Impact of heat stress on the fitness outcomes of symbiotic infection in aphids: a meta-analysis

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Abstract

Beneficial microorganisms shape the evolutionary trajectories of their hosts, facilitating or constraining the colonization of new ecological niches. One convincing example entails the responses of insect-microbe associations to rising temperatures. Indeed, the insect resilience to stressful high temperatures depends on the genetic identity of the obligate symbiont and the presence of heat protective facultative symbionts. With accumulating empirical evidence, there is a need of integrative studies to draw general patterns about the thermal sensitivity of insect-microbe associations, from an eco-evolutionary perspective. Focusing on aphid-bacteria mutualisms, this meta-analysis aims to quantify the context-dependent impacts of symbionts on host phenotype in benign or stressful heat conditions, across fitness traits, types of heat stress, and symbiont species. We found that warming lowered the benefits (parasitoid resistance) and costs (development, fecundity) of infection by facultative symbionts, which was overall mostly beneficial to the aphids under short-term heat stress (heat shock) rather than extended warming. Heat tolerant genotypes of the obligate symbiont *Buchnera aphidicola* and some facultative symbionts (*Rickettsia* sp., *Serratia symbiotica*) improved or maintained aphid fitness under heat stress. As phytophagous insects are central to terrestrial ecosystems, symbiont-mediated responses to increasing mean temperatures and frequency of heat waves in the context of climate change are key elements that may have cascading effects on food webs and there is an urgent need to continue accumulating data on other models. We discuss the implications of these conclusions for the general understanding of the cost-benefits balance and eco-evolutionary dynamics of insect-microbe associations faced with climate change.

Keywords: fitness; symbiosis; secondary symbiont; temperature; warming; life-history traits

Introduction

The ecology and evolution of most insect species are now understood through the lens of their mutualistic associations with diverse microorganisms (*e.g.*, bacteria, fungi, viruses) (1–3). These beneficial microbes shape the life histories and the evolutionary trajectories of their hosts, providing opportunities as well as imposing constraints for colonization of new ecological niches, depending on the nature of the association and the pace of environmental change. On the one hand, acquisitions of obligate microbial partners involved in nutritional complementation are envisioned as key evolutionary innovations that enabled several insect taxa to specialize on unbalanced diets like plant sap and vertebrate blood (4–6). However, these obligate symbionts underwent severe genome degradation (in terms of size, function and structural dynamic) as a product of their ancient coevolution history with their hosts, limiting their potential to adapt to rapidly changing environmental conditions and the one of their hosts alike, since they are required for insect successful growth and reproduction (7–9). On the other hand, insects harbor facultative symbionts responsible for the expression of ecological traits such as plant exploitation and defense against parasitic enemies (10–12). These symbiont-mediated traits might be adaptive under specific ecological contexts like those involving harsh biotic or abiotic stress, but infection by facultative symbionts is also known for inducing fitness costs under more benign environments. Hence, the overall impacts of facultative symbionts on the fitness and adaptive capacity of their hosts can be viewed as a cost-benefit balance tipping in a costly or beneficial state in relation with ecological contingency (13–16).

Temperature has been rapidly identified as a key environmental parameter determining the net fitness consequences of carrying a particular symbiont genotype (for obligate and facultative symbionts) or species (for facultative symbionts) (for reviews, see 17–20). Indeed, the insect resilience to stressful high temperatures can be curtailed by a single mutation affecting a gene encoding the production of heat protective molecules by the obligate symbiont (21, 22), while improved by the presence of some facultative symbionts bestowing physiological tolerance to heat (23, 24). Conversely, temperature can mediate the expression of ecological traits displayed by symbiont-infected individuals, such as levels of protection against parasitoids, thereby affecting the adaptive value of hosting these defensive facultative symbionts in contexts of high parasitism pressure (25–27). These different lines of evidence raise concern about the short- and long-term maintenance of symbiotic associations enduring changing thermal conditions, such as increased mean temperature and frequency of heat events forecasted under climate change (28). Several putative evolutionary scenarios have been proposed to anticipate the fate of insect-microbe mutualisms in this climatic context, depending on the potential for symbiotic relationships to maintain sustainable insect performance and evolutionary capacity under heat stress (7, 19, 29). As empirical evidence has accumulated over the last two decades, it would be now valuable to draw first general patterns about the thermal sensitivity of insect-microbe partnerships and their implications for understanding the ecological and evolutionary trajectories of these mutualistic associations under a changing climate.

Here, we propose a meta-analysis intended to quantify the impacts of microbial symbionts on the phenotype of their insect hosts under different thermal settings (control permissive *versus* stressful heat conditions). We focused our literature survey on aphid-bacteria associations because the vast majority of the data about the thermal ecology of insect-microbe alliances were gathered on these biological models, with a diversity of traits measured, temperature treatments applied, and microbial species identified. This also enabled to standardize the host background from a taxonomic and biological viewpoint, because insect lineages have formed very different types of associations with microbial symbionts in terms of symbiont tissue tropism and degree of mutual dependence (30, 31). Our goal was to provide an integrative, analytical synthesis allowing to examine the generality and quantify the intensity of the thermal modulation of symbiont-related alterations of insect phenotype among (i) fitness components (for facultative symbionts), (ii) nature of the heat stress applied through experimental procedures (for facultative symbionts) and (iii) symbiont species involved (for obligate and facultative symbionts). Indeed, we included studies that controlled for aphid symbiotic state using natural genetic variation in obligate symbiont (comparing heat sensitive *versus* heat tolerant genotypes) and/or natural/artificial infection with different facultative symbionts (comparing individuals deprived of *versus* individuals hosting a particular species). This design allowed testing if the effects of symbiotic infection on aphid fitness differed under various biological and thermal contexts. Specifically, we wanted to test for the broadening of the assumption that microbial players should predominantly modulate the ability of their aphid hosts to withstand a heat stress, or conversely, that temperature should act as an important driver of the expression of traits mediated by endosymbionts.

Material and Methods

Literature search

We collected data within a framework that allowed us to evaluate the interplay between the temperature treatment and the host symbiotic state on the set of aphid traits mentioned below. We searched for appropriate literature using the following key words in Google Scholar: ("aphid*" AND (symbiont* OR facultative OR secondary OR obligat*) AND "temperature*" AND (trait* OR fitness OR development OR growth OR fecundity OR morpholog* OR longevity OR survival OR defens*)), bounded between 1991 and 2020. Indeed, the first historical report of the experimental impacts of temperature on an aphid-bacterium mutualism comes from the study of Ohtaka and Ishikawa (1991) (32).

Our main inclusion criteria were: (i) data with measures of at least one of the focus traits, reporting mean, variation (variance, standard error or confidence intervals) and sample size, (ii) data with at least two temperature treatments, including a control (unheated conditions), (iii) data with at least two aphid symbiotic states, including a control (either an individual hosting a heat sensitive genotype of the obligate symbiont or an individual not infected by a given facultative symbiont). After an additional manual screening, we excluded articles which investigated the effect of cold stress (because they were deemed in insufficient number to be analyzed independently), those which addressed purely microbiological issues without presenting any data on aphid traits (*e.g.*, transcriptomic or genomic analyses, symbiont transmission patterns and titres), qualitative review papers, and those which did not present a proper negative control in a crossed-design experiment required to test for the significance of the interplay (including both a control temperature treatment and a control symbiotic state).

Following our inclusion criteria, we retrieved data from a total of 18 relevant articles, published between 2000 and 2020 (list included as a **Supplementary Material** file). Pairs of datapoints were extracted manually from (i) text and tables, (ii) raw data made available by the authors of