

# Defensive symbionts provide high protection against natural enemies at low cost to hosts: a meta-analysis

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## eLife Assessment

This study makes a **valuable** advance in our understanding of defensive symbionts in insects. It uses a meta-analysis to quantify the magnitude of change in host fitness components when symbionts are present in hosts exposed to natural enemies. The evidence supporting the study conclusions is **solid**, with analyses confirming common assumptions that symbionts generally provide defence at low cost to hosts.

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## Abstract

Defensive symbionts (i.e., symbionts that protect hosts against natural enemies) are common in nature, infecting many arthropod species. Defensive symbioses likely arose from host-symbiont interactions that are costly for hosts and evolved over time to a facultative mutualism. Hence, the role symbionts play in protecting hosts against natural enemies might be one of the reasons for their successful spread in nature. Here, we conducted a meta-analysis to investigate the costs and benefits of hosting symbionts in arthropods. We gathered approximately 3,000 effect sizes from 226 studies. Our results show that in the presence of natural enemies (i.e., hosts infected with pathogens or parasites), symbionts provide protection, positively affecting the fitness of their hosts. In the absence of natural enemies, symbionts caused little reduction in host fitness. Overall, protection was six times higher than the cost to hosts, indicating that symbionts provide high protection at low cost to hosts. We also found that natural enemies attacking hosts infected with symbionts have a significant reduction in their fitness. Moreover, the level of protection and cost for both hosts and natural enemies varies between host families, fitness components, and between natural and introduced symbionts (i.e., if the symbiont is natural of that species or if it was transinfected into a new species). Our results reveal a broad generality of protection at little cost for hosts, which may explain the successful spread of symbionts in wild arthropod populations.

# 1. Introduction

Symbiotic interactions (i.e., the living together of different organisms) are a ubiquitous part of most life stages of individuals and profoundly affect the ecology and evolution of all organisms (Thompson, 2015 [↗](#); Werren et al., 2008 [↗](#)). Symbiosis is extremely common in nature and comprises interactions that varies from harmful (i.e., parasitism) to advantageous (i.e., mutualism) (Oliver et al., 2003 [↗](#); Werren et al., 2008 [↗](#); Zug and Hammerstein, 2015 [↗](#)). A specific type of symbiosis is endosymbiosis, which occurs when microorganisms live within the body or cells of their hosts (Wernegreen, 2012 [↗](#)). Endosymbionts can be obligate or facultative and are maternally inherited (Vorbürger and Perlman, 2018 [↗](#); Werren et al., 2008 [↗](#); Zug and Hammerstein, 2015 [↗](#)). Defensive endosymbionts (symbionts hereafter) are widely spread among arthropods and many abiotic and biotic factors can influence the abundance and distribution of symbionts (Frago et al., 2020; Oliver et al., 2014 [↗](#)). For example, the bacterial symbiont *Wolbachia* is known for manipulating the reproduction of its hosts (i.e., reproductive parasitism), increasing the proportion of infected females across generations (Werren et al., 2008 [↗](#)). Until recently it was believed that reproduction manipulation was the main reason for the successful spread of *Wolbachia* within and among host populations (Werren et al., 2008 [↗](#); Zug and Hammerstein, 2015 [↗](#)) as it infects approximately 50% of all sampled terrestrial arthropod species (Shropshire et al., 2018 [↗](#)). However, reproductive parasitism alone may not be sufficient to guarantee that a new host species will be successfully invaded as certain *Wolbachia* strains, such as wMel, have incomplete maternal transmission and weak levels of reproductive parasitism (Fenton et al., 2011 [↗](#); Kriesner et al., 2013 [↗](#); Zug & Hammerstein, 2015 [↗](#)). There are also *Wolbachia* strains, such as wAu, that does not present any type of reproductive parasitism and can still increase in frequency within host populations (Kriesner et al., 2013 [↗](#)). Recent studies revealed that specific *Wolbachia* strains can protect hosts against RNA viruses (Hedges et al., 2008 [↗](#); Teixeira et al., 2008 [↗](#)). Besides *Wolbachia*, there are many other facultative symbionts commonly found infecting wild arthropod populations that do not present any type of reproductive parasitism and can protect their hosts against natural enemies (i.e., any organism – pathogens, parasites or predators – that negatively affects the individuals they are interacting with) (Oliver et al., 2014 [↗](#)). Thus, the successful spread of symbionts among arthropods can be related to the beneficial effect of symbionts to the fitness of their hosts when they are facing harmful environmental conditions, such as the presence of natural enemies (Oliver et al., 2014 [↗](#); Zug & Hammerstein, 2015 [↗](#)). Therefore, the outcome of the interaction – mutualism or parasitism – may depend on the environmental conditions that hosts are experiencing.

The interaction with symbionts can be costly for hosts (Martinez et al., 2016 [↗](#); Vorbürger et al., 2013 [↗](#); Zytynska et al., 2021 [↗](#)), yet the cost of the interaction can be overcome in the presence of natural enemies, evolving towards facultative mutualism. In facultative mutualism, the symbiont provides fitness benefits to the host, such as increased survival or fecundity, which may facilitate the infection and spread of symbionts into new hosts and within populations (Werren et al., 2008 [↗](#); Zug & Hammerstein, 2015 [↗](#); Zytynska et al., 2021 [↗](#)). On the other hand, in the absence of natural enemies, the interaction may remain costly to the host, evolving towards parasitism, where only the symbiont benefits from the interaction (Martinez et al., 2014 [↗](#); Oliver et al., 2014 [↗](#); Werren et al., 2008 [↗](#); Zug & Hammerstein, 2015 [↗](#)). For instance, aphids are commonly infected with bacterial symbiont *Hamiltonella defensa*, which provides protection against parasitoid wasps. Aphids infected with *H. defensa* under attack of parasitoids have increased protection with direct fitness benefits, but in the absence of parasitism, *H. defensa* infection can become costly (Martinez et al., 2018 [↗](#)). Other examples are *Wolbachia* strains wMel and wMelCS, which provide strong antiviral protection by substantially reducing the viral titer of two RNA viruses in *Drosophila simulans* (Martinez et al., 2014 [↗](#); Martinez et al., 2015 [↗](#)), but at the same time, when hosts are not infected with virus, both strains reduce the fitness of the flies (Martinez et al., 2014 [↗](#); Martinez et al., 2015 [↗](#)). However, there are cases where the interaction with

symbionts causes mild negative effects on the fitness of hosts (Martinez et al., 2015; Vorburger et al., 2009). Therefore, it is expected that in most cases, in the absence of natural enemies, symbionts will reduce the fitness of their hosts, while in the presence of natural enemies, symbionts will increase the fitness of their hosts and reduce the fitness of the natural enemy.

Because of the advances in molecular techniques and tools to answer ecological questions (Rowe et al., 2017), many studies focused on investigating defensive symbiosis in the past decades. More recently, many studies looked at the possible application of symbionts to control insect-borne human diseases such as dengue, chikungunya and malaria (Bian et al., 2010; Hughes et al., 2011; Moreira et al., 2009; van den Hurk et al., 2012; Werren et al., 2008; Xue et al., 2018), and as an agricultural pest control (Bourtzis, 2008; Gong et al., 2020; Werren et al., 2008). Among arthropod hosts, the families most frequently studied are Aphididae, Culicidae and Drosophilidae. In aphids, symbionts protect its hosts against parasitoid wasps (Cayetano et al., 2015; Ferrari et al., 2004; Oliver et al., 2003; Oliver et al., 2014) and fungal pathogens (Ferrari et al., 2004; Lukasik et al., 2013; Oliver et al., 2014; Scarborough et al., 2005). In Drosophilidae, symbionts protect flies against parasitoid wasps (Bian et al., 2010; Xie et al., 2011; Xie et al., 2014), nematodes (Haselkorn et al., 2013), viruses (Cogni et al., 2021; Hedges et al., 2008; Pimentel et al., 2021; Teixeira et al., 2008) and fungi (Panteleev et al., 2007). Finally, in Culicidae, *Wolbachia* protects mosquitoes against viruses (Bian et al., 2010; Moreira et al., 2009; van den Hurk et al., 2012), nematodes (Andrews et al., 2012; Kambris et al., 2009), protozoans (Hughes et al., 2011; Kambris et al., 2010; Zélé et al., 2012) and bacteria (Kambris et al., 2009; Ye et al., 2013). Although most studies report protective effects against natural enemies, there is also evidence of symbionts providing no protection against pathogens (Fytrou et al., 2006; Longdon et al., 2012; Martinez et al., 2012; Rottschaefer & Lazzaro, 2012) and even increasing the host susceptibility to natural enemies (Baton et al., 2013; Graham et al., 2012; Van Nouhuys et al., 2016). Thus, we do not know how general the protection provided by symbionts is as it may differ among host families as well as protection may be specific to certain symbiont and natural enemy species and/or strains (Cayetano & Vorburger, 2015; Coggi et al., 2021; Martinez et al., 2014; Martinez et al., 2015; Parker et al., 2013). Therefore, although many studies have investigated defensive symbiosis, we still cannot confirm its generality among different study systems.

