (i: Wald $\chi_1 = 5.321$, $P =$
389 0.021, ii: Wald $\chi_1 = 10.956$, $P < 0.001$, and similarly for iii-v). The effect of host plant
390 (the biotype's natural species versus *V. faba*) was significant in all cases (i: Wald $\chi_1 =$
391 26.949, $P < 0.001$, ii: Wald $\chi_1 = 5.705$, $P = 0.017$, and similarly for iii-v) and the effect of
392 control survival was never significant (i: Wald $\chi_1 = 0.049$, $P = 0.824$, ii: Wald $\chi_1 = 2.201$,
393 $P = 0.138$, and similarly for iii-v). Our conclusions are therefore robust to different
394 possible definitions of intrinsic resistance (presence of sporulating cadavers vs. aphid
395 survival).

396 We extended the sporulation analysis using a phylogenetic method that allowed us to
397 include a measure of the genetic distance between aphids in the four biotypes (Fig. 4).
398 We still found a negative correlation between sporulation and extrinsic resistance (DIC =
399 1425, $P = 0.040$; with the *Ononis* native host plant data excluded and control survival
400 included). The analysis incorporating genetic distance suggests that only a small part of
401 the negative correlation is due to relatedness.

402 ***Experiment 2***

403 In experiment 2 we asked whether individual clones that did or did not carry a protective
404 symbiont differed in their intrinsic susceptibility to *Pandora*. Overall, 51.3% of aphids
405 survived the second experiment, 25.2% sporulated and 23.5% died without sporulation.
406 We assayed ten clones, all from the *Medicago* biotype, six of which carried a protective
407 symbiont (prior to its removal) and four which did not. We found no difference in the
408 intrinsic susceptibility of the two types of aphid (Fig. 5, Wald $\chi_1 = 0.600$, $P = 0.440$) and

control survival had no significant effect on intrinsic susceptibility (Wald $\chi^2_1 = 0.137$, $P = 0.711$). We included a vulnerable *Lotus* clone as a positive control for pathogen spore dose and as expected observed a high sporulation rate of 64.6% compared to the average of 21.9% for *Medicago* clones (range 6.3-38.9%). We repeated the above analyses with survival as a response variable and there was again no difference in the intrinsic susceptibility between aphids that did or did not carry protective symbionts (Wald $\chi^2_1 = 0.547$, $P = 0.459$), but control survival had a significant effect on survival (Wald $\chi^2_1 = 12.998$, $P < 0.001$).

We extended the sporulation analysis by incorporating measures of the genotypic distance between the different clones but again found no relationship between symbiont carriage and the strength of intrinsic susceptibility (DIC = 416.795, $P = 0.082$). Control survival was included in the model and was not significant ($P = 0.712$).

Discussion

We set out to investigate the nature of the relationship between intrinsic and extrinsic (symbiont-conferred) resistance to natural enemies, and in particular to ask whether hosts ‘outsource’ resistance to their symbionts (Altincicek et al. 2008; Hurst and Darby 2009; Boughton et al. 2011; Parker et al. 2011; Martinez et al. 2016). We did this using a tractable model system: the pea aphid, its facultative symbionts, and the natural aphid-specialist fungal pathogen *Pandora neoaphidis*. We found that aphids from host-plant

adapted populations ('biotypes') that more commonly harbour protective symbionts also show lower intrinsic susceptibility (the inverse of intrinsic resistance). Intrinsic and extrinsic resistance are thus positively correlated. Within a biotype, we found no association between the level of intrinsic susceptibility in a clone and whether it carried a protective symbiont or not. We therefore do not find evidence that hosts outsource resistance to protective symbionts. Variation in aphid susceptibility to fungal pathogens is more likely to be driven by biotypes differing in their risk of exposure to fungus. Where the risk is high, aphids employ both intrinsic and extrinsic resistance mechanisms. The phylogenetic analysis shows that relatedness may also contribute to some variation in intrinsic resistance.

The experimental design controlled for spore dose, humidity, observation bias, maternal effects and adaptation of the pathogen strain to specific biotypes. We extended the analyses by statistically controlling for non-independence of aphid lines due to relatedness, incorporating control survival and by exploring the effect of our incomplete knowledge of protective effects of some symbiont species. Additional analyses using survival as a response variable instead of sporulation lead to the same conclusions as the main analysis based on sporulation. Our results appear to be robust in these additional analyses. Experiment 2 also confirmed on an extended set of aphid clones that intrinsic susceptibility of the *Medicago* biotype aphids is low compared to the *Lotus* biotype.

Aphid fungal pathogens can reach very high prevalence in the wild (Smith et al. 2015; Hrček et al. 2016), but we do not yet know whether aphid biotypes that have high levels

of intrinsic and extrinsic resistance are more frequently exposed to fungal pathogens. Of the four biotypes included in our experiments, *Lotus corniculatus* and *Ononis spinosa* occur at relatively low densities growing in mixed vegetation in naturally managed meadows and grasslands, while *Trifolium* spp. and *Medicago sativa* are also commonly planted in fields as monoculture or in pastures in mixed fodder crops. It is possible that aphids feeding on high density host plants suffer more frequent or more severe fungal epidemics, either because their population densities are higher or because their more uniform spatial distribution facilitates pathogen spread (Jousimo et al. 2014). Further data are needed to test this hypothesis, but the collection of field data on aphid fungal pathogens is challenging because pathogen prevalence is highly variable both in space and time (Smith et al. 2015; Hrček et al. 2016).

