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RESEARCH ARTICLE

Benefits and costs of hosting facultative symbionts in plant-sucking insects: A meta-analysis

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Abstract

Many animals have evolved associations with symbiotic microbes that benefit the host through increased growth, lifespan, and survival. Some interactions are obligate (essential for survival) while others are facultative (usually beneficial but not essential). Not all individuals host all facultative symbionts in a population, and thus there is probably a trade-off between the cost of hosting these symbionts and the benefits they confer to the host. Plant-sucking insects have been one of the most important models to test these costs and benefits experimentally. This research is now moving beyond the description of symbiont effects towards understanding the mechanisms of action, and their role in the wider ecological community. We present a quantitative and systematic analysis of the published evidence exploring this question. We found that whitefly and true bugs experience benefits through increased growth and fecundity, whereas aphids experience costs to their fecundity but benefits through increased resistance to natural enemies. We also report the lack of data in some plant-sucking groups, and explore variation in effect strengths and directions across aphid host, symbiont and plant species thus highlighting the importance of considering the context dependency of these interactions.

KEYWORDS
aphid, defensive symbiont, hemiptera, heteroptera, life-history, meta-analysis, parasitoid, stink bug, trade-off, whitefly

1 | INTRODUCTION

Animal associations with microbes are widespread across the natural world and can play key roles in the biology of their hosts. Thanks to important innovations in molecular techniques, the last two decades have provided deep insights into these diverse and often intricate host-microbe interactions (McFall-Ngai et al., 2013). Insects are the most abundant group of species in terrestrial systems and have evolved symbiotic associations with various microbes (Brownlie & Johnson, 2009; Feldhaar, 2011; Frago et al., 2020). Herbivorous insects in particular host complex communities of bacteria and fungi in their guts that enable them to feed on low-quality and toxin-laden leaf material (Dillon & Dillon, 2004; Hammer & Bowers, 2015). Many insects acquire beneficial symbionts from their surroundings each generation (Kikuchi et al., 2011), while others host more permanent endosymbionts within specialised insect cells, which are transferred vertically from mother to offspring (Douglas, 1998). Plant-sucking insects feed on impoverished diets, and have co-evolved with specialised bacteria that synthesise essential nutrients they cannot acquire directly from the plant (Bennett & Moran, 2015). Over time, this coevolution can lead to genomic erosion resulting in an obligate

mutualistic relationship where neither partner can survive without the other (Bennett & Moran, 2015). These associations with nutritional symbionts has enabled the plant-sucking lifestyle to evolve multiple times among Hemiptera and includes most Sternorrhyncha (whiteflies, mealybugs, aphids and psyllids), many Auchenorrhyncha (planthoppers and leafhoppers) and most herbivorous Heteroptera (lygaeids, pentatomids, and coreids among others) (Johnson et al., 2018).

Many plant-sucking insects have further formed facultative associations with various bacteria, which are not essential for survival but do provide benefits to the host through additional nutrition, increased host tolerance to environmental stressors, or protecting them against natural enemies like fungal pathogens or parasitic wasps (Guo et al., 2017; Haine, 2008; Kikuchi et al., 2012; Oliver et al., 2014; Vorburger, 2018; Zytynska & Weisser, 2016). Studies with these facultative symbionts have provided recent breakthroughs on the role of microbial symbionts of animals in modulating the impact of abiotic conditions on their hosts, insecticide resistance, and in mediating interactions with other members of their communities (Brownlie & Johnson, 2009; Feldhaar, 2011; Frago et al., 2020; Kikuchi et al., 2012). Vertical (maternal) transmission of facultative symbionts passes these to the next generation, with laboratory studies showing almost 100% transmission, yet a study on pea aphids suggested this could be much lower in the field (Rock et al., 2018). However, in many species they are also horizontally transmitted among individuals (Ahmed et al., 2013; Caspi-Fluger et al., 2012; Chrostek et al., 2017; Haine, 2008). Horizontal transmission among insects means that beneficial symbionts can be passed onto others within the same generation, immediately releasing them from their natural enemies, or allowing them to colonise previously inhospitable habitats.

The role that facultative symbionts play in the insect host has been studied for more than a decade, particularly in well established model systems like aphids and whiteflies (Ferrari et al., 2006; Frago et al., 2012; McLean et al., 2011; Oliver et al., 2006; Russell & Moran, 2006; Scarborough et al., 2005; Simon et al., 2007; Tsuchida et al., 2006; Vorburger & Gouskov, 2011; Zchori-Fein et al., 2014), but increasingly for other plant-sucking Hemipterans, particularly heteropterans (Hosokawa et al., 2006; Kashkouli et al., 2019; Kikuchi et al., 2012; Lee et al., 2017). These three different groups differ remarkably on the type of symbioses they engage in. While aphid and whitefly facultative associates are endosymbionts hosted within host tissues, heteropterans mostly host their symbionts in specialized structures associated to the gut (Kikuchi et al., 2011). In addition, in this latter group, the boundary between facultative vs obligatory association is not always clear. While a 100% mortality rate without a symbiont indicates it is obligatory, to what extent a symbiont can influence insect survival and still be considered facultative has not been determined.

Another question that has yet to be resolved is why facultative symbionts are often found in only a fraction of the individuals of a population, or why they are more abundant in some populations throughout the distribution range of a given species (Smith et al.,

2015). One explanation is that in the absence of the environmental stress or of natural enemy pressure, facultative symbionts can incur fitness costs (Russell & Moran, 2006; Vorburger et al., 2013; Vorburger & Gouskov, 2011); unless they also provide a benefit to the host they may be more likely be lost from a population, especially if reacquired each generation or vertical transmission rates are low. Experimental work on the potential fitness costs and benefits of hosting bacterial symbionts indicate that these effects are variable across symbiont and host species, and also strains or genotypes within these (Rouchet & Vorburger, 2012; Russell & Moran, 2006). Since herbivorous insects generally host limited microbial diversity (especially compared to mammals) (Sugio et al., 2015), this variation can be due to highly specialized insect-microbe associations, microbe-microbe interactions, or the selection of specific microbes within insect hosts via drift (Mathé-Hubert et al., 2019).