Although the most relevant study systems have their commonalities, there are differences between them. Aphids are infected by a wide range of defensive symbionts such as *Hamiltonella defensa*, *Serratia symbiotica*, *Regiella insecticola*, among others (Guo et al., 2017). Some of these symbionts infect only aphids and whiteflies and they appear in intermediate frequencies in nature (Oliver et al., 2014). They can protect their hosts against parasitoid wasps or fungi, and they do not present any reproductive parasitism phenotype (Guo et al., 2017; Oliver et al., 2014). *Wolbachia* and *Spiroplasma* are also found infecting aphid species (Guo et al., 2017). However, despite both symbionts being reported as reproductive parasites, there is no confirmation of reproductive parasitism in the strains infecting aphids. While *Spiroplasma* can protect aphids against fungi and parasitoids, the role of *Wolbachia* in aphids remains elusive, although it may be related to nutrition or enhanced parthenogenesis (De Clerck et al., 2015, 2014). Drosophilids are often found infected with *Wolbachia* and *Spiroplasma*. Both symbionts have been found infecting flies with strains that induces reproductive parasitism and can be found in high frequencies in nature (Gerth et al., 2021). While *Spiroplasma* can protect flies against parasitoids and nematodes (Gerth et al., 2021), *Wolbachia* can protect flies against many RNA viruses (Pimentel et al., 2021; Zug & Hammerstein, 2015). Mosquitoes are infected with *Wolbachia*, which offers protection against a wide range of natural enemies, such as RNA viruses, protozoans, pathogenic bacteria, and nematodes (Pimentel et al., 2021; Zug & Hammerstein, 2015). Thus, aphids are infected by a wide range of symbionts that are usually found at intermediate frequencies in nature and can protect their hosts against a small group of natural enemies. On the other hand, flies and mosquitoes are infected by a small group of symbionts that can be found in high frequency in nature and protect their hosts from a wide range of natural enemies.

Here, we used a meta-analytical approach to investigate the costs and benefits of hosting symbionts in different study systems. Specifically, we addressed the following questions: (1) what is the magnitude of effect of the protection provided by symbionts to their hosts?, (2) what is the magnitude of effect of the cost of carrying symbionts to hosts?, (3) does the presence of symbionts in hosts affect the fitness of natural enemies? Furthermore, we tested if the outcome of the host-symbiont interaction varies between host families, natural and introduced symbionts (i.e., if symbiont is natural of that species or if it was artificially infected into a non-native species), symbiont species, natural enemy group, and fitness components.

## 2. Methods

### 2.1. Literature search

We conducted a literature search using all databases of *ISI Web of Science* and *Scopus* platforms. Our data search was last updated in January 2021. We used the following combinations of keywords: “(protect\* OR defens\*) AND (symbio\*) AND (fitness)” and “(endosymbio\*) AND (fitness)”. We also searched for grey literature at the advanced search (subject areas: “evolutionary biology” and “ecology”) in the *bioRxiv* platform using the same keywords. Additionally, we conducted a backward search of studies cited in the reference list of the following review articles: [Oliver et al. \(2014\)](#), [Vorburger & Perlman \(2018\)](#) and [Zug & Hammerstein \(2015\)](#). This allowed us to identify 45 additional studies.

### 2.2. Inclusion criteria

For the title and abstract screening, studies must have: (1) been performed on arthropod hosts, (2) mentioned that they measured the fitness of hosts and/or natural enemies, and (3) mentioned the presence of one or more defensive symbionts infecting hosts. For the full text screening, studies must have: (1) been empirical, (2) been performed in whole organisms (cell culture/in vitro experiments were excluded), (3) measured fecundity, egg hatch, longevity, survival or any other fitness measure (i.e., response variable) of the host in the presence and/or absence of a natural enemy, (4) measured viral load, parasitoid survival or any other fitness measure, which varies according to the natural enemy, (5) compared treatment (host with symbiont) and control (host without symbiont), (6) reported sample size, mean and standard deviation (S.D.) or standard error (S.E.), or proportion/number of events of the response variables. If descriptive data were missing, study must have reported inferential statistics (F, t, and degree of freedom) to be included; and (7) the symbiont used in the study had to be a defensive symbiont – therefore, previous studies or the study from which the data were extracted must have reported that the symbiont conferred protection against at least one natural enemy. Criteria (3) and (4) did not have to be attended at the same time because we had distinct datasets for the host and the natural enemy data. After full text screening, our sample included 226 studies with a great variety of study systems (Tables S1, S2, and S3). The full screening process is summarized in a PRISMA diagram ([Moher et al., 2009](#)) (Figure S1). A list with all studies included in our meta-analyses is provided in the Supplementary information.

### 2.3. Data extraction

We extracted different fitness measures and grouped similar data types under six broad categories of arthropod performance: survival, fecundity, body size, development time, uninfected hosts (i.e., % or number of individuals that were unsuccessfully parasitized or infected by natural enemies. We used this measure as a proxy for fitness as hosts that are non-infected tend to have higher survival), and natural enemy load (i.e., the amount of natural enemy replicating inside the host such as viruses and bacteria) (Table S4). When possible, we extracted the raw data from tables and graphs. Otherwise, we collected data from inferential statistics (F, t, and degree of freedom). When data were reported as mean  $\pm$  S.E., we calculated the S.D. based on the S.E. In the case of missing

S.D. or S.E., we estimated the S.D. using the metric proposed by Bracken (1992) (Lajeunesse, 2013 [↗](#)). When data were reported in figures, we extracted it using *PlotDigitizer* (Huwaldt, 2005 [↗](#)). In the case of data reported in box plots, we estimated the mean and S.D. using the metric proposed by Hozo et al. (2005) [↗](#). When authors measured the performance for more than one generation, we extracted only the first generation reported as it was a restricted number of studies and we were not interested in the effect of time (i.e., repeated measures). If the figure reported was a survival curve, we extracted the median and calculated the estimated S.D., using the Bracken (1992) metric (Lajeunesse, 2013 [↗](#)). We considered the median equal to the mean when the sample size was  $\geq 25$  (Hozo et al., 2005 [↗](#)).

We gathered the data in three distinct datasets: (1) protection - fitness measures of hosts with and without symbionts in the presence of natural enemies, (2) cost to the host - fitness measures of hosts with and without symbionts in the absence of natural enemies, and (3) cost to the natural enemy - fitness measures of natural enemies after infecting hosts with and without symbionts. Besides the fitness measures as response variables to calculate effect sizes, we collected aspects of the original studies that could be used as moderators in our models, such as the family that the host belongs to, symbiont type (i.e., natural, if the symbiont is natural of that species; or introduced, if the symbiont was artificially infected into a non-native species), symbiont species, natural enemy group (i.e., bacteria, fungus, nematode, parasitoid, protozoan, and virus), and the six broad categories of fitness measure (Table S5). We decided to exclude studies with predators as natural enemies, as in this specific scenario, symbionts do not confer a direct benefit to their hosts. This is because the fitness of the natural enemy is negatively affected only after the predator consumes the host and becomes infected with the symbiont (Noriyuki et al., 2014 [↗](#)).

## 2.4. Effect size calculation

For data reported as proportion or number of events, we used the odds ratio (OR) to calculate effect sizes. For data reported as mean  $\pm$  S.D., we used the Hedges' *g* to calculate effect sizes (Freeman et al., 1986 [↗](#)). We converted the OR estimates to Hedges' *g* to have a common metric (Polanin and Snijlsteit, 2016 [↗](#)). The calculation of effect sizes, including the conversion of OR to Hedges' *g*, was performed using the function *escalc* in the *metafor* package in R (Team R Development Core, 2022 [↗](#); Viechtbauer, 2017 [↗](#)). We calculated and converted the data collected from inferential statistics to Hedges' *g* with the Practical Meta-Analysis Effect Size Calculator, which uses pre-established equations to estimate effect sizes from different inferential statistics (Wilson, 2014 [↗](#)). Therefore, all the different types of data that we collected were converted into the same metric (Hedges' *g*), making them comparable. We calculated and adjusted the direction of our effect sizes in a way that positive values of Hedges' *g* indicate that symbionts have a positive effect on the fitness of either the host or the natural enemy. Hence, positive values mean higher fecundity, survival, body size, number of uninfected hosts, natural enemy load, but decreased development time. Consequently, negative values indicate that symbionts have a negative effect on fitness.

## 2.5. Statistical analyses

We divided our analyses in two parts: the main analyses, where we included all the study groups together, and two separate analyses for two subgroups as they were the majority of our datapoints: 1) a subgroup including data only from aphid hosts, and 2) a subgroup including data only from hosts harboring *Wolbachia* as a symbiont.

### Main analyses

Because we had many cases of multiple effect sizes coming from the same study, we built multilevel meta-analytical models with two random effects: study ID (between study variance) and effect size ID (within study variance). This allowed us to consider the non-independence of our data (Nakagawa and Santos, 2012 [↗](#)). After running an overall model with random effects only, we

included the moderators as fixed factors, totalizing 17 models (Table S6). Because the number of effect sizes recommended for each moderator level is at least 10 (Nakagawa et al., 2017 [↗](#)), when a moderator level had less than 10 effect sizes, we excluded it from the model. An exception to this is in the arthropod family and symbiont species models, where families and species levels with less than 10 effect sizes were gathered into a single moderator level – “other families” and “other species”, respectively. The overall models were run before and after exclusion of effect sizes to check if it affected the main results (Table S9). All analyses were performed using the *rma.mv* function in the *metafor* package in R (Team R Development Core, 2022 [↗](#); Viechtbauer, 2017 [↗](#)).