Another possibility was that chemical or physical differences amongst host plants might affect the susceptibility of different aphid biotypes to fungal pathogens or their ability to defend themselves. Effects of host plant on insect-pathogen interactions are frequently observed (Cory and Hoover 2006). We therefore tested aphid resistance on both the native host plant for each biotype and on *Vicia faba*, which appears to be acceptable to all biotypes. Our results show that intrinsic resistance to fungal infection is not directly influenced by host plant chemistry or morphology (with the possible exception of *Ononis*, which we believe is an artefact as discussed above). This is surprising, as we found host plant to strongly affect body size of the aphids (Fig. S3): a major life-history trait related to nutrition that affects immune function in other systems (Siva-Jothy and Thompson 2002).

Animal immune systems often include multiple redundant mechanisms, and this arrangement increases robustness of the defensive response. In turn, pathogens have evolved counter-adaptations to some host defences (Schmid-Hempel 2009; Nish and Medzhitov 2011). Symbiont conferred protection represents an additional and independent set of defense mechanisms that may improve both the strength and redundancy of resistance (Kitano and Oda 2006; Schulenburg et al. 2009; Martinez et al. 2017).

When will a host that can already defend itself be selected to acquire symbionts that provide further resistance? If there is no cost to a host of carrying symbionts, symbiont-mediated defence will be free and any individual carrying symbionts will be at an advantage due to its superior ability to resist infection. If symbionts are highly effective and spread through a host population then there may be selection on hosts to reduce their own costly investment in intrinsic resistance, and thus reap a further indirect benefit from symbiont carriage (this is illustrated in a model in Fig. 6). However, there may be costs to carrying symbionts, as is suggested by recent evidence (Russell and Moran 2006; Oliver et al. 2008; Martinez et al. 2015; Hrček et al. 2016). The benefit to a host of acquiring symbionts would then depend on the costs as well as the extent of the additional protection it provides and the probability of pathogen infection. Again, it may be possible that some of the costs of carrying protective symbionts can be mitigated by reducing investment in costly intrinsic resistance (Fig. 6). Future analysis of the interaction of intrinsic and extrinsic resistance would benefit from investigating the possibility of

redundancy and synergy in the action of the two processes, and the possibility of an evolutionary response in symbionts and hosts.

References

- Akman, L., A. Yamashita, H. Watanabe, K. Oshima, T. Shiba, M. Hattori, and S. Aksoy. 2002. Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nat. Genet.* 32:402–407.
- Altincicek, B., J. Gross, and A. Vilcinskas. 2008. Wounding-mediated gene expression and accelerated viviparous reproduction of the pea aphid *Acyrtosiphon pisum*. *Insect Mol. Biol.* 17:711–716.
- Barribeau, S. M., B. J. Parker, and N. M. Gerardo. 2014. Exposure to natural pathogens reveals costly aphid response to fungi but not bacteria. *Ecol. Evol.* 4:488–493.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67:1–48.
- Bennett, G. M., and N. A. Moran. 2015. Heritable symbiosis: The advantages and perils of an evolutionary rabbit hole. *Proc. Natl. Acad. Sci.* 112:10169–10176.
- Blouin, M., M. Parsons, V. Lacaille, and S. Lotz. 1996. Use of microsatellite loci to classify individuals by relatedness. *Mol. Ecol.* 5:393–401.

- 511 Boughton, R. K., G. Joop, and S. A. O. Armitage. 2011. Outdoor immunology:
512 methodological considerations for ecologists: Advancing ecological immunology
513 methods. *Funct. Ecol.* 25:81–100.
- 514 Brownlie, J. C., and K. N. Johnson. 2009. Symbiont-mediated protection in insect hosts.
515 *Trends Microbiol.* 17:348–354.
- 516 Caragata, E. P., H. L. C. Dutra, and L. A. Moreira. 2016. Exploiting intimate
517 relationships: controlling mosquito-transmitted disease with *Wolbachia*. *Trends*
518 *Parasitol.* 32:207–218.
- 519 Cory, J. S., and K. Hoover. 2006. Plant-mediated effects in insect–pathogen interactions.
520 *Trends Ecol. Evol.* 21:278–286.
- 521 Douglas, A. E. 2014. Molecular dissection of nutrient exchange at the insect-microbial
522 interface. *Curr. Opin. Insect Sci.* 4:23–28.
- 523 Ferrari, J., J. A. West, S. Via, and H. C. J. Godfray. 2012. Population genetic structure
524 and secondary symbionts in host-associated populations of the pea aphid complex.
525 *Evolution* 66:375–390.
- 526 Fox, J., and S. Weisberg. 2011. *An R companion to applied regression*. 2nd edn.
527 Thousand Oaks, CA: Sage.
- 528 Gottlieb, Y., E. Zchori-Fein, N. Mozes-Daube, S. Kontsedalov, M. Skaljic, M. Brumin, I.
529 Sobol, H. Czosnek, F. Vavre, F. Fleury, and M. Ghanim. 2010. The transmission
530 efficiency of tomato yellow leaf curl virus by the whitefly *Bemisia tabaci* is
531 correlated with the presence of a specific symbiotic bacterium species. *J. Virol.*
532 84:9310–9317.