We aim to provide a broad analysis of symbiont effects in plant-sucking insects, yet we cannot escape that an important amount of the experimental work on symbiont effects has been performed on aphids, predominantly on the model pea aphid species. After more than two decades of research, experimental work directly measuring the costs and benefits of hosting symbionts in aphids is being replaced by work aiming to understand the ecological and evolutionary consequences of these interactions, and the mechanisms behind specific effects (Oliver & Higashi, 2019; Tian et al., 2019). For whitefly, most of the work is on a single species and its symbionts, with more recent focus on understanding mechanisms underlying these interactions (Santos-Garcia et al., 2020; Wang, Ren, et al., 2020; Zchori-Fein et al., 2014). For other plant-sucking insects, work to identify a symbiotic microbiome is just starting, particularly for many Heteropterans that rely on diverse gut bacteria for digestion, nutrient synthesis or toxin degradation (Moran et al., 2019). Since hemipteran microbiomes are more diverse than in the other groups, an increasing accessibility to molecular tools (including molecular cytogenetic techniques such as FISH) are likely to be behind this trend. Hence it is timely to present a quantitative and systematic analysis of the published evidence exploring these costs and benefits to assist decisions for the future direction of this research, and to provide solid conclusions based on currently available data.

We present results from a meta-analysis study to understand the generality of the costs and benefits of hosting facultative symbionts across plant-sucking insects. We focused on studies that compare insects that host a symbiont to those that do not host a symbiont for any measured life history trait. For these 'control versus treated' comparisons we included experiments both where a symbiont had been artificially cured using antibiotics or infected (e.g., using microinjection), and those using naturally collected infected/uninfected lines. While several recent reviews have highlighted the importance of these symbionts in understanding their hosts' biology (most recently Frago et al., 2020), we currently lack a quantitative and systemic analysis of the published evidence. A systemic analysis will provide insights into the role of these microbial symbionts in different plant-sucking insect groups and will identify knowledge gaps at the level of both research questions and insect (or symbiont) taxa.

In this meta-analysis we address the following general questions: (a) Symbionts are widespread in plant-sucking insects, but which groups of species have been sufficiently experimentally examined for the effects of symbionts on the host? (b) Facultative symbionts are expected to be costly, particularly those that protect their hosts against natural enemies or adverse abiotic conditions, is this trade-off similar across the different groups of species studied?

Most data came from aphids as they are a classic group for the study of insect symbiosis. We can thus ask additional questions within this data subset to further explore: (a) Do facultative symbionts confer general costs and benefits across host and symbiont species? (b) Are facultative symbionts involved in host-plant specialisation or host-switching to novel hosts? (c) Insects can host multiple symbiont species: does addition of a second symbiont increase costs or benefits?

2 | MATERIALS AND METHODS

The majority of experimental work on facultative symbionts has been done using aphids (Hemiptera: Sternorrhyncha: Aphidoidea), but there are studies on whiteflies (Hemiptera: Sternorrhyncha: Aleyrodoidea) and, increasingly on Heteropterans (Hemiptera: Heteroptera). We searched for relevant literature using keyword searches in Web of Science finding articles published until the end of 2019 (details for each group below). Literature and data pertaining to other herbivorous sucking insects was searched for, but with few articles relevant to our meta-analyses; data for three Auchenorrhyncha species hosting symbionts was extracted but this taxa does not belong to any of the three identified groups and was insufficient to analyse independently (Li et al., 2019; Shentu et al., 2019; Yoshida et al., 2019).

The main inclusion criteria across the groups were: (a) data on at least one insect species, (b) an experimental test of facultative symbiont effects on insects, either experimentally cured of a symbiont, or inoculated with one (Experimental), or a comparison of field-collected infected and uninfected aphids (Natural), (c) any of the following types of variables tested: any behaviour, growth, fecundity, survival, or parasitism-resistance related variable, and (d) data on means, an estimation of variation, and sample size. In addition, we only included studies that used artificial curing methods (e.g., antibiotics) with demonstrated negative effects on symbionts, but not their insect hosts. If novel methods to remove symbionts were used, we only included studies that demonstrated that the technique used had no direct negative effects on insect fitness.

2.1 | Heteroptera meta-analysis (Hemiptera: Heteroptera)

We used the search terms: (["scale insect*" OR mealybug* OR cicad* OR leafhopper* OR treehopper* OR planthopper* OR stink bug* OR stinkbug OR froghopper* OR psyllid* OR sharpshoot* OR mealybug*

OR pentatomid* OR coreidae* OR lygaeid*] AND symbio*). This search resulted in 590 potential articles, and although it included planthoppers, leafhoppers, treehoppers, froghoppers, mealybugs, and psyllids, only articles related to stink bugs, shield bugs and true bugs (hemipteran suborders Heteroptera) were relevant for this meta-analysis because in the other groups no studies (except three planthopper articles) satisfied the main meta-analysis inclusion criteria as stated above. In stink bugs, shield bugs and true bugs, symbionts are located in the gut, and often provide nutritional services, which means that the boundary between obligatory and facultative symbiosis is less clear than in aphids and whiteflies. In most studies, whether the symbiont is obligatory or not was unveiled by the study itself (e.g., in Hosokawa et al., 2006; Karamipour et al., 2016). We found 38 articles that tested symbiont effects on insect fitness by artificially removing the microbe. Among them, 16 articles included obligatory associations as suggested by the authors, or as revealed by total nymph mortality, or adult infertility when the symbiont was removed (Supporting Information Appendix 1b). We therefore exclude these obligatory associations; this criterion inherently requires studies to report data on insect survival after symbiont removal. The final data set consisted of 22 articles (from 2002 to 2019, see Appendix 1a), and the only variables of interest with sufficient data were adult mass (fresh or dry weight), adult size (length of abdomen, thorax, tibia or total body), time to adulthood, fecundity and survival.