For each model, we quantified the total amount of heterogeneity ( $I^2$ ) and the percentage of contribution of each random effect – study ID and effect size ID – for the variance in the models. We used a modified version of  $I^2$  metric to calculate heterogeneity (Nakagawa and Santos, 2012 [↗](#)).

We also estimated the publication bias for each model. Publication bias occurs when the outcomes reported in the published literature deviate from those observed in all conducted studies and statistical analyses, leading to an incomplete and potentially skewed representation of the available evidence, which can lead to erroneous conclusions (Jennions et al., 2013 [↗](#)). We checked for potential publication bias with Egger’s regression (Egger et al., 1997 [↗](#)), using the *lm* function in R, using the residuals from the multilevel meta-analytic models (Team R Development Core, 2022 [↗](#)).

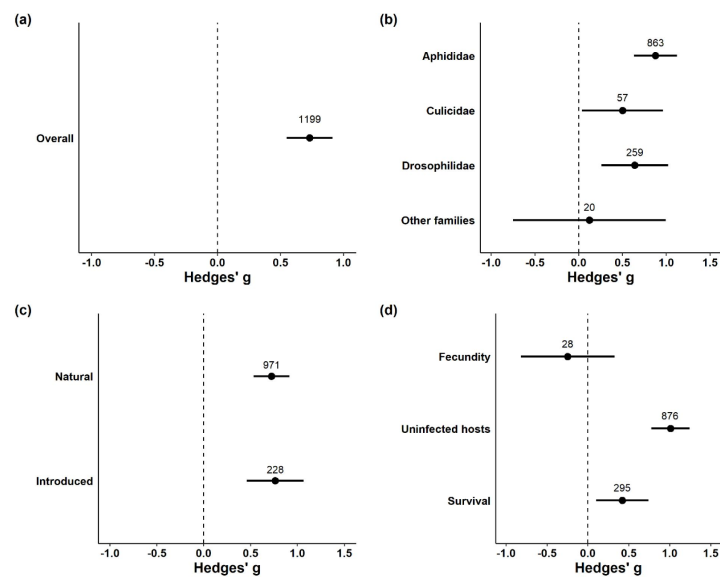
## Subgroup analyses

We followed the same methodology and criteria to run the analyses for subgroups “*Wolbachia*” (i.e., analyses including only datapoints where hosts were infected with the bacterial symbiont *Wolbachia*) and “Aphids” (i.e., analyses including only datapoints where hosts belonged to the Aphididae family). After running an overall model with random effects only, we included the same moderators as fixed factors, totalizing 14 models in the “*Wolbachia*” analyses (Table S7) and 12 models in the “Aphids” analyses (Table S8). We also quantified the heterogeneity and estimated the publication bias for each model as described above.

# 3. Results

## 3.1. Protection: effect of symbionts on the fitness of hosts in the presence of natural enemies (main analyses)

Mean overall effect size was moderate to high and positive, indicating that symbionts offer high protection against natural enemies, increasing host fitness (Table 1 [↗](#); Figure 1a [↗](#)). Fitness of hosts from Aphididae, Culicidae, and Drosophilidae families were positively influenced by the presence of symbionts (Table 1 [↗](#), Figure 1b [↗](#)). For other arthropod families, mean effect size was positive, but the lower confidence interval overlapped zero, indicating little evidence of protection (Table 1 [↗](#), Figure 1b [↗](#)). Both natural and introduced symbionts positively affected the fitness of hosts (Table 1 [↗](#); Figure 1c [↗](#)), indicating that both types of symbionts offer protection. While hosts harboring symbionts had higher survival and a higher number of individuals uninfected by natural enemies (uninfected hosts) (Table 1 [↗](#); Figure 1d [↗](#)), the presence of symbionts had no apparent effect on host fecundity (Table 1 [↗](#); Figure 1d [↗](#)). All symbiont species conferred protection against natural enemies, except for *Regiella* and *Spiroplasma* simultaneously infecting hosts (Table S10; Figure S2a). Overall, symbionts were able to offer protection against viruses, parasitoids and fungi, but not against bacteria, nematodes and protozoans (Table S11; Figure S2b).



**Figure 1.**

Main analyses: effect of defensive symbionts on the fitness of hosts that are under attack of natural enemies. (a) overall model, (b) model including host family as moderator, (c) model including symbiont type (i.e., if the symbiont naturally infects the host species or if it was artificially introduced) as moderator, and (d) model including fitness measure as moderator. Positive values indicate a positive effect on the fitness of hosts. Therefore, positive values indicate higher fecundity, survival, and number of individuals that were uninfected by their natural enemies (i.e., uninfected hosts - number of individuals that were unsuccessfully parasitized or infected by their natural enemies). Negative values indicate that symbionts have a negative effect on fitness. Points are the weighted mean effect sizes  $\pm$  95% confidence intervals. Numbers above points are the number of effect sizes.

Table 1.

Results of the meta-analysis of the costs and benefits of carrying a symbiont and test of moderators for overall, host family, symbiont type, and fitness measure.

| Data                      | Model and moderator levels | Meta-analytical models |     |                  |                  | Test of moderators |        |               |
|---------------------------|----------------------------|------------------------|-----|------------------|------------------|--------------------|--------|---------------|
|                           |                            | k                      | m   | Mean effect size | 95% C.I.         | p-value            | QM     | p-value       |
| Protection                | Overall                    | 1199                   | 92  | 0.73             | 0.547 to 0.913   | <0.0001            |        |               |
|                           | Host Family                |                        |     |                  |                  |                    | 64.75  | <0.0001       |
|                           | Aphididae                  | 863                    | 49  | 0.876            | 0.631 to 1.120   | <0.0001            |        |               |
|                           | Culicidae                  | 57                     | 18  | 0.500            | 0.037 to 0.962   | <b>0.034</b>       |        |               |
|                           | Drosophilidae              | 259                    | 22  | 0.639            | 0.259 to 1.019   | <b>0.001</b>       |        |               |
|                           | Other families             | 20                     | 4   | 0.122            | -0.751 to 0.995  | 0.783              |        |               |
|                           | Symbiont type              |                        |     |                  |                  |                    | 61.21  | <0.0001       |
|                           | Natural                    | 971                    | 78  | 0.723            | 0.531 to 0.914   | <0.0001            |        |               |
|                           | Introduced                 | 228                    | 21  | 0.762            | 0.458 to 1.066   | <0.0001            |        |               |
|                           | Fitness measure            |                        |     |                  |                  |                    | 80.87  | <0.0001       |
| Cost to the host          | Fecundity                  | 28                     | 10  | -0.247           | -0.820 to 0.325  | 0.397              |        |               |
|                           | Uninfected hosts           | 876                    | 57  | 1.007            | 0.773 to 1.241   | <0.0001            |        |               |
|                           | Survival                   | 295                    | 33  | 0.418            | 0.099 to 0.738   | <b>0.010</b>       |        |               |
|                           | Overall                    | 1625                   | 157 | -0.121           | -0.239 to -0.002 | <b>0.045</b>       |        |               |
|                           | Host Family                |                        |     |                  |                  |                    | 17.21  | <b>0.002</b>  |
|                           | Aphididae                  | 801                    | 46  | -0.352           | -0.553 to -0.150 | <b>0.0006</b>      |        |               |
|                           | Culicidae                  | 256                    | 29  | -0.215           | -0.484 to 0.053  | 0.116              |        |               |
|                           | Drosophilidae              | 287                    | 38  | -0.067           | -0.307 to 0.173  | 0.584              |        |               |
|                           | Other families             | 281                    | 44  | 0.193            | -0.036 to 0.422  | 0.098              |        |               |
|                           | Symbiont type              |                        |     |                  |                  |                    | 6.59   | <b>0.037</b>  |
| Cost to the natural enemy | Natural                    | 1170                   | 127 | -0.075           | -0.206 to 0.056  | 0.2616             |        |               |
|                           | Introduced                 | 455                    | 44  | -0.260           | -0.467 to -0.052 | <b>0.014</b>       |        |               |
|                           | Fitness measure            |                        |     |                  |                  |                    | 110.63 | <0.0001       |
|                           | Body size                  | 105                    | 29  | 0.546            | 0.281 to 0.810   | <0.0001            |        |               |
|                           | Development time           | 284                    | 54  | 0.486            | 0.299 to 0.672   | <0.0001            |        |               |
|                           | Fecundity                  | 635                    | 129 | -0.213           | -0.362 to -0.064 | <b>0.005</b>       |        |               |
|                           | Survival                   | 601                    | 105 | -0.349           | -0.499 to -0.200 | <0.0001            |        |               |
|                           | Overall                    | 379                    | 58  | -0.909           | -1.277 to -0.542 | <0.0001            |        |               |
|                           | Host Family                |                        |     |                  |                  |                    | 22.96  | <b>0.0001</b> |
|                           | Aphididae                  | 73                     | 12  | -0.821           | -1.647 to 0.005  | 0.0514             |        |               |
|                           | Culicidae                  | 101                    | 25  | -0.961           | -1.532 to -0.390 | <b>0.0010</b>      |        |               |
|                           | Drosophilidae              | 193                    | 16  | -1.000           | -1.705 to -0.295 | <b>0.0054</b>      |        |               |
|                           | Other families             | 12                     | 5   | -0.511           | -1.861 to 0.839  | 0.4584             |        |               |
|                           | Symbiont type              |                        |     |                  |                  |                    | 25.89  | <0.0001       |
|                           | Natural                    | 246                    | 39  | -0.763           | -1.186 to -0.341 | <b>0.0004</b>      |        |               |
|                           | Introduced                 | 133                    | 24  | -1.135           | -1.626 to -0.644 | <0.0001            |        |               |
|                           | Fitness measure            |                        |     |                  |                  |                    | 26.87  | <0.0001       |
|                           | Body size                  | 20                     | 5   | -1.668           | -2.788 to -0.549 | <b>0.0035</b>      |        |               |
|                           | Natural enemy load         | 193                    | 36  | -0.738           | -1.193 to -0.284 | <b>0.0015</b>      |        |               |
|                           | Survival                   | 166                    | 20  | -1.043           | -1.637 to -0.448 | <b>0.0006</b>      |        |               |

p-values that are statistically significant are in bold ( $\alpha = 0.05$ )

k = number of effect sizes

m = number of studies

The overall model presented high heterogeneity (97.25%). The effect size ID explained 61.82% of the variance, followed by the study ID (35.43%). Although we included moderators, heterogeneity remained high in all models. In all models the effect size ID explained most of the variance, followed by the study ID ([Table 2](#)).