- 533 Hadfield, J. D. 2010. MCMC methods for multi-response generalized linear mixed
534 models: the MCMCglmm R package. *J. Stat. Softw.* 33:1–22.
- 535 Haine, E. R. 2008. Symbiont-mediated protection. *Proc. R. Soc. B Biol. Sci.* 275:353–
536 361.
- 537 Hajek, A. E., B. Papierok, and J. Eilenberg. 2012. Methods for study of the
538 Entomophthorales. Pp. 285–315 *in* L. A. Lacey, ed. *Manual of techniques in*
539 *invertebrate pathology*. Academic Press, London.
- 540 Heath, K. D., and J. R. Stinchcombe. 2014. Explaining mutualism variation: a new
541 evolutionary paradox? *Evolution* 68:309–317.
- 542 Henry, L. M., J. Peccoud, J.-C. Simon, J. D. Hadfield, M. J. C. Maiden, J. Ferrari, and H.
543 C. J. Godfray. 2013. Horizontally transmitted symbionts and host colonization of
544 ecological niches. *Curr. Biol.* 23:1713–1717.
- 545 Heyworth, E. R., and J. Ferrari. 2015. A facultative endosymbiont in aphids can provide
546 diverse ecological benefits. *J. Evol. Biol.* 28:1753–1760.
- 547 Hrčák, J., A. H. C. McLean, and H. C. J. Godfray. 2016. Symbionts modify interactions
548 between insects and natural enemies in the field. *J. Anim. Ecol.* 85:1605–1612.
- 549 Hurst, G. D. D., and A. C. Darby. 2009. The inherited microbiota of arthropods, and their
550 importance in understanding resistance and immunity. P. *in* J. Rolff and S. E.
551 Reynolds, eds. *Insect infection and immunity: evolution, ecology, & mechanisms*.
552 Oxford Biology.
- 553 Jaenike, J. 2012. Population genetics of beneficial heritable symbionts. *Trends Ecol.*
554 *Evol.* 27:226–232.

- 555 Jousimo, J., A. J. M. Tack, O. Ovaskainen, T. Mononen, H. Susi, C. Tollenaere, and A.-
556 L. Laine. 2014. Ecological and evolutionary effects of fragmentation on infectious
557 disease dynamics. *Science* 344:1289–1293.
- 558 King, K. C., and M. B. Bonsall. 2017. The evolutionary and coevolutionary consequences
559 of defensive microbes for host-parasite interactions. *BMC Evol. Biol.* 17.
- 560 Kitano, H., and K. Oda. 2006. Robustness trade-offs and host–microbial symbiosis in the
561 immune system. *Mol. Syst. Biol.* 2.
- 562 Koch, H., and P. Schmid-Hempel. 2011. Socially transmitted gut microbiota protect
563 bumble bees against an intestinal parasite. *Proc. Natl. Acad. Sci.* 108:19288–
564 19292.
- 565 Koga, R., T. Tsuchida, and T. Fukatsu. 2003. Changing partners in an obligate symbiosis:
566 a facultative endosymbiont can compensate for loss of the essential endosymbiont
567 *Buchnera* in an aphid. *Proc. R. Soc. B Biol. Sci.* 270:2543–2550.
- 568 Kraaijeveld, A. R., and H. C. J. Godfray. 1997. Trade-off between parasitoid resistance
569 and larval competitive ability in *Drosophila melanogaster*. *Nature* 389:278–280.
- 570 Kraemer, P., and G. Gerlach. 2013. Demerelate: Functions to calculate relatedness on
571 diploid genetic data. R package version 0.8-1.
- 572 Łukasik, P., M. van Asch, H. Guo, J. Ferrari, and H. Charles J. Godfray. 2013. Unrelated
573 facultative endosymbionts protect aphids against a fungal pathogen. *Ecol. Lett.*
574 16:214–218.

- 575 Martinez, A. J., M. R. Doremus, L. J. Kraft, K. L. Kim, and K. M. Oliver. 2017. Multi-
576 modal defences in aphids offer redundant protection and increased costs likely
577 impeding a protective mutualism. *J. Anim. Ecol.*, doi: 10.1111/1365-2656.12675.
- 578 Martinez, J., R. Cogni, C. Cao, S. Smith, C. J. R. Illingworth, and F. M. Jiggins. 2016.
579 Addicted? Reduced host resistance in populations with defensive symbionts. *Proc.*
580 *R. Soc. B Biol. Sci.* 283:20160778.
- 581 Martinez, J., S. Ok, S. Smith, K. Snoeck, J. P. Day, and F. M. Jiggins. 2015. Should
582 symbionts be nice or selfish? Antiviral effects of *Wolbachia* are costly but
583 reproductive parasitism is not. *PLoS Pathog.* 11:e1005021.
- 584 McFall-Ngai, M., M. G. Hadfield, T. C. Bosch, H. V. Carey, T. Domazet-Lošo, A. E.
585 Douglas, N. Dubilier, G. Eberl, T. Fukami, S. F. Gilbert, and others. 2013.
586 Animals in a bacterial world, a new imperative for the life sciences. *Proc. Natl.*
587 *Acad. Sci.* 110:3229–3236.
- 588 McLean, A. H. C., B. J. Parker, J. Hrček, L. M. Henry, and H. C. J. Godfray. 2016. Insect
589 symbionts in food webs. *Philos. Trans. R. Soc. B Biol. Sci.* 371:20150325.
- 590 McLean, A. H. C., M. van Asch, J. Ferrari, and H. C. J. Godfray. 2011. Effects of
591 bacterial secondary symbionts on host plant use in pea aphids. *Proc. R. Soc. B*
592 *Biol. Sci.* 278:760–766.
- 593 Nish, S., and R. Medzhitov. 2011. Host defense pathways: role of redundancy and
594 compensation in infectious disease phenotypes. *Immunity* 34:629–636.
- 595 Oliver, K. M., J. Campos, N. A. Moran, and M. S. Hunter. 2008. Population dynamics of
596 defensive symbionts in aphids. *Proc. R. Soc. B Biol. Sci.* 275:293–299.