2.2 | Whitefly meta-analysis (Hemiptera: Sternorrhyncha: Aleyrodoidea)

We used the search terms: ([whitefl* OR Bemisia* OR Siphoninus* OR Trialeurodes OR Aleurodicus OR Aleuronudus OR Dialeurodicus OR Metaleurodicus OR Palaealeurodicus OR Paraleyrodes] AND symbio*), resulting in 260 potential articles. For the whitefly data, two studies were eliminated because the antibiotic treatment also eliminated the obligatory symbiont. We also included a study that used introgression of the symbiont via crossing to achieve infection. The final data set consisted of eight articles (from 1993 to 2019, see Appendix 2), and the only variables of interest with sufficient data were adult body size, time to adulthood, fecundity, and survival.

2.3 | Aphid meta-analysis (Hemiptera: Sternorrhyncha: Aphidoidea)

We used the terms: ("aphid*" AND ("Hamiltonella" OR "Regiella" OR "Serratia" OR "Rickettsia" OR "Rickettsiella" OR "Sprioplasma" OR "Arsenophonus" OR "Wolbachia" OR "X-type" OR "PAXS")). This resulted in 512 potential articles. Following the inclusion criteria, we extracted data from 68 articles (1997–2019; Appendix 3) and were able to analyse the effect of bacterial symbionts on fresh adult weight, age at first reproduction, lifespan, number of offspring, and the proportion of aphids surviving parasitic wasp attack. We were

also able to extract data on the experimental host plant and plant from which the aphid was originally collected (related to aphid biotype in pea aphids). We pooled data within studies across aphid genotypes and symbiont strains removing effects of within-species genetic variation, yet data remained separated by aphid species, symbiont species, and host plant (experimental and of original collection). No further cross-comparison of common aphid or symbiont genetic lines was possible due to insufficient replication within these groups. Most of the articles reported single symbiont infections, but 14 of them also reported effects of multiple symbionts in aphids, and these were analysed separately.

2.4 | Estimating costs and benefits across insect taxa

To estimate overall effects that are comparable among insect groups, traits were classified into the following six main categories. (a) Body size, which included fresh or dry adult body mass and adult size (length of the body, tibia or abdomen), (b) Development time, which included age at first reproduction and time to adulthood, (c) Lifespan, which included days survived and age of death, (d) Fecundity, which included the number of eggs or nymphs produced (the latter in the case of viviparous aphids), (e) Survival, which includes the proportion of individuals surviving to adulthood for whitefly and Heteroptera only, and (f) Resistance to parasitism, which includes the proportion of individuals surviving after attack by a parasitic wasp for aphids only. Symbiont impacts on insects were transformed into effect sizes ("yi", see below) and we aimed at these effects to represent either a benefit or a cost to the insect, with values significantly larger or smaller than zero, respectively. Effect sizes were thus multiplied by –1 in the case of development time based on the slow-growth-high-mortality hypothesis because slower growing herbivores suffer greater mortality due to a prolonged window of vulnerability (Chen & Chen, 2018). The proportion of aphids parasitized was also multiplied by –1 so that this variable was transformed into resistance to parasitic wasp attack and therefore represented a benefit for the aphid when values were larger than zero.

2.5 | Statistical analysis

The meta-analysis was conducted in R v3.6.3 (R Core Team, 2020) in RStudio v1.2.5033 (RStudio Team, 2020) using the package metafor (Viechtbauer, 2010); all data and R scripts can be accessed in Dryad (Zytynska et al., 2021). The standardised mean difference was used with unbiased estimates of the sampling variances (SMDH, giving Hedges' g). This measure was used since it gives a direct effect size comparison of the treated (infected with a symbiont) to untreated (no symbiont control) data. For the analysis of the main variables, we only present data where there are at least three data points from three independent studies (i.e., not from the same research group). A priori power analyses (medium effect size d = 0.5) (Table S1) were

performed in R following the script of Quintana and Tiebel (2019) for the main variables, and subsets of data by insect and symbiont species. For this, we used the number of effect sizes from the collected data (data points), the average number of replicates used to calculate each effect size (control vs treated), against a predicted effect size of d = 0.5 (medium) and low heterogeneity (h = 0.33).

We used a meta-analytic linear mixed effects model (rma.mv) to test the effect of hosting symbionts on the different insect response traits. "Study" was included as a random effect to account for multiple data points across insect and symbiont species within individual studies. In the main analyses (for each data set), response (levels: body size, development time, lifespan, fecundity, and survival or resistance to parasitism) was used as a fixed effect moderator to determine the effect of hosting facultative symbionts on the different insect groups. Data bias was assessed by testing the funnel-plot asymmetry for a random-effects model and Eggers test for publication bias in the metafor R package (Figures S1, S2). We also used P-curve analysis in the R package dmetar (Harrer et al., 2019) as an alternative to test for bias that shows the distribution of significant effect sizes to identify potential p-hacking (Figure S1). P-hacking occurs when data or experimental design is manipulated to produce significant results below significant p-values (usually 0.05) and can be detected when there is a large proportion of values close to the significance level so that P-curves are skewed towards these values (Figure S1). For the results figures, the mean effect size and 95% confidence intervals are presented; the mean effect size was considered significantly different from 0 if its 95% CI did not include zero, and level of significance given from model outputs.

For the aphid data set, data were also subset into those where insect lines had been directly compared through experimental curing/infecting (Experimental) or a comparison of field-collected infected and uninfected insects (Natural), and analyses run as above on each separate data set. We further subset the data by aphid trait (i.e., one model for each trait) and explored differences across aphid or symbiont species within these by including "aphid species" or "symbiont species" as fixed effect moderators. An interaction model was considered but in no case was there sufficient data for this to be meaningful, thus we analysed aphid species and symbiont species separately. For cases where there was sufficient data (here deemed as ≥3 data points), we also analysed the effect of symbiont species within aphid species; however, as the data subsets get smaller the interpretation needs to be done with caution.