### 3.2. Cost to hosts: effect of symbionts on the fitness of hosts in the absence of natural enemies (main analyses)

Mean overall effect size was low and negative, indicating that hosts suffered little cost on their overall fitness in the absence of natural enemies ([Table 1](#), [Figure 2a](#)). Hosts from the family Aphididae had a negative effect on their fitness due to the presence of symbionts. Culicidae also had their mean overall fitness reduced and although the upper confidence interval slightly overlapped zero, we found little evidence of a positive effect on fitness. We found little evidence of substantial associated costs in Drosophilidae and other arthropod families ([Table 1](#), [Figure 2b](#)). Introduced symbionts had a negative effect on the fitness of hosts ([Table 1](#), [Figure 2c](#)). Natural symbionts had a negative mean effect size and although the upper confidence interval overlapped zero, we found little evidence of a positive effect on the fitness of hosts ([Table 1](#), [Figure 2c](#)). Body size and development time were positively affected by the presence of symbionts ([Table 1](#); [Figure 2d](#)) while fecundity and survival were negatively affected ([Table 1](#); [Figure 2d](#)). Additionally, we found that only *Hamiltonella* and *Spiroplasma* significantly reduced the fitness of their hosts ([Table S10](#); [Figure S3](#)).

The overall model had high heterogeneity (98.17%). Study ID explained 21.98% of the variance and the effect size ID explained 76.19%. Although we included moderators, heterogeneity remained high in all models. In each model the effect size ID explained most the variance, followed by the study ID ([Table 2](#)).

### 3.3. Cost to natural enemies: effect of symbionts on the fitness of natural enemies (main analyses)

Mean overall effect size was high and negative, indicating that natural enemies suffered a strong reduction in overall fitness when infecting hosts carrying symbionts ([Table 1](#), [Figure 3a](#)). Natural enemies infecting insects from families Culicidae and Drosophilidae carrying symbionts had a negative effect on their fitness ([Table 1](#); [Figure 3b](#)). For natural enemies infecting hosts from Aphididae family, although the upper confidence interval slightly overlapped zero, we found little evidence of positive effects on fitness. For natural enemies infecting hosts from other arthropod families, we found little evidence of associated costs on fitness ([Table 1](#); [Figure 3b](#)). Both natural and introduced symbionts had negative effect on the fitness of natural enemies ([Table 1](#); [Figure 3c](#)). The presence of symbionts in hosts had a negative effect on body size, survival and load of natural enemies ([Table 1](#); [Figure 3d](#)). Additionally, we found that only *Hamiltonella* was not able to significantly reduce the fitness of natural enemies ([Table S10](#); [Figure S4a](#)). We also found that nematodes, parasitoids, protozoans and viruses had their fitness significantly reduced due to the presence of symbionts in hosts, although the upper confidence interval from the virus overlapped zero. However, we found little evidence of symbionts substantially reducing the fitness of pathogenic bacteria infecting their hosts ([Table S11](#); [Figure S4b](#)).

The overall model had high heterogeneity (97.91%). Study ID explained 45.49% of the variance and the effect size ID explained 52.42%. Although we included moderators, heterogeneity remained high in all models. In each model the effect size ID explained most the variance, followed by the study ID ([Table 2](#)).

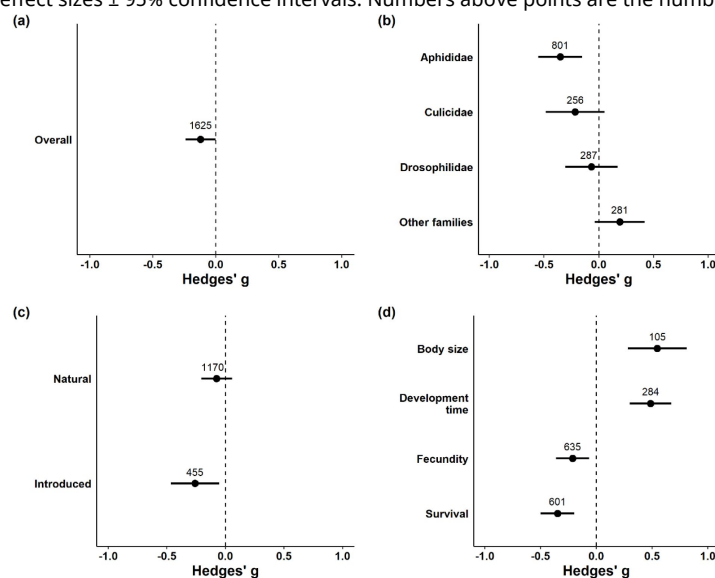
Table 2.

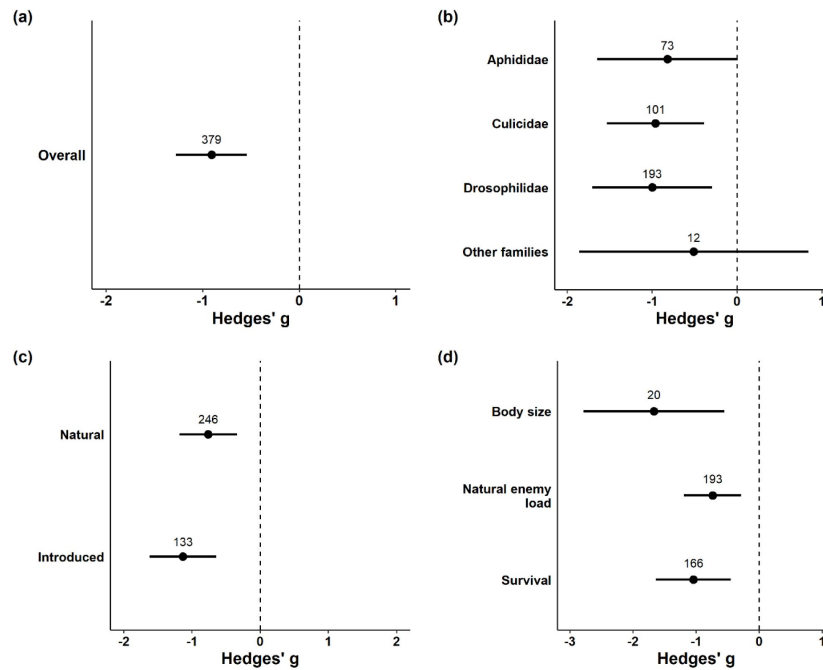
Test of heterogeneity (total, between studies and within studies) for each of the 17 meta-analytical models included in the main analyses.

| Data                      | Model               | I <sup>2</sup> (%) | I <sup>2</sup> between studies (%) (study ID) | I <sup>2</sup> within studies (%) (effect size ID) |
|---------------------------|---------------------|--------------------|-----------------------------------------------|----------------------------------------------------|
| Protection                | Overall             | 97.25              | 35.43                                         | 61.82                                              |
|                           | Host family         | 97.27              | 36.02                                         | 61.25                                              |
|                           | Symbiont type       | 97.26              | 35.56                                         | 61.70                                              |
|                           | Symbiont species    | 97.29              | 37.35                                         | 59.94                                              |
|                           | Natural enemy group | 97.18              | 34.04                                         | 63.14                                              |
|                           | Fitness measure     | 97.28              | 37.17                                         | 60.11                                              |
| Cost to the host          | Overall             | 98.17              | 21.98                                         | 76.19                                              |
|                           | Host family         | 98.14              | 20.77                                         | 77.37                                              |
|                           | Symbiont type       | 98.16              | 21.84                                         | 76.32                                              |
|                           | Symbiont species    | 98.15              | 21.49                                         | 76.66                                              |
|                           | Fitness measure     | 98.14              | 27.28                                         | 70.86                                              |
| Cost to the natural enemy | Overall             | 97.91              | 45.49                                         | 52.42                                              |
|                           | Host family         | 97.97              | 46.87                                         | 51.10                                              |
|                           | Symbiont type       | 97.88              | 44.63                                         | 53.25                                              |
|                           | Symbiont species    | 97.42              | 49.74                                         | 47.68                                              |
|                           | Natural enemy group | 97.81              | 42.87                                         | 54.94                                              |
|                           | Fitness measure     | 97.87              | 44.07                                         | 53.80                                              |

Figure 2.