- 597 Oliver, K. M., P. H. Degnan, G. R. Burke, and N. A. Moran. 2010. Facultative symbionts
598 in aphids and the horizontal transfer of ecologically important traits. *Annu. Rev.*
599 *Entomol.* 55:247–266.
- 600 Oliver, K. M., J. A. Russell, N. A. Moran, and M. S. Hunter. 2003. Facultative bacterial
601 symbionts in aphids confer resistance to parasitic wasps. *Proc. Natl. Acad. Sci.*
602 100:1803–1807.
- 603 Parker, B. J., S. M. Barribeau, A. M. Laughton, J. C. de Roode, and N. M. Gerardo. 2011.
604 Non-immunological defense in an evolutionary framework. *Trends Ecol. Evol.*
605 26:242–248.
- 606 Parker, B. J., J. R. Garcia, and N. M. Gerardo. 2014. Genetic variation in resistance and
607 fecundity tolerance in a natural host-pathogen interaction. *Evolution* 68:2421–
608 2429.
- 609 Parker, B. J., J. Hrčák, A. H. C. McLean, and H. C. J. Godfray. 2017. Genotype
610 specificity among hosts, pathogens, and beneficial microbes influences the
611 strength of symbiont-mediated protection. *Evolution* 71:1222–1231.
- 612 Parker, B. J., C. J. Spragg, B. Altincicek, and N. M. Gerardo. 2013. Symbiont-mediated
613 protection against fungal pathogens in pea aphids: a role for pathogen specificity?
614 *Appl. Environ. Microbiol.* 79:2455–2458.
- 615 Peccoud, J., A. Ollivier, M. Plantegenest, and J.-C. Simon. 2009. A continuum of genetic
616 divergence from sympatric host races to species in the pea aphid complex. *Proc.*
617 *Natl. Acad. Sci.* 106:7495–7500.

- 618 R Core Team. 2015. R: A language and environment for statistical computing. R
619 Foundation for Statistical Computing, Vienna, Austria.
- 620 Rolff, J., and M. T. Siva-Jothy. 2003. Invertebrate ecological immunology. *Science*
621 301:472–475.
- 622 Rothacher, L., M. Ferrer-Suay, and C. Vorburger. 2016. Bacterial endosymbionts protect
623 aphids in the field and alter parasitoid community composition. *Ecology* 97:1712–
624 1723.
- 625 Russell, J. A., and N. A. Moran. 2006. Costs and benefits of symbiont infection in aphids:
626 variation among symbionts and across temperatures. *Proc. R. Soc. B Biol. Sci.*
627 273:603–610.
- 628 Russell, J. A., S. Weldon, A. H. Smith, K. L. Kim, Y. Hu, P. Łukasik, S. Doll, I.
629 Anastopoulos, M. Novin, and K. M. Oliver. 2013. Uncovering symbiont-driven
630 genetic diversity across North American pea aphids. *Mol. Ecol.* 22:2045–2059.
- 631 Sassaera, D., S. Epis, M. Pajoro, and C. Bandi. 2013. Microbial symbiosis and the control
632 of vector-borne pathogens in tsetse flies, human lice, and triatomine bugs. *Pathog.*
633 *Glob. Health* 107:285–292.
- 634 Scarborough, C. L., J. Ferrari, and H. C. J. Godfray. 2005. Aphid protected from
635 pathogen by endosymbiont. *Science* 310:1781.
- 636 Schmid-Hempel, P. 2009. Immune defence, parasite evasion strategies and their
637 relevance for “macroscopic phenomena” such as virulence. *Philos. Trans. R. Soc.*
638 *B Biol. Sci.* 364:85–98.