For the model pea aphid (*Acyrthosiphon pisum*) we also analysed the effect of symbionts across host plants (experimental host plant and host-plant from which the aphid was originally collected) as pea aphids belong to genetically differentiated host-plant associated populations and these particularly associate to specific symbiont species. The effect of experimental host plant could only be analysed for plant hosts *Medicago sativa* and *Vicia faba* (a universally-accepted host plant for all pea aphid biotypes (Ferrari et al., 2006)). We restricted our analyses to aphid lines collected from *M. sativa* (*Medicago*/alfalfa biotype) to reduce variation due to aphid biotypes. We analysed the effect of all symbionts (combined due to

lack of sufficient replication of individual symbionts) on aphid fecundity and parasitism, for which there was more than three data points from at least two independent studies. The effect of host-plant of collection was analysed for pea aphids hosting *Hamiltonella defensa* symbionts for aphids collected from *M. sativa* and *Ononis spinosa* plants. We restricted the analysis to experiments using the universal *V. faba* plant as the experimental host plant to reduce confounding effects of unbalanced replication across other host plants. There was sufficient data to analyse aphid fecundity and parasitism, with at least three data points from three independent studies for each comparison.

Lastly, the effect of hosting multiple symbionts was also possible to analyse for pea aphids. This was possible for data where either a second symbiont was added by microinjection (N = 14 data points), or where collected clones of aphids varied in symbiont status (N = 12 data points). Due to lack of data, it was not possible to analyse an interaction between any two symbionts. Therefore, we approached this by analysing the effect of adding any additional symbiont to a particular symbiont species, and similarly the effect of adding a particular symbiont species as the second one. For the meta-analysis, this means the control was the single infection and treatment was the double infection, with the first analysis observing the effect of the focal (single) symbiont species and the second the effect of the single (paired) symbiont species.

3 | RESULTS

This meta-analysis includes a total of 453 data points: 60% from aphids, 30% from Heteropterans and the remaining 10% from whitefly. In aphids the most measured variables were fecundity and parasitism, while body size and development time were the most measured in Heteropterans and whitefly (Table 1; Figure 1). There was insufficient data to analyse symbiont effects on lifespan in both Heteropterans and whitefly. The number of studies has steadily

increased since year 2000, initially with aphids but early studies also included effects on shield bugs and whitefly (Figure S1).

We found that facultative associations in Heteroptera have been experimentally studied in 18 insect species from six families and 14 genera (*Acrosternum, Adomerus, Blissus, Brachynema, Dolycoris, Elasmostethus, Eurydema, Eurygaster, Graphosoma, Halyomorpha, Megacopta, Murgantia, Nezara, Riptortus*). These studies primarily test the effect of the symbiont *Burkholderia*, with some studies on *Pantoea and Pandoraea*, all of them located in specialized structures in the gut; however, in most studies the symbiont species was not fully identified. The whitefly data (years 2011–2015) contains studies on different biotypes of a single whitefly species (*Bemisia tabaci*), hosting *Hamiltonella, Rickettsia* and *Wolbachia* symbionts; we found no additional relevant studies since 2016. Experimental manipulation of aphid symbionts increased strongly in 2010 and, while new studies are still being published, the numbers per year have remained similar in the last four years (probably due to research now focusing on understanding the mechanism of effects). The aphid data set included 13 aphid species, from eight genera (*Acyrthosiphon, Aphis, Macrosiphum, Megoura, Myzus, Obtusicauda, Rhopalosiphum, Sitobion*) and eight symbiont species (*Hamiltonella defensa, Regiella insecticola, Serratia symbiotica, Rickettsia, Ricketsiella, Spiroplasma, Fukatsia symbiotica* (X-type, or PAXS), and *Arsenophonus*). Within each data set, we found no evidence for bias due to publication of only significant results or p-hacking (Figure S1).

3.1 | Costs and benefits of hosting facultative symbionts in plant-sucking insects

Beneficial effects of hosting facultative symbionts were observed in all groups of insects studied, whereas costs were mainly observed in aphids (Figure 1a). General effects (across all insect and symbiont species) of hosting facultative symbionts showed these did not confer the same benefits for all groups studied. Heteropterans experienced benefits from increased body size (Hedges' g = 2.96, p = .001, N = 68),

TABLE 1 Summary of number of data points included in the three meta analyses datasets, separated by the five commonly measured life history traits.

| Dataset | Species number | Number of data points (number of articles) | | | | |
		Body size	Development time	Lifespan	Fecundity	Survival[d]/parasitism[e]
Heteroptera[a]	18	68 (13)	31 (10)	4 (1)	6 (5)	14 (8)
Whitefly[b]	1	18 (4)	16 (4)	3 (2)	8 (5)	4 (4)
Aphids[c]	13	32 (16)	34 (16)	34 (15)	124 (47)	57 (29)

[a]*Acrosternum heegeri, Adomerus rotundus, Adomerus triguttulus, Blissus insularis, Brachynema germari, Dolycoris baccarum, Elasmostethus humeralis, Eurydema rugosa, Eurygaster integriceps, Graphosoma italicum, Graphosoma lineatum, Halyomorpha halys, Megacopta cribaria, Megacopta punctatissima, Murgantia histrionica, Nezara viridula, Riptortus clavatus, Riptortus pedestris.*

[b]*Bemisia tabaci.*

[c]*Acyrthosiphon kondoi, Acyrthosiphon pisum, Aphis craccivora, Aphis fabae, Aphis glycines, Aphis gossypii, Macrosiphum euphorbiae, Megoura crassicauda, Myzus persicae, Obtusicauda frigidae, Rhopalosiphum padi, Sitobion avenae, Sitobion miscanthi.*

[d]Survival to adulthood for Heteroptera and whitefly data sets.