Main analyses: effect of defensive symbionts on the fitness of hosts that are in the absence of natural enemies. (a) overall model, (b) model including host family as moderator, (c) model including symbiont type (i.e., if the symbiont naturally infects the host species or if it was artificially introduced) as moderator, and (d) model including fitness measure as moderator. Positive values indicate a positive effect on the fitness of hosts. Therefore, positive values indicate higher fecundity, survival, body size and decreased development time. Negative values indicate that symbionts have a negative effect on fitness. Points are the weighted mean effect sizes  $\pm$  95% confidence intervals. Numbers above points are the number of effect sizes.





**Figure 3.**

Main analyses: effect of defensive symbionts on the fitness of natural enemies attacking hosts that are infected with symbionts. (a) overall model, (b) model including host family as moderator, (c) model including symbiont type (i.e., if the symbiont naturally infects the host species or if it was artificially introduced) as moderator, and (d) model including fitness measure as moderator (i.e., natural enemies' body size, survival and load). Positive values indicate a positive effect on the fitness of hosts. Therefore, positive values indicate higher body size, survival, and natural enemy load (the amount of natural enemy replicating inside the host such as viruses and bacteria). Negative values indicate that symbionts have a negative effect on fitness. Points are the weighted mean effect sizes  $\pm$  95% confidence intervals. Numbers above points are the number of effect sizes.

## Publication bias (main analyses)

We found evidence for publication bias in 8 out of the 17 models. All models from protection data, one model from the cost to the natural enemy data, and one model from the cost to the host data had evidence for publication bias (Table 3 [↗](#)).

## 3.4. Subgroup analyses: *Wolbachia* and aphids

### Wolbachia

Protection data showed that hosts harboring *Wolbachia* increased their fitness when they were under attack of natural enemies as mean overall effect size was positive and moderate (Table S12). Drosophilidae and Culicidae hosts had their fitness increased when infected with *Wolbachia*, although the lower confidence interval from Culicidae slightly overlapped zero (Table S12). *Wolbachia* increased the fitness of hosts infected with viruses, but not with bacteria, protozoans and parasitoids (Table S12).

Overall, *Wolbachia* showed little evidence of reducing the fitness of their hosts in the absence of natural enemies as the mean effect size was close to zero (Table S12). *Wolbachia* had a negative effect on body size and survival of their hosts, but not on fecundity and development time (Table S12). *Wolbachia* strains that naturally infected their hosts did not cause costs to their fitness, however, *Wolbachia* strains that were artificially introduced into new hosts reduced their fitness (Table S12).

Regarding the cost to natural enemies that infected hosts harboring *Wolbachia*, their fitness were substantially reduced as mean overall effect size was negative and moderate to high (Table S12). This was observed specifically in Culicidae and introduced *Wolbachia* strains (Table S12).

### Aphids

Protection data showed that hosts from family Aphididae harboring symbionts had their fitness increased when they were under attack of natural enemies as mean overall effect size was high and positive (Table S13). This could be observed when aphids were harboring any symbiont species, except *Spiroplasma*, and when aphids were attacked by either fungus or parasitoids (Table S13). However, when we looked separately at each of the fitness measures, only the number of uninfected hosts (i.e., not parasitized) contributed to the overall positive effect on fitness (Table S13).

Overall, symbionts reduced the fitness of aphids in the absence of natural enemies as mean overall effect size was moderate and negative (Table S13). However, the only symbiont species that caused this reduction on aphid fitness was *Hamiltonella* and *Spiroplasma*, and this was observed only in symbionts that naturally infected aphids. All fitness measures contributed to the overall reduction in fitness that was observed in aphids, except for body size (Table S13).

Regarding the cost to natural enemies, overall, there was a substantial reduction on the fitness of natural enemies attacking aphids harboring symbionts, as mean effect size was high and negative. However, when we looked separately at each fitness component, only survival contributed to the overall negative effect observed (Table S13).

All results from subgroup analyses for “*Wolbachia*” and “Aphids” data, including heterogeneity test and publication bias are in Tables S12 and S13.

| Data                      | Model               | Intercept | t      | p-value         |
|---------------------------|---------------------|-----------|--------|-----------------|
| Protection                | Overall             | 0.763     | 13.61  | < <b>0.0001</b> |
|                           | Host family         | 0.694     | 12.61  | < <b>0.0001</b> |
|                           | Symbiont type       | 0.762     | 13.6   | < <b>0.0001</b> |
|                           | Symbiont species    | 0.776     | 13.99  | < <b>0.0001</b> |
|                           | Natural enemy group | 0.696     | 12.61  | < <b>0.0001</b> |
|                           | Fitness measure     | 0.647     | 12.03  | < <b>0.0001</b> |
| Cost to the host          | Overall             | -0.167    | -1.84  | 0.064           |
|                           | Host family         | -0.098    | -1.09  | 0.276           |
|                           | Symbiont type       | -0.162    | -1.79  | 0.073           |
|                           | Symbiont species    | -0.015    | -1.65  | 0.098           |
|                           | Fitness measure     | -0.199    | -2.20  | <b>0.027</b>    |
| Cost to the natural enemy | Overall             | -0.200    | -1.766 | 0.078           |
|                           | Host family         | -0.168    | -1.494 | 0.136           |
|                           | Symbiont type       | -0.209    | -1.839 | 0.066           |
|                           | Symbiont species    | 0.105     | 0.945  | 0.345           |
|                           | Natural enemy group | -0.245    | -2.224 | <b>0.026</b>    |
|                           | Fitness measure     | -0.201    | -1.795 | 0.073           |

p-values that are statistically significant are in bold ( $\alpha=0.05$ )

**Table 3.**

**Summary of the Egger's regression test for publication bias of the models included in the main analyses. The results for each of the 17 meta-analytical models is presented below.**

## 4. Discussion

In this meta-analysis we were able to collect data from 226 studies, adding up to approximately 3,000 effect sizes from many different study systems which allowed us to make a broad analysis on the generality of symbiont effects on host fitness. The overall models from our main analyses revealed that the protection provided by symbionts (mean effect size = 0.73) is around six times higher than the cost in the absence of natural enemies (mean effect size = -0.12), indicating a high level of protection at little cost to hosts. We also found that the outcome of the host-symbiont interaction can vary among host families, fitness components, and between symbionts that were transinfected into a new species and the ones that naturally infect the species. Moreover, not all fitness components contribute equally to our overall conclusions. For example, by looking at the fitness components separately, we can see that under natural enemy attack, symbionts have a substantial effect on host survival but not on fecundity. Below we delve into a more comprehensive discussion of our results.

Zytynska et al. (2021) [performed](#) a meta-analysis on the costs and benefits of harboring facultative symbionts in aphids, whiteflies and heteropterans. Overall, they found that whiteflies have higher fecundity and reduced development time whereas aphids have increased resistance to natural enemies, but lower fecundity when infected by symbionts. This is in accordance with our findings where aphids harboring symbionts have strong protection against natural enemies such as parasitoids and fungi, and a substantial cost to their fitness, especially their fecundity and survival. By comparing our datasets with their studies with defensive symbiosis, which is the focus of our study, we have 48 overlapping studies. Therefore, considering that our dataset has 226 studies included, we only share around 21% of the entire data, meaning that around 79% of our study is composed of new data on defensive symbiosis, which is expected since we included all terrestrial arthropods, including two major study systems: *Drosophila-Wolbachia-virus* and mosquito-*Wolbachia-virus* associations. Hence, here we expand their important findings on the interaction between hosts and defensive symbionts.

We found that in the presence of natural enemies, hosts harboring symbionts have higher fitness than hosts without symbionts, indicating that there is a general pattern of symbiont protection against natural enemies in insects. We also found that in the absence of natural enemies, hosts carrying symbionts have lower fitness than those not carrying symbionts. However, although the interaction with symbionts is costly for the hosts, our results indicate that this cost is low. Collectively, these results suggest that over the coevolutionary course, selection may lead to the evolution of symbiont strains that provide moderate to high protection against natural enemies with low costs associated. Our findings support results and predictions from prior studies. In pea aphids, it has been reported mild fitness costs associated with *Hamiltonella* infection (Leclair et al., 2016 [; Martinez et al., 2018 \[; Oliver et al., 2006\]\(#\)](#) ), which have strains that provide moderate to high protection against parasitoids (Doremus & Oliver, 2017 [; Doremus et al., 2018 \[; Martinez et al., 2018 \\[; Oliver et al., 2005\\]\\(#\\)\]\(#\) \). Martinez et al. \(2015\) \[observed\]\(#\) a natural strain of \*D. simulans\*, wAu, that is highly protective and causes little cost on egg hatch rates in the absence of viruses and suggested that, over the long term of host-symbiont association, natural selection might be able to break the trade-off between antipathogenic protection and cost. Therefore, in some cases, because of the presence of natural enemies, the host-symbiont interaction may evolve to a facultative mutualism rather than parasitism.](#)

Our results showed no evidence of protection against natural enemies in arthropod families other than Aphididae, Culicidae and Drosophilidae. Protection against natural enemies may be one of the reasons for symbionts being so widely spread among arthropods (Pimentel et al., 2021 [; Zug and Hammerstein, 2015](#) ). However, studies on protection against natural enemies are still limited mostly to aphid and diptera hosts. Besides studies on different species from the families

Aphididae, Culicidae and Drosophilidae, we only found studies that tested for symbionts protecting against natural enemies in Diptera: Glossinidae (Schneider et al., 2019), Hemiptera: Aleyrodidae (Ghosh et al., 2018), Trombidiformes: Tetranychidae (Zélé et al., 2020), Isopoda: Armadillidiidae (Braguart-Varnier et al., 2015), and Isopoda: Porcellionidae (Braguart-Varnier et al., 2015). Thus, more studies on different arthropod families are essential to know how protection from natural enemies is correlated with symbiont prevalence in natural populations of terrestrial arthropods.