- 639 Schulenburg, H., J. Kurtz, Y. Moret, and M. T. Siva-Jothy. 2009. Introduction.
640 Ecological immunology. *Philos. Trans. R. Soc. B Biol. Sci.* 364:3–14.
- 641 Siva-Jothy, M. T., and J. J. Thompson. 2002. Short-term nutrient deprivation affects
642 immune function. *Physiol. Entomol.* 27:206–212.
- 643 Smith, A. H., P. Łukasik, M. P. O'Connor, A. Lee, G. Mayo, M. T. Drott, S. Doll, R.
644 Tuttle, R. A. Disciullo, A. Messina, K. M. Oliver, and J. A. Russell. 2015.
645 Patterns, causes and consequences of defensive microbiome dynamics across
646 multiple scales. *Mol. Ecol.* 24:1135–1149.
- 647 Tsuchida, T., R. Koga, and T. Fukatsu. 2004. Host plant specialization governed by
648 facultative symbiont. *Science* 303:1989–1989.
- 649 Venables, W. N., and B. D. Ripley. 2002. *Modern applied statistics with S*. Fourth
650 edition. Springer, New York.
- 651 Zipperer, A., M. C. Konnerth, C. Laux, A. Berscheid, D. Janek, C. Weidenmaier, M.
652 Burian, N. A. Schilling, C. Slavetinsky, M. Marschal, M. Willmann, H.
653 Kalbacher, B. Schitteck, H. Brötz-Oesterhelt, S. Grond, A. Peschel, and B.
654 Krismer. 2016. Human commensals producing a novel antibiotic impair pathogen
655 colonization. *Nature* 535:511–516.
- 656
- 657

Figure legends

Figure 1. Stylised model of the evolution of intrinsic resistance. We assume that allocating more resources to intrinsic resistance (investment) that could be used for growth and reproduction is costly and so reduces the organism's baseline fitness in the absence of disease (upper panel, line A) but increases the probability of survival after infection (line B). The product of these two quantities is the realised fitness of an infected aphid (line C). It can be shown that the optimum investment in intrinsic resistance occurs when its marginal costs (lower panel, B) equal the marginal benefits (A) which depends on the probability of infection (four different levels are graphed). X, Y & Z are the points of intersection when this probability is 0.4, 0.5 or 0.6 respectively and the optimum investment can be read from the x-axis. Note the curves do not cross when the probability is 0.3 and no investment in defence is selected, here the host in effect "gambling" on not being infected.

If symbionts neutralise pathogens then this is equivalent to reducing the probability of infection experienced by the host. Successively more efficient symbionts would therefore cause host adaptation in the sequence ZYX and a negative correlation between intrinsic and extrinsic resistance would be observed. Alternatively, compare a host in a low pathogen incidence (0.4) environment selected to invest at level X, with one in a high (0.6) incidence environment selected to invest at level Z. If the latter host acquired a symbiont that reduced effective pathogen level to 0.5 then optimum intrinsic investment is at Y which is still higher than X. If hosts are more likely to acquire

symbionts in more dangerous environments then a positive correlation between intrinsic resistance and the presence of symbionts will be found. Mathematical equations on which this model is based are available in the Supporting Information.

Figure 2. Frequency of carriage of symbionts protective against the fungal pathogen *Pandora* in different pea aphid biotypes. The total height of the bars shows the proportion of aphids carrying at least one symbiont of the following five species which have been shown to protect against *Pandora*: *Regiella insecticola*, *Spiroplasma*, *Rickettsia*, *Rickettsiella* and X-type. The fraction of aphids carrying one, two or three protective symbionts is shown in different colours and is only a complementary information which is not used in the analysis. Data are a subset from Henry et al. (2013) and include biotypes collected in the UK, France and Germany, with at least 50 genotypes per biotype (range: 50-159, mean: 84). Biotypes included in our intrinsic resistance study are marked with a star. Because not all strains of the five symbionts mentioned above are protective, we use also three conservative scenarios and present them in Fig. S1.

Figure 3. A) Sporulation of pea aphid biotypes following infection by *Pandora neoaphidis* fungal pathogen (\pm SE of the mean of five genotypes per biotype). Sporulation of symbiont-free aphids was assessed on universal host plant *Vicia faba* and on natural host plant of each biotype as proportion of sporulating aphids from all aphids alive on day 2 of the experiment. B) Correlation of sporulation on *V. faba* (a measure of intrinsic

susceptibility) and field frequency of protective symbionts (a measure of extrinsic resistance, see Fig. 2) for four pea aphid biotypes.

Figure 4. Correlation between aphid genotypic relatedness and sporulation on *Vicia faba*. Relatedness is represented by distance in XY plane and sporulation by the height of the cylinders along the Z-axis. Relatedness distances were obtained by genotype sharing algorithm of Blouin et al. (1996) from seven microsatellite loci. The distances were compressed to two dimensions by Principal Coordinate Analysis (the two axes together represent 38% of the variability in relatedness distances).

Figure 5. Sporulation following *Pandora* infection of aphid genotypes that at collection did or did not carry symbionts protective against *Pandora* (\pm SE of the mean). All ten *Medicago sativa* biotype lines were cured from secondary symbionts prior to the experiment. Sporulation was assessed as proportion of sporulating aphids from all aphids alive on day 2 of the experiment.

Figure 6. Evolutionary response by the host to the presence of a protective symbiont. An evolutionary model predicts that if intrinsic resistance is costly then host investment in this function will decline to nothing as the strength of extrinsic resistance increases (upper panel). The lower panel illustrates how host fitness can be influenced by the

720 strength of extrinsic resistance, the costs of symbiont carriage, and by whether the host
721 adjusts investment in intrinsic resistance in the presence of a symbiont. The model
722 assumes that in the absence of any symbionts the prevailing risk of natural enemy attack
723 and optimum host investment in intrinsic resistance leads to host fitness given by the
724 horizontal line X. If a host acquires a protective cost-free symbiont its fitness is given by
725 line a while lines b, c & d show host fitness as the costs of carrying the symbiont
726 increases (note that costly symbionts providing poor protection reduce fitness). If hosts
727 optimally adjust their investment in costly intrinsic resistance then host fitness increases
728 from a, b, c & d to A, B, C & D respectively. Mathematical equations on which this
729 model is based are available in the Supporting Information.