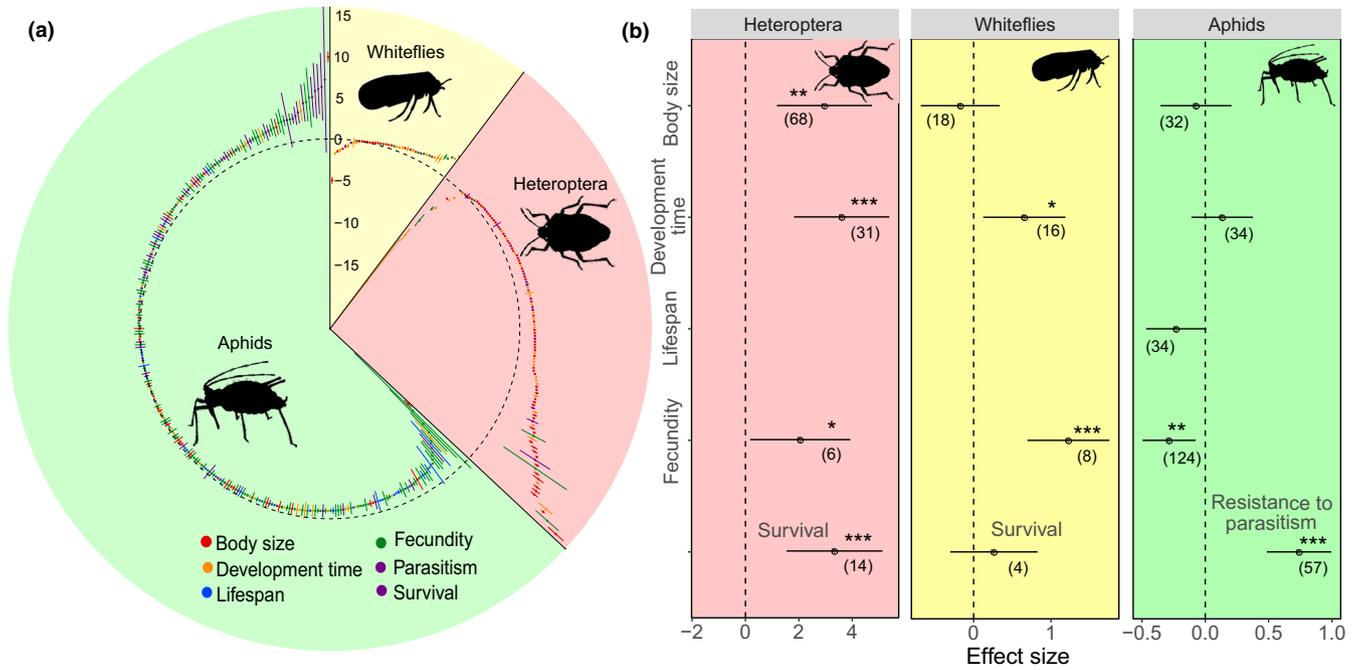[e]Resistance to parasitism for aphid data set.

(a)

(b)

FIGURE 1 Overall effects of facultative symbionts on Heteroptera (Hemiptera: Heteroptera), Whitefly (Hemiptera: Aleyrodidae) and Aphids (Hemiptera: Aphididae). (a) circle plot shows all individual effect sizes extracted in our study (Hedges' g and 95% Confidence Interval) across the life history traits measured for the three taxon groups. (b) Overall effect sizes for the six main measured variables. Traits are considered a benefit (above zero) or cost (below zero) and significant when the 95% CI does not include zero. Body size includes size and mass traits, development time is measured in days for days until adulthood or first reproduction, lifespan is measured in days from birth or hatching until death, and fecundity is the number of offspring produced in a given time. Survival is measured as the proportion of individuals that survive to adulthood, and resistance to parasitism is measured as proportion survival after attack by a parasitoid wasp.*p < .05, **p < .01, ***p < .001.

reduced development time of three days (Hedges' g = .60, p < .001, N = 31), increased fecundity (Hedges' g = 2.05, p = .032, N = 6) and increased survival (22% more individual surviving to adulthood with a symbiont; Hedges' g = 3.33, p < .001, N = 14) (Figure 1b). Whiteflies benefited from reduced development time of a day (Hedges' g = 0.70, p = .011, N = 16) and increased fecundity (Hedges' g = 1.22, p < .001, N = 8) (Figure 1b). For the aphids, the main benefit was via symbiont-conferred resistance to parasitoid wasps (Hedges' g = 0.75, p < .001, N = 57, Figure 1b); the proportion of aphids that were resistant to an attack by a parasitic wasp was increased from 0.43 for uninfected aphids to 0.60 for aphids infected by a symbiont. There was a cost to aphids through reduced fecundity (Hedges' g = −0.28, p = .007, N = 124) and marginally nonsignificant overall effect on reduced lifespan (Hedges' g = −0.23, p = .056, N = 34).

We continue to explore the aphid data with respect to variation among aphid and symbiont species in the following section. There was insufficient replication across different species or symbionts to analyse subsets of the Heteroptera or whitefly data.

3.2 | In-depth exploration of the effect of facultative symbionts in aphids

Most aphid studies used experimental methods to artificially inoculate or cure aphids of symbionts (N = 217) while others compared

naturally-collected infected and uninfected clones of aphids collected from the same location and host-plant (N = 64). Increased resistance to parasitism was observed in both experimental (Hedges' g = 0.86, p < .001, N = 36) and natural (Hedges' g 0.76, p < .001, N = 21) data sets, while significant costs via reduced fecundity were only observed in the experimental studies (Experimental: Hedges' g = −0.36, p = .003, N = 98; Natural: Hedges' g = 0.01, p = .954, N = 26; Figure S3); despite smaller data sets for the natural lines, statistical power was over 90% and no publication bias was observed.