We found that the interaction is substantially costly for aphids. This is in accordance with Zytynska et al. (2021), that found that the interaction with symbionts reduced fecundity and lifespan of aphids, but not of whiteflies. When we looked at the subgroup analyses for hosts from the family Aphididae only, we observed that this cost is associated with host fecundity and survival and specifically with symbionts *Hamiltonella* and *Spiroplasma*. Although defensive symbionts have successfully spread among insects, the level of colonization vary between symbiont and host species. For example, while *Wolbachia* frequency in natural insect populations varies from 10% to 100% (Kajtoch and Kotásková, 2018; Kriesner et al., 2016), aphid symbiont *Serratia symbiotica* occurs at low frequencies (approximately 20%) in the wild, despite offering protection against parasitoids (Pons et al., 2022). This suggests that harboring certain symbionts species and strains involves a moderate to high cost to aphid fitness in the absence of selection by parasitoids. In addition to the balance between cost and protection, some symbionts have other strategies to increase in frequency in nature. For instance, certain strains of *Wolbachia* have successfully colonized insects and arthropods populations via reproductive parasitism (Himler et al., 2011; Martinez et al., 2015; Zug & Hammerstein, 2015). Therefore, in the absence of natural enemies, some symbiont strains can cease to be a facultative mutualist and act as a reproductive parasite. This is the case for some *Wolbachia* strains that causes cytoplasmic incompatibility but also have an antipathogenic effect at the same time (Martinez et al., 2015). Thus, our results suggest that one possible explanation for aphid symbionts having lower frequencies in natural host populations might be due to relatively moderate to high costs associated with the interaction, and the lack of other strategies to spread among and within populations, such as reproductive parasitism.

Looking at our main analyses we found that both natural and introduced symbionts confer protection against natural enemies, but only the ones that are introduced to a new host species are costly in the absence of pathogens. When we looked at the subgroup analyses for hosts infected with *Wolbachia*, we found the same pattern. Both natural and introduced *Wolbachia* strains protected their hosts against natural enemies, but only the *Wolbachia* strains that were recently transinfected into a new species caused a substantial reduction on the fitness of their hosts. Among flies and mosquitoes hosts, there is an overall trend that symbiont strains that provide stronger protection have the highest densities inside the cells of hosts (Martinez et al., 2014; Martinez et al., 2015; Zug & Hammerstein, 2015). Therefore, it is common to transinfect high density strains into new hosts to test for protection against natural enemies (Moreira et al., 2009; van den Hurk et al., 2012; Zug & Hammerstein, 2015), especially in the Culicidae family to control insect-borne human diseases (Bian et al., 2010; Hughes et al., 2011; Moreira et al., 2009; van den Hurk et al., 2012; Werren et al., 2008; Xue et al., 2018). However, hosts have a reduction in their fitness when they are not infected with natural enemies but are carrying these high-density strains (Martinez et al., 2014; Martinez et al. 2015; Martinez et al. 2016). For example, Martinez et al. (2014) transferred 19 *Wolbachia* strains with different levels of density into *D. simulans* to test them for antiviral protection. After transinfecting *Wolbachia* strains into a new recipient species, usually they keep the same level of density as in their original host. Flies infected with high density strains wMel and wMelCS, natural from *D. melanogaster*, had higher survival and lower viral load when infected with virus. However, when flies were not infected with virus, these same strains caused reduction in fecundity and lifespan of flies. Interestingly, when flies were infected with natural and highly protective strain wAu, fitness costs were lower (Martinez et al., 2015). Another interesting study using wAu showed that despite this strain not

presenting a reproductive parasitism phenotype – which is one of the known mechanisms that *Wolbachia* uses to spread in arthropod populations – this strain is still able to spread in field populations of its natural host, *D. simulans*. This alone suggests that symbionts have different mechanisms to propagate, one of them potentially being protection against natural enemies (Kriesner et al., 2013 [DOI](#)). Thus, the level of protection and cost to the host depends on the symbiont strain that is infecting the hosts, and this might influence the ability of symbionts to spread in nature. Moreover, one possible explanation is that early on the host-symbiont interaction the cost of harboring symbionts is high, but it decreases over evolutionary time, facilitating the propagation of symbionts.

Although it is known that *Wolbachia* density plays an important role in the level of protection in mosquitoes and flies (Martinez et al., 2014 [DOI](#); Martinez et al., 2015 [DOI](#); Zug & Hammerstein, 2015 [DOI](#)), in aphid symbionts there is no evidence that higher density is the main cause of higher protection (Doremus et al., 2018 [DOI](#)). For example, *H. defensa* have strains that provide moderate to high protection against parasitoids (Doremus & Oliver, 2017 [DOI](#); Doremus et al., 2018 [DOI](#); Martinez et al., 2018 [DOI](#); Oliver et al., 2005 [DOI](#)). Aphids can harbor different *H. defensa* strains that are associated with bacteriophages called APSE, which encode putative toxins that are responsible for defense against parasitoids. Different types of APSE phages are associated with different levels of protection (Doremus et al., 2018 [DOI](#); Martinez et al., 2018 [DOI](#); Vorburger & Gouskov, 2011 [DOI](#)). Besides symbiont-based defenses, hosts may have endogenous defenses against natural enemies that also vary in their level of protection, depending on host genotype (Martinez et al., 2018 [DOI](#)). Under natural enemy pressure, the immune system of insect hosts allocates a lot of resources, leading to a trade-off between immune defense and reproductive output (Schwenke et al., 2016 [DOI](#)). It is interesting to note that in some cases the presence of symbionts weakens the host endogenous defenses. An experiment performed in *Drosophila* hosts showed that the presence of *Wolbachia* in the populations along generations weakened the selection on the host endogenous antiviral defenses (Martinez et al., 2016 [DOI](#)). This could mean that, in some cases, the cost of harboring a defensive symbiont can be lower than the cost associated with activating their own immune defense. Therefore, the level of protection against natural enemies depends on many factors such as host genotype, symbiont presence, symbiont strain and on the balance between the costs and benefits of harboring a symbiont or depending solely on immune defenses.

We found that, in the presence of natural enemies, symbionts were able to increase survival and the likelihood of hosts not becoming infected by natural enemies but were unable to recover fecundity. These results were expected since there is little evidence of symbionts recovering fecundity of their hosts, such as *Spiroplasma* when *D. neotestacea* is infected by nematodes of the genus *Howardula*, which sterilizes the female fly (Haselkorn et al., 2013 [DOI](#); Jaenike et al., 2010 [DOI](#)). We also found that in the absence of natural enemies, symbionts decreased host fecundity and survival, but increased body size and decreased development time. This potentially have a positive impact on the overall fitness, since it is expected that larger individuals have higher reproductive success (Beukeboom, 2018 [DOI](#)), and individuals that develop faster spend less time being exposed to natural enemies such as parasitoids (Nijhout et al., 2010 [DOI](#)). Thus, despite the cost in fecundity and survival, the benefit in development time and body size may be reducing the overall cost of the interaction.

Our results showed that natural enemies from all host families had low survival, load, and smaller body size when they infected hosts with symbionts. Also, both natural and introduced symbionts reduced natural enemies' fitness. Symbionts can compete for resources within the host, release toxins or activate the host's immune response, directly affecting the chance of success of the natural enemy (Vorburger and Perlman, 2018 [DOI](#)). In aphids, *H. defensa* releases toxins that are produced by bacteriophages present in its genome and kill the early life stages of parasitoids (Vorburger and Gouskov, 2011 [DOI](#); Weldon et al., 2013 [DOI](#)). Although we do not know exactly which mechanism is adopted by *Wolbachia*, *Spiroplasma* and other aphid symbionts (Zug and Hammerstein, 2015 [DOI](#)), our results suggest a general pattern of symbionts reducing the fitness of

natural enemies that attack their hosts. However, it is important to notice that there is a wide functional diversity among natural enemies. For example, when parasitoid wasps successfully parasitize their host, the host dies. In the case of *Drosophila* and mosquitoes hosts, viruses are a common pathogen in nature, but their virulence (i.e., the capacity to cause damage in a host) varies greatly. Looking specifically at the viruses that infect *Drosophila* for example, DCV is frequently studied in the laboratory but rarely found in nature due to its virulence, killing the flies within days (Kapun et al., 2010 [↗](#)). Other viruses that are more frequently found in nature such as DAV and Nora similarly affect the fitness of their hosts, albeit to a lesser extent than DCV. DAV reduces the fecundity of flies (Brosh et al., 2022 [↗](#)), and both Nora and DAV kill their host, but at a much slower rate than DCV (Ambrose et al., 2009 [↗](#); Habayeb et al., 2006 [↗](#)). If the natural enemy has only mild effects on the host fitness, there may be correspondingly smaller fitness benefits to any protection that symbionts can provide. Hence, the level of virulence of natural enemies can directly influence the outcome of the host-symbiont-natural enemy interaction.