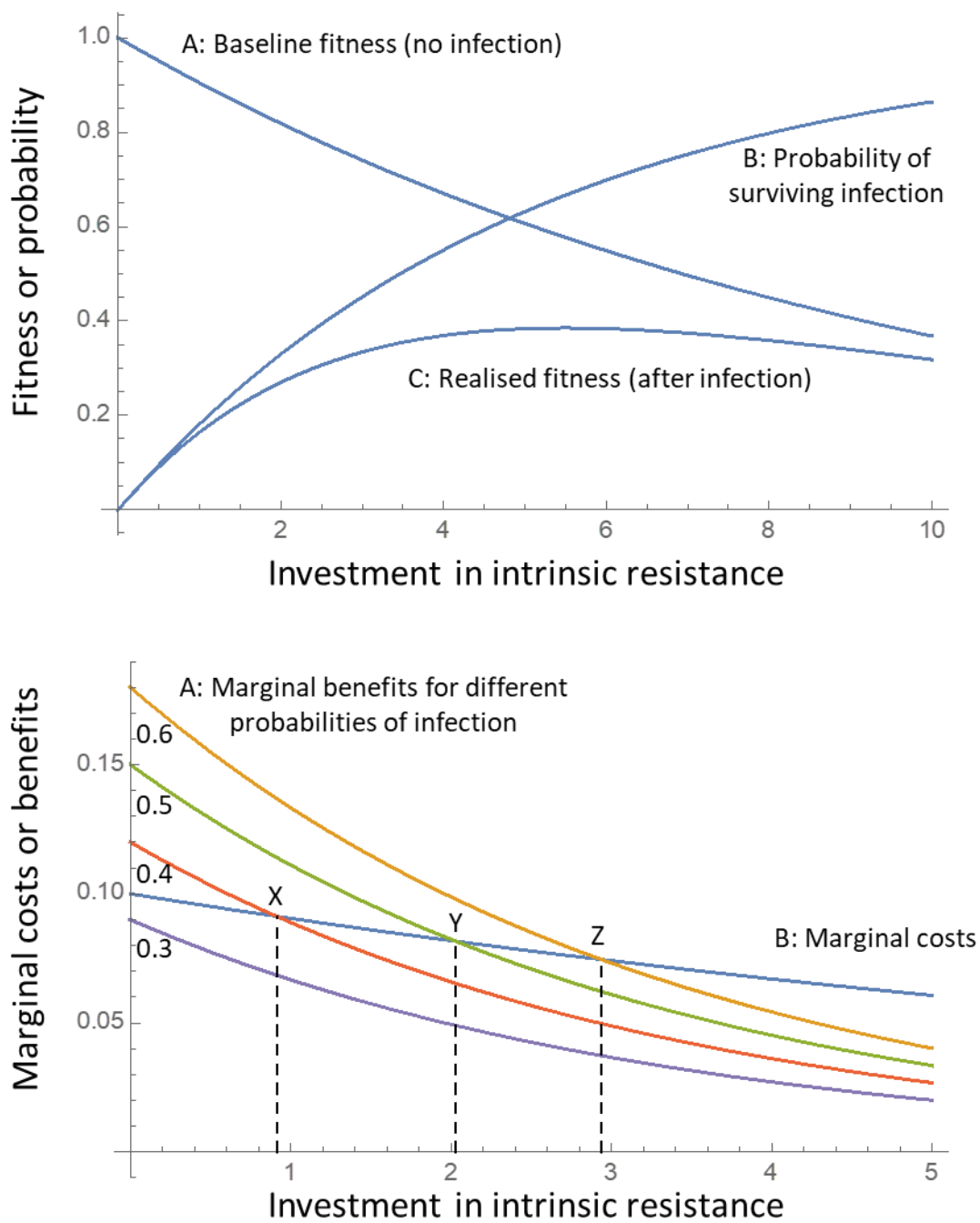


Figure 1

730

731

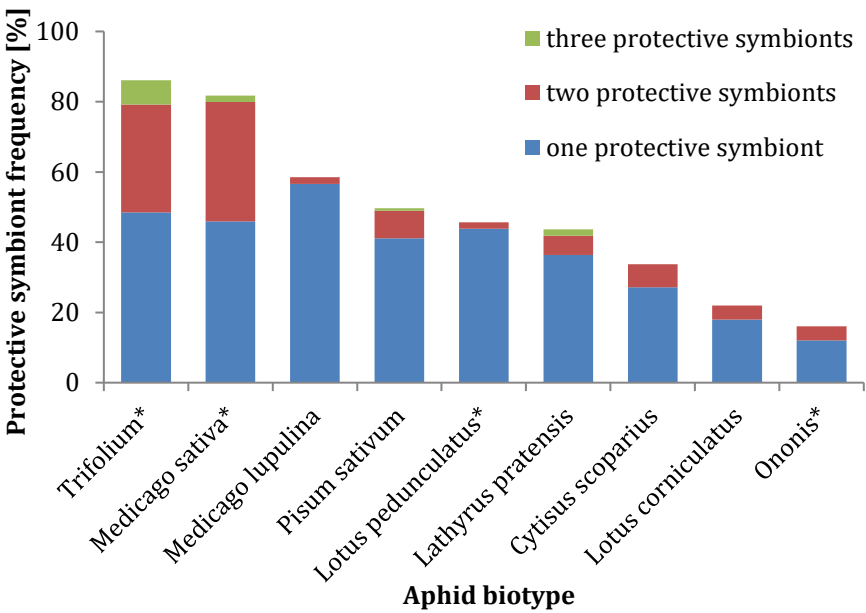