3.2.1 | Effects across symbiont and aphid species

There was variation in the benefits and costs conferred by facultative symbionts across aphid species (Figure 2a) and symbiont species (Figure 2b). We were unable to analyse effects of individual symbiont strains or aphid genotypes due to lack of replication. Here, we briefly detail those differences for aphid lifespan, fecundity, and resistance to parasitism with further details available in Supporting Information Appendix 4 for all traits. Symbiont reduction of aphid lifespan was observed in the three most studied aphid species *A. pisum* (Hedges' g = −0.63, p = .001, N = 21), *A. fabae* (Hedges' g = −1.66, p < .001, N = 5), and *A. kondoi* (Hedges' g = −0.89, p < .001, N = 4), with no effect on the other four aphid species studied taken together (one data point per species; Hedges' g = −0.40, p = .170, N = 4). This reduction
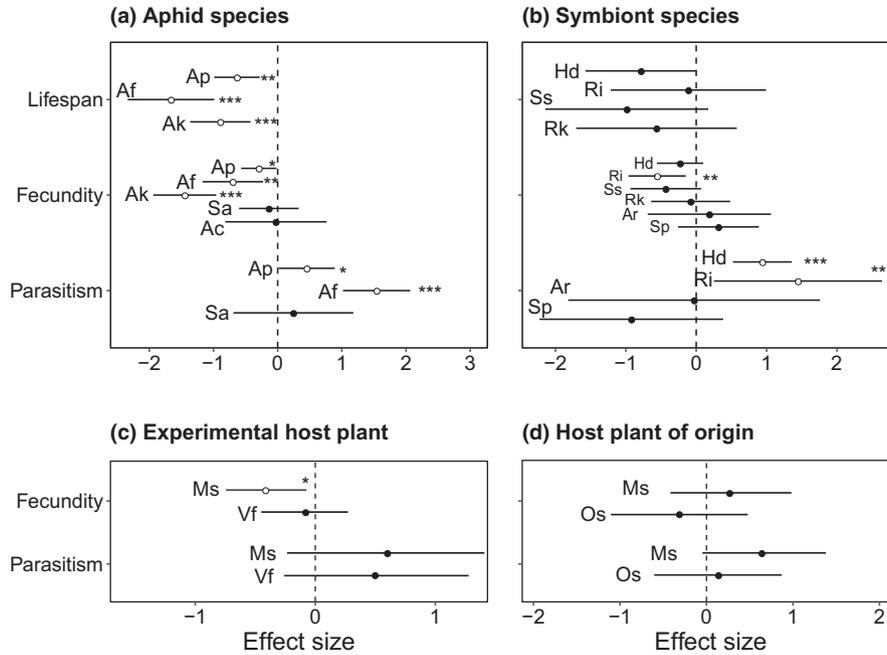
FIGURE 2 Effect of symbionts on aphid lifespan, fecundity, and resistance to parasitism across (a) aphid species, (b) symbiont species, (c) experimental host plant, and (d) host plant of origin (i.e., where the aphid line was collected). The experimental host plant analysis is restricted to *Medicago* biotype aphids, tested on the two host plants within the experiment. The host plant of origin analysis compares two aphid biotypes (*Medicago* and *Ononis*) tested on *Vicia faba* experimental plants. Effect sizes are Hedges' g and 95% confidence interval. Abbreviations: (a) *Ac, Aphis craccivora; Af, Aphis fabae; Ak, Acyrthosiphon kondoi; Ap, Acyrthosiphon pisum; Sa, Sitobion avenae.* (b) *Ar, Arsenophonus; Hd, Hamiltonella defensa; RI, Regiella insecticola; Rk, Rickettsia; Sp, Spiroplasma; Ss, Serratia symbiotica.* (c–d) *Ms, Medicago sativa; Os, Ononis spinosa; Vf, Vicia faba.* Associated sample sizes are given in the main text. *p < .05, **p < .01, ***p < .001.

in lifespan was driven by the symbiont *H. defensa* (Hedges' g = −0.79, p = .051, N = 11; Figure 2b); however, pea aphids (21/34 data points) also experienced reduced lifespan when infected by *Rickettsia* sp., *S. symbiotica* and *Spiroplasma* symbionts while the other aphid species did not (Supporting Information Appendix 4). Aphid fecundity was reduced in three aphid species (*A. kondoi*: Hedges' g = −1.45, p < .001, N = 4; *A. pisum*: Hedges' g = −0.29, p = .037, N = 68; *A. fabae*: Hedges' g = −0.70, p = .004, N = 10, Figure 2a), driven by *R. insecticola* symbionts (Hedges' g = −0.55, p = .008, N = 32; Figure 2b), but had no effect on two aphid species (*A. craccivora*: Hedges' g = −0.03, p = .942, N = 9; *S. avenae*: Hedges' g = −0.14, p = .564, N = 23; Figure 2a).

Symbiont-conferred resistance to parasitic wasps was found for two aphid species (*A. pisum*: Hedges' g = 0.45, p = .047, N = 29; *A. fabae*: Hedges' g = 1.41, p < .001, N = 16; Figure 2a) but not for *S. avenae* (Hedges' g = 0.25, p = .613, N = 3; Figure 2a). The majority of these studies used *H. defensa* symbionts (43/57 data points) (Hedges' g = 0.90, p < .001, N = 43; Figure 2b), yet there is some evidence that hosting *R. insecticola* also conferred resistance to parasitoid wasps (Hedges' g = 1.45, p = .015, N = 4; Figure 2b).

3.2.2 | Host-plant effects on symbiont costs and benefits in pea aphids

The effect of the experimental host plant compared aphids reared on either *Medicago sativa* or the universal host *Vicia faba* and was

restricted to aphids collected from *M. sativa* (*Medicago*/alfalfa biotype). Aphids experienced reduced fecundity in response to hosting symbionts on their original host *M. sativa* (Hedges' g = −0.41, p = .017, N = 6) but not on the universal host *V. faba* (Hedges' g = −0.08, p = .640, N = 11) (test of moderators QM = 5.75, df = 2, p = .057) (Figure 2c). While symbionts increased aphid resistance to parasitism on both experimental plants when data were combined (Hedges' g = 0.88, p < .001, N = 8), there was reduced power, and increased variation within the smaller subsets with no significant difference in effect size across the two experimental host plants (*M. sativa*: Hedges' g = 0.61, p = .157, N = 3; *V. faba*: Hedges' g = 0.51, p = .195, N = 5) (test of moderators QM = 2.94, df = 2, p = .230) (Figure 2c).