## Caveats

The great majority of studies addressing symbiont protection against natural enemies are performed under laboratory conditions, minimizing biotic and abiotic factors that could influence the outcome of the host-symbiont-natural enemy interactions in nature. For aphids, it has been shown that under laboratory conditions, *H. defensa* is able to increase host resistance against parasitoids and that the level of protection varies among host and parasitoid genotypes (Cayetano and Vorburger, 2015 [↗](#)). Few studies have tested *H. defensa* protection against parasitoids in the field, and some suggested that *H. defensa* reduces aphid mortality against specific parasitoid strains but there was no overall fitness benefit for the hosts possibly because of the cost of the host-symbiont interaction (Hrček et al., 2016 [↗](#); Rothacher et al., 2016 [↗](#)). A recent study has shown no significant relationship between the presence of parasitoid wasps and *H. defensa* prevalence in the field as well as no strong evidence for symbiont cost in the absence of parasitoids. Instead, an important factor modulating symbiont prevalence in the field could be the temperature (Smith et al., 2021 [↗](#)). In dipterans, only very recently it has been shown that *Wolbachia* protects flies against virus infection in nature (Cogni et al., 2021 [↗](#)). This study showed that in a natural *D. melanogaster* population, flies infected with *Wolbachia* are less likely to be infected by viruses (Cogni et al., 2021 [↗](#)). Regarding the effect of temperature, it has been demonstrated in the laboratory that the *Wolbachia* antiviral effect observed when flies develop at high temperatures is reduced when flies develop at low temperatures (Chrostek et al., 2021 [↗](#); Martins et al., 2023 [↗](#)). This gives a hint that not only defensive symbiosis can be a factor modulating symbiont prevalence in the field, but temperature might be an important factor as well. The lack of studies performed in the field is a major caveat and more studies investigating protection against natural enemies in natural populations are essential for understanding the ecological importance of defensive symbiosis and what factors modulate the prevalence of symbionts in field populations.

Concerning heterogeneity and publication bias, all our meta-analytical models had high heterogeneity, which is expected in biological meta-analysis. However, even with the inclusion of moderators in the models, the amount of heterogeneity remained high, suggesting that there are other factors (i.e., moderators not included in our meta-analysis) that may influence the results. Moreover, in our main analyses we found evidence for publication bias in 12 out of our 17 models, which requires caution in interpreting the results. However, it does not invalidate our findings, since adding more effect sizes to our data could reduce the mean effect sizes but maintain the same results. Such bias can occur because studies with positive or statistically significant results are more likely to be published than studies with negative or inconclusive findings. Several factors can lead to publication bias: (1) difficult access to all existing studies due to the language of the publication, impact factor of the journal or number of citations of the article (Jennions et al., 2013 [↗](#)), (2) failure to present the results, since researchers can report only data based on the significance of the results and direction of effect sizes that corroborate their hypothesis (Jennions et al., 2013 [↗](#); Thornton and Lee, 2000 [↗](#)), (3) reviewers and editors of journals who tend to deny

negative results (Thornton and Lee, 2000 [↗](#)), and (4) there is a possibility that these studies simply do not exist yet. Thus, we encourage researchers, reviewers, and editors to publish any results obtained in high quality studies, regardless of their significance or direction of effect size. The dissemination of this practice can help to reduce publication bias in systematic and narrative reviews.

## Conclusion

In summary, this study shows that: (1) in the presence of natural enemies, symbionts provide high protection to their hosts, (2) in the absence of natural enemies, the interaction with symbionts has low cost to hosts, and (3) natural enemies attacking hosts harboring symbionts have a high cost on their fitness. Our results reveal a broad generality of protection at little cost for hosts, which helps explain the successful spread of symbionts among terrestrial arthropods, especially insects. It suggests that symbionts play a crucial role for hosts in natural populations, acting as a complement of the hosts' endogenous defenses. However, it is important to notice that when we look separately at different study systems, the level of protection and cost for both hosts and natural enemies varies between the fitness components, natural and introduced symbionts, natural enemy group, symbiont species and host family.

Defensive symbionts vary from intermediate to high frequencies in wild insect populations, suggesting that there are many factors that can influence the spread of symbionts in nature such as if the symbiont strain causes reproductive parasitism, as well as the strength of the reproductive parasitism phenotype and the efficiency of maternal transmission; how strong is the protection that symbionts offer against natural enemies (which may vary with symbiont density and natural enemy virulence, for example); how high is the cost of the interaction with symbionts; and if the natural enemies' frequencies vary temporally in nature. Furthermore, temperature and nutritional provisioning can also be an important factor in modulating the prevalence of symbionts in nature.

We hope our study stimulates researchers to expand the investigation on defensive symbiosis, collecting information on protection in other terrestrial arthropod families. Hopefully, in the future, this will allow us to investigate if the same pattern we found here occurs in other arthropods.

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## Data availability statement

All data and script are deposited at <https://github.com/cassiasqr/MetaSymbiont> [↗](#)

## Author Contributions

Designed research: Rodrigo Cogni, Cássia S. Cesar

Performed research: Cássia S. Cesar

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## Editors

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## Reviewer #1 (Public review):

Summary:

Cesar, Santos & Cogni use a meta-analysis to report on the direction and magnitude of three fundamental fitness components in defensive symbioses. Specifically, the work focuses on interactions between three arthropod host families (Aphididae, Culicidae, Drosophilidae, and others) and common bacterial endosymbionts (Wolbachia, Serratia, Hamiltonella, Spiroplasma, Rickettsia, Regiella X-type and Arsenophonus). The results of the overall analysis confirm common assumptions and previous work on such fitness components, showing that defensive symbionts provide strong protection to hosts and cause detectable costs to both hosts and the enemy. The analysis provides insight into the extent of the cost/benefit tradeoff for hosts, reporting that the cost is six times lower than the protective effect. The confirmation that natural enemies attacking hosts infected with symbionts have a reduction in their fitness is also an interesting one, as this shows that the majority of defensive symbionts provide protection by resisting enemy infection, as opposed to tolerating it. This finding has important consequences for evolutionary counter-responses in the enemy species. Of course, this result has less relevance for certain types of enemies (such as parasitoids) where successful infection is dependent upon host killing.

Interesting results also emerge from the subgroup analysis. For the full dataset, both natural and introduced symbionts were similarly effective in positively influencing the fitness of hosts. However, in the Wolbachia-specific analysis, the artificially introduced symbionts caused costs to the hosts where the natural strain did not. These findings have potentially important ramifications for schemes that use endosymbionts for biocontrol or vector competence, suggesting that (in some cases) natural strains may be the more stable choice for deploying (as they are associated with lower costs).

The analysis draws from an impressively large dataset, but the interpretation of the full impact of the results would be helped by greater detail on the species/strain level systems included, the data extraction approach, and inclusion criteria. Accounting for phylogenetic nonindependence and alternative coding of one of the moderator variables could also strengthen the biological relevance of the models. Suggestions and thoughts are outlined below.

#### Strengths & Potential Improvements:

An impressively large number of effect sizes (3000) from only 226 studies is collected, robustly confirming common assumptions on the magnitude of fundamental fitness components. However the paper would benefit from a clear breakdown in the main text of the specificities of each system included (e.g. a table at the host species/symbiont strain level, where it is possible). Currently, there is not enough detail for those who want a deep dive to understand what data was extracted for the analysis from these 226 studies, or those who want to understand the underlying diversity in the dataset.

Currently, when the 'natural enemy group' is tested as a moderator it is coded broadly by type of organism (e.g. virus, bacterium, fungi, parasitoid). But this doesn't adequately capture the mode of killing/fitness reduction by the enemy, which would be the much more biologically relevant categorisation for your questions. For example, parasitoid infection is dependent upon host death (thus host fecundity is not relevant, because the host either survived or did not). Among bacterial and viral pathogens antagonists there is scope for both fecundity and survival to be affected. This in turn may be a very influential factor for the outcome. You could consider recoding this enemy moderator.

The analysis is restricted to arthropod hosts and defensive symbionts that are also classed as endosymbionts. This focus should be made clear early on in the paper, as there are many systems (that are classed by many as defensive symbioses) that are not part of the analysis.

There is fairly minimalistic testing of moderators/sub-groups (which probably has its statistical strengths) but perhaps there are also some missed opportunities for testing other ecological contributors to variance, including coinfection (although perhaps limited by power) and other approaches to coding enemy group (as detail above).

Looking at the overview of systems included, there's likely a high degree of phylogenetic non-independence in the dataset. Where it is possible, using phylogenetically controlled models could strengthen this analysis.

Looking at your included systems (Table S5), you might be able to test the effect of coinfection on the 3 variables of interest. For example, it would be particularly important to see if the effects of two symbionts are additive or not.

No code for the analysis is provided for review at this stage and full details of the dataset are also not available. This slightly limits the ability to assess the full scope and robustness of the study. It would be helpful to have an extensive table in the supplementary detailing (minimum) the reference, study, experiment, host species, symbiont strain, and a description of the exact data extraction source (e.g. table/figure/in text), and method of extraction.