Figure 2

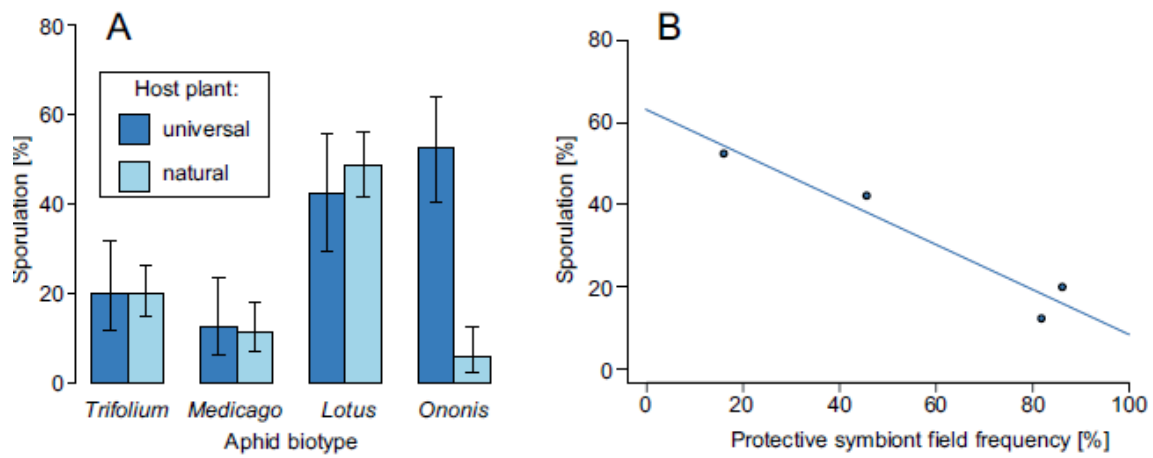
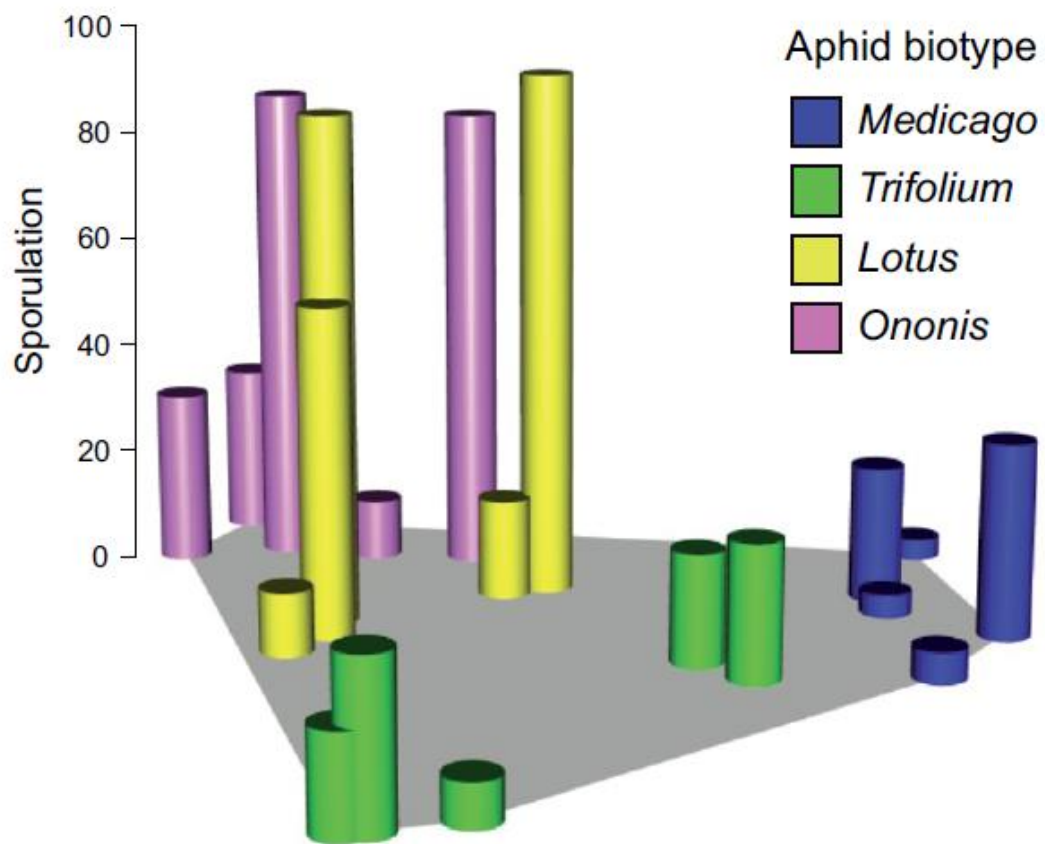