The effect of host-plant of collection (*M. sativa* or *Ononis spinosa* for studies using *V. faba* as the experimental plant) did not significantly alter *H. defensa*-mediated resistance to parasitism (test of moderators QM = 3.40, df = 2, p = .183). However, there was some evidence that aphids collected from *M. sativa* may experience increased symbiont-mediated resistance to parasitism (Hedges' g = 0.66, p = .069, N = 5) than those from *O. spinosa* (Hedges' g = 0.15, p = .687, N = 5). Again, there was no significant difference in the aphid fecundity response to hosting *H. defensa* across host plants (test of moderators QM = 3.82, df = 2, p = .148). Yet similarly, while not significant and with very low replication, data may suggest the potential for a cost for aphids collected from *O. spinosa* (Hedges' g = −0.31, p = .450, N = 3) with no cost for those collected from *M.*

sativa (Hedges' g = 0.29, *p* = .425, *N* = 6). These interactions should be investigated further with continued experiments to enable a full analysis to be conducted.

3.2.3 | Multiple hosting of symbionts within aphids

For this data set we only had 45 data points, from eight symbiont pairs (5/8 include *H. defensa*). For aphids infected by *H. defensa* symbionts, the addition of a second symbiont (not specified by symbiont species) increased aphid lifespan (Hedges' g = 0.62, *p* = .013, *N* = 4), with no effect on fecundity (Hedges' g = −0.24, *p* = .595, *N* = 8), or resistance to parasitism (Hedges' g = −0.01, *p* = .976, *N* = 5). When the second symbiont was *Fukatsia symbiotica* (previously X-type) aphid lifespan was increased by ~3 days (Hedges' g = 0.74, *p* < 001, *N* = 5), but fecundity decreased (Hedges' g = −0.90, *p* = .020, *N* = 6). In natural pea aphid populations, this symbiont was almost always in a co-infection with *H. defensa* or *Spiroplasma* (we extracted insufficient data to analyse this symbiont in single infections). When the second symbiont was *H. defensa* the symbiont-mediated resistance to parasitism was increased (Hedges' g = 2.76, *p* = .006, *N* = 6) highlighting the strong protective effect of this species even in coinfection with other symbionts.

4 | DISCUSSION

In this meta-analysis we have explored the benefits and potential costs associated with carrying facultative symbionts in Heteroptera, whitefly and aphids. Across all species and symbionts, Heteroptera and whitefly experienced strong fitness benefits, but minimal costs from hosting facultative symbionts. Benefits included reduced development time and increased fecundity in both, and increased body size and higher survival additionally for Heteroptera. In contrast, pooling together all aphid and symbiont species, aphids only benefitted through increased resistance to parasitic wasps while experiencing fecundity costs, the trade-off we hypothesised. At the species level, some aphid species experienced greater costs and benefits than others, and this varied due to symbiont species, but there was no clear evidence of host-plant effects on aphid responses to symbionts. Hosting of multiple symbionts was expected to increase costs, but despite the limited amount of data available at least one symbiont pairing (*Hamiltonella defensa* + *Fukatsia symbiotica*) maintained benefits while potentially reducing overall fitness costs.

Aphid studies contributed 60% of the total data points, with a strong bias towards a few well-studied species, such as the model pea aphid (*Acyrthosiphon pisum*), black-bean aphids (*Aphis fabae*), and cereal aphids (*Sitobion avenae*). All whitefly studies were performed on the model species *Bemisia tabaci*, despite this group containing many other economically important pest species (Martin et al., 2000). In contrast, the Heteroptera data set represents a broader taxonomic scope comprising six different families and 14 genera despite making up just less than a third of all data points in our analyses. Other than

lack of research effort, one reason for these biases might be due to the difficulty of artificially removing symbionts in some insect species. In aphids, for instance, using antibiotics to "cure" them from facultative symbionts is simple and well documented, albeit time-consuming (Simon et al., 2007); this technique, however, does not work with other species like the potato aphid *Macrosiphum euphorbiae* because the antibiotic treatment eliminates both facultative and the obligatory *Buchnera* symbionts resulting in aphid death (Hackett et al., 2013). One work around, as done by Hackett et al., 2013 for the aphid *M. euphorbiae*, is by testing symbiont effects in various field-collected genotypes with and without the bacterium (termed 'Natural' aphid lines in this meta-analysis), creating infected lines via introgression (as done in whiteflies, e.g., Asiimwe et al., 2014), or exploring novel chemicals to remove symbionts as done in some Heteropteran studies (e.g., Kashkouli et al., 2019).