<https://doi.org/10.7554/eLife.100079.1.sa2>

#### Reviewer #2 (Public review):

Summary:

In this exciting study, Cesar and co-authors perform a meta-analysis on the influence of arthropod symbionts on the fitness of their hosts when they are exposed or not to natural enemies. These so-called defensive symbionts are increasingly recognized as key elements in arthropod survival against natural enemies, with effects that ripple through entire terrestrial ecosystems. The topic is timely, the approach is sound, and the manuscript is well-written. I believe this manuscript will attract the attention of entomologists and of microbiologists interested in symbiosis. This study builds on a previous meta-analysis that I was involved in, which was based on phloem-feeding insects. This novel data set is much larger and includes flies (including the model system *Drosophila*) and mosquitoes (a group of high medical interest). While the previous meta-analysis considered only parasitoids as natural enemies, this study also includes fungi, bacteria, and viruses.

#### Strengths:

The authors compile a very large dataset and provide a broad quantitative overview of the effects of defensive symbionts in insects. By measuring symbiont effects in the presence and absence of natural enemies, the authors are able to infer whether a trade-off between defense and the costs of mutualism in the absence of enemy pressure exists. Defensive symbioses are an important research topic that had its initial "momentum" a decade ago, so the timing for such a systematic review is very appropriate.

#### Weaknesses:

I think the manuscript could be improved by clarifying several sections, particularly the introduction and methods. The introduction section is too specific and heavily reliant on particular examples. In my view, the theoretical background of the study could be made clearer, and the knowledge gap identified more explicitly. A focus on how widespread defensive symbioses are, along with a brief, up-to-date review of the groups possessing such symbionts, would help. This lack of focus is also observed in the methods section, where more details are needed in many instances to better understand how data was collected and analyzed. Regarding the analyses, the multi-level analysis contains many moderators, but it's unclear why these moderators were included. While this may seem a minor issue, it highlights a disconnection between the analyses, the conceptual background, and the hypotheses tested. Another important weakness is that the analyses are too general, and much-hidden information is not immediately apparent. For instance, readers cannot easily identify which species of symbionts are studied (and the effects they have), or which natural enemies are involved. Although this information is found in the supplementary material, including it in the main body would significantly improve the manuscript.

<https://doi.org/10.7554/eLife.100079.1.sa1>

#### Author response:

##### **Reviewer #1 (Public review):**

##### *Summary:*

*Cesar, Santos & Cogni use a meta-analysis to report on the direction and magnitude of three fundamental fitness components in defensive symbioses. Specifically, the work focuses on interactions between three arthropod host families (Aphididae, Culicidae, Drosophilidae, and others) and common bacterial endosymbionts (Wolbachia, Serratia, Hamiltonella, Spiroplasma, Rickettsia, Regiella X-type and Arsenophonus). The results of the overall analysis confirm common assumptions and previous work on such fitness components, showing that defensive symbionts provide strong protection to hosts and*

cause detectable costs to both hosts and the enemy. The analysis provides insight into the extent of the cost/benefit tradeoff for hosts, reporting that the cost is six times lower than the protective effect. The confirmation that natural enemies attacking hosts infected with symbionts have a reduction in their fitness is also an interesting one, as this shows that the majority of defensive symbionts provide protection by resisting enemy infection, as opposed to tolerating it. This finding has important consequences for evolutionary counter-responses in the enemy species. Of course, this result has less relevance for certain types of enemies (such as parasitoids) where successful infection is dependent upon host killing.

Interesting results also emerge from the subgroup analysis. For the full dataset, both natural and introduced symbionts were similarly effective in positively influencing the fitness of hosts. However, in the *Wolbachia*-specific analysis, the artificially introduced symbionts caused costs to the hosts where the natural strain did not. These findings have potentially important ramifications for schemes that use endosymbionts for biocontrol or vector competence, suggesting that (in some cases) natural strains may be the more stable choice for deploying (as they are associated with lower costs).

The analysis draws from an impressively large dataset, but the interpretation of the full impact of the results would be helped by greater detail on the species/strain level systems included, the data extraction approach, and inclusion criteria. Accounting for phylogenetic nonindependence and alternative coding of one of the moderator variables could also strengthen the biological relevance of the models. Suggestions and thoughts are outlined below.

We sincerely thank Reviewer #1 for the time and effort dedicated to reviewing our manuscript. The suggestions provided are highly constructive and will greatly assist us in improving both our analyses and the manuscript overall.

#### *Strengths & Potential Improvements:*

An impressively large number of effect sizes (3000) from only 226 studies is collected, robustly confirming common assumptions on the magnitude of fundamental fitness components. However the paper would benefit from a clear breakdown in the main text of the specificities of each system included (e.g. a table at the host species/symbiont strain level, where it is possible). Currently, there is not enough detail for those who want a deep dive to understand what data was extracted for the analysis from these 226 studies, or those who want to understand the underlying diversity in the dataset.

We thank the reviewer for the suggestion, and we will add this information to our revised manuscript.

Currently, when the 'natural enemy group' is tested as a moderator it is coded broadly by type of organism (e.g. virus, bacterium, fungi, parasitoid). But this doesn't adequately capture the mode of killing/fitness reduction by the enemy, which would be the much more biologically relevant categorisation for your questions. For example, parasitoid infection is dependent upon host death (thus host fecundity is not relevant, because the host either survived or did not). Among bacterial and viral pathogens antagonists there is scope for both fecundity and survival to be affected. This in turn may be a very influential factor for the outcome. You could consider recoding this enemy moderator.

We agree, and we will implement this in the analysis to our revised manuscript.

The analysis is restricted to arthropod hosts and defensive symbionts that are also classed as endosymbionts. This focus should be made clear early on in the paper, as

*there are many systems (that are classed by many as defensive symbioses) that are not part of the analysis.*

We agree, and we will implement this to our revised manuscript.

*There is fairly minimalistic testing of moderators/sub-groups (which probably has its statistical strengths) but perhaps there are also some missed opportunities for testing other ecological contributors to variance, including coinfection (although perhaps limited by power) and other approaches to coding enemy group (as detail above).*

We agree, and we will implement this in the analysis to our revised manuscript.

*Looking at the overview of systems included, there's likely a high degree of phylogenetic non-independence in the dataset. Where it is possible, using phylogenetically controlled models could strengthen this analysis.*

We thank the reviewer for the suggestion. We will explore the possibility of using phylogenetically controlled models in our analyses, although we recognize the challenges associated with their implementation, particularly in the case of the natural enemies, given the great diversity of distant related groups included in our study - viruses, bacteria, fungi, protozoans, nematodes and parasitoids wasps.

*Looking at your included systems (Table S5), you might be able to test the effect of coinfection on the 3 variables of interest. For example, it would be particularly important to see if the effects of two symbionts are additive or not.*

We agree, and we will implement this in the analysis to our revised manuscript.

*No code for the analysis is provided for review at this stage and full details of the dataset are also not available. This slightly limits the ability to assess the full scope and robustness of the study. It would be helpful to have an extensive table in the supplementary detailing (minimum) the reference, study, experiment, host species, symbiont strain, and a description of the exact data extraction source (e.g. table/figure/in text), and method of extraction.*

The code for the analysis and the full raw data with the suggested information are available at <https://github.com/cassiasqr/MetaSymbiont> (The link is available at the end of the manuscript).

**Reviewer #2 (Public review):**

**Summary:**

*In this exciting study, Cesar and co-authors perform a meta-analysis on the influence of arthropod symbionts on the fitness of their hosts when they are exposed or not to natural enemies. These so-called defensive symbionts are increasingly recognized as key elements in arthropod survival against natural enemies, with effects that ripple through entire terrestrial ecosystems. The topic is timely, the approach is sound, and the manuscript is well-written. I believe this manuscript will attract the attention of entomologists and of microbiologists interested in symbiosis. This study builds on a previous meta-analysis that I was involved in, which was based on phloem-feeding insects. This novel data set is much larger and includes flies (including the model system *Drosophila*) and mosquitoes (a group of high medical interest). While the previous metaanalysis considered only parasitoids as natural enemies, this study also includes fungi, bacteria, and viruses.*

*Strengths:*

*The authors compile a very large dataset and provide a broad quantitative overview of the effects of defensive symbionts in insects. By measuring symbiont effects in the presence and absence of natural enemies, the authors are able to infer whether a trade-off between defense and the costs of mutualism in the absence of enemy pressure exists. Defensive symbioses are an important research topic that had its initial "momentum" a decade ago, so the timing for such a systematic review is very appropriate.*

We sincerely thank Reviewer #2 for dedicating their time and effort to reviewing our manuscript. The suggestions are very insightful and will significantly contribute to improving our manuscript.

*Weaknesses:*

*I think the manuscript could be improved by clarifying several sections, particularly the introduction and methods. The introduction section is too specific and heavily reliant on particular examples. In my view, the theoretical background of the study could be made clearer, and the knowledge gap identified more explicitly. A focus on how widespread defensive symbioses are, along with a brief, up-to-date review of the groups possessing such symbionts, would help. This lack of focus is also observed in the methods section, where more details are needed in many instances to better understand how data was collected and analyzed. Regarding the analyses, the multi-level analysis contains many moderators, but it's unclear why these moderators were included. While this may seem a minor issue, it highlights a disconnection between the analyses, the conceptual background, and the hypotheses tested.*

We thank the reviewer for the suggestions, and we will try to make the introduction and the methods section clearer.

*Another important weakness is that the analyses are too general, and much-hidden information is not immediately apparent. For instance, readers cannot easily identify which species of symbionts are studied (and the effects they have), or which natural enemies are involved. Although this information is found in the supplementary material, including it in the main body would significantly improve the manuscript.*

We agree, and we will implement this to our revised manuscript.

<https://doi.org/10.7554/eLife.100079.1.sa0>