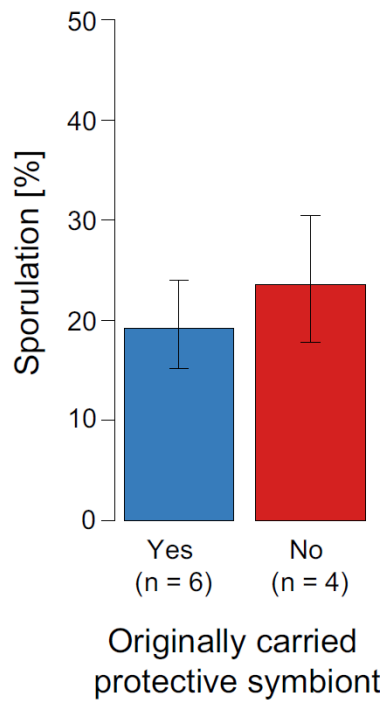
Figure 3



740

741 Figure 4

742



743

744 Figure 5

745

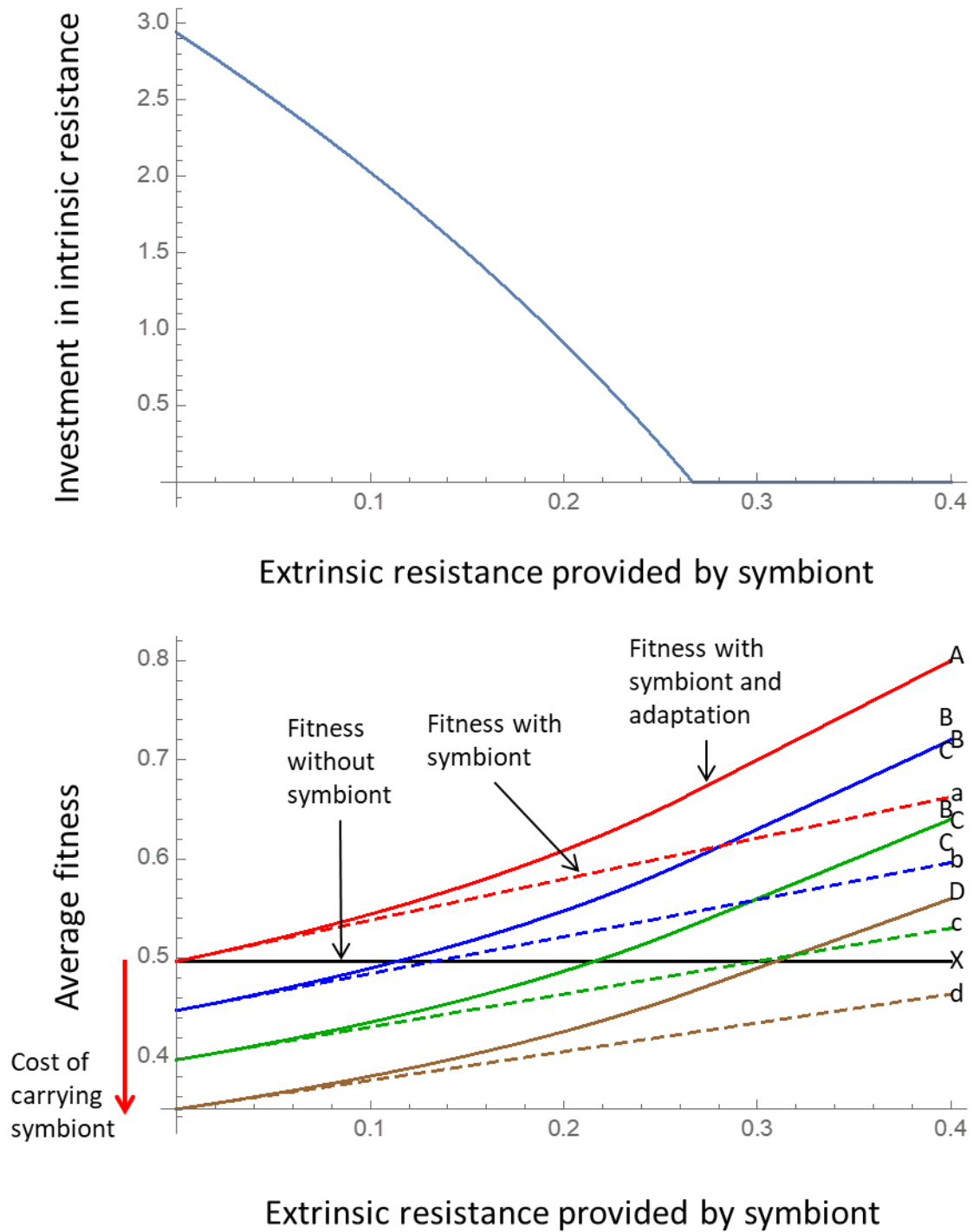


Figure 6