We found multiple beneficial effects for plant-sucking insects that host facultative symbionts, only for aphids did we find a cost. The lack of observed costs in Heteroptera may be attributed to the more transient nature of these (predominantly) gut symbionts that are reacquired each generation in many insect species (Kikuchi et al., 2011). Each generation could thus filter out those microbes which would constitute a cost in their current environment, thereby only acquiring beneficial ones. In Heteropterans, another important question that needs to be clarified is how often symbiont associations are either facultative or obligatory. In our meta-analysis, 16 studies were not included because symbiont removal led to 100% egg-to-adult mortality (e.g., Ohbayashi et al., 2019), suggesting that many of these symbionts are obligatory, even if in many taxa a recent and ongoing process of symbiont acquisition and loss is also likely. For both whiteflies and heteropterans, the lack of evidence for symbiont costs may also be a result of just not measuring the traits for which there would be such a cost, or not measuring these under the right ecological conditions (Kikuchi et al., 2012). It would be interesting to continue to compare the effect of different microbiomes across novel environments or environmental gradients (e.g., Guay et al., 2009; Heyworth & Ferrari, 2016; Zytynska et al., 2016). For both Heteroptera and whitefly, fewer studies measured fecundity (than body size or development time) highlighting difficulties in accurately assessing reproduction for nonviviparous insects (Ridley, 1988). For asexual viviparous aphids, measuring fecundity has been one of the most used methods for assessing fitness, while their small soft-bodies can make measuring body size and development time more difficult (Lamb et al., 2009). As far as we are aware, benefits related to resistance to natural enemies have never been tested in whiteflies or Heteropterans (Flórez et al., 2015), but it is likely that such defensive symbionts are yet to be discovered. The lack of defensive symbionts in whiteflies is surprising because they often carry *Hamiltonella defensa* (Gueguen et al., 2010) that provides strong protection to aphids. In whiteflies, however, this symbiont has been found to participate in the transmission of plant viruses (Su et al., 2013). Plant viruses trigger deep changes in plant resourse allocation and defenses, so the symbiont may modulate insect fitness via changes in the physiology of the plant. The last few years

have revealed exciting evidence of the role that symbionts play in modulating insect fitness through changes in plant physiology or defensive state with consequences that cascade up to natural enemies (Frago et al., 2020). It is therefore possible that many indirect costs associated to such changes are yet to be discovered.

We show that aphids may experience a cost-benefit trade-off when hosting certain symbionts, or at least tolerate lifespan or fecundity costs when there are important survival benefits for example under strong natural enemy pressure. While other benefits such as resistance to entomopathogenic fungi or heat stress have been highlighted in reviews on aphid symbiont effects (Guo et al., 2017; Oliver et al., 2014), these traits lacked sufficient data across multiple aphid species and symbionts to be included in a meta-analysis. Experimental laboratory studies have revealed that defensive symbionts are costly mostly in the absence of natural enemy pressure in aphids (Oliver et al., 2008; Vorburger & Gouskov, 2011) and *Drosophila* (Jaenike & Brekke, 2011). However, from the meta-analysis we can only infer general patterns, but not comprehensively conclude that this occurs for individual symbionts within specific aphid species. The species-specific costs and benefits we identified in aphids have the potential to contribute to the variation in symbiont-hosting frequencies observed in the field within and among populations (reviewed in Zytynska & Weisser, 2016). In the field both infected and uninfected aphids coexist, thus any reduced fitness of aphids hosting a symbiont means they will be outcompeted by the uninfected aphids when there is no benefit (e.g., through natural enemy resistance) (Vorburger & Gouskov, 2011). Our comparison of experimental curing versus naturally occurring infected/uninfected lines showed higher fecundity costs for the experimental lines but no difference in parasitism resistance levels. This suggests that naturally occurring combinations are less costly while maintaining the benefits, probably reflecting a strong purifying selection against novel and more costly aphid-symbiont infections (Oliver et al., 2006). Further, many insects host more than one symbiont in the field and our analyses find that aphids cohosting another symbiont alongside *Hamiltonella defensa* experienced similar benefits but with reduced costs; particularly with *Fukatsia symbiotica*, which is commonly observed with *H. defensa* in the field (Guay et al., 2009). Understanding interactions among aphid genotypes and symbiont strains, and the impact of drift within small populations hosting multiple symbionts (Mathé-Hubert et al., 2019) may further uncover why lower costs are observed for naturally occurring combinations. These results may also help to understand why there is limited evidence for symbiont horizontal transmission in aphids (Chrostek et al., 2017) as many successful transmission events may probably to be lost under natural conditions (Rock et al., 2018).

An ongoing question in symbiont work on pea aphids is the importance of the host plant, since pea aphids form distinct biotypes associated with different host plants and different prevalent symbionts (Ferrari et al., 2004; McLean et al., 2011; Sochard et al., 2019). Despite the interest in this question, we were able to extract little data to test this and had to restrict analyses to specific combinations. The results suggested that *Medicago*/alfalfa biotype aphids experienced higher fecundity costs when tested on *Medicago* plants than on the universally-accepted *V. faba* bean plants. Whether this is common across other biotypes for their native plants, and/or due to the aphids with symbionts eliciting reduced plant defences in the universal plant, thereby mediating any fecundity cost, is to be determined (Sanchez-Arcos et al., 2016; Wang, Yuan, et al., 2020). Two aphid biotypes (collected from *Medicago* and *Ononis*) hosting *H. defensa* symbionts were also able to be compared on *V. faba*, with no difference in fecundity but potentially higher parasitism resistance for *Medicago* aphids (not statistically significant due to low sample sizes). We were not able to account for symbiont strain or aphid genotype (within biotype) variation and shows the lack of empirical data to comprehensively conclude any impact of host plant at this stage.

5 | CONCLUSIONS

In general, we found that facultative symbionts provide variable benefits to their insect hosts that range from lifetime fitness benefits to increased survival chances. Aphids were the only group to experience fitness costs (reduced lifespan and fecundity), seemingly a trade-off for strong effects of parasitism resistance. An expectation that facultative symbionts must incur a cost, otherwise all individuals would host them, was not met in non-aphid Hemiptera suggesting a rather grey area between facultative and obligate symbioses that needs to be further studied. Despite the growing number of studies on insect symbionts, we highlight several areas where limited replication within and among species reduces our ability to make clear and general statements on the impact of insect symbionts. In particular, more species need to be studied for a broader taxonomic reference and increased replication within certain host-species to understand the impact of host-plant species and multiple hosting of symbionts.

AUTHOR CONTRIBUTIONS

Sharon E. Zytynska and Enric Frago conceived the idea and designed the study. All authors extracted data, Sharon E. Zytynska and Enric Frago performed the analysis and wrote the manuscript with comments from Karim Tighiouart.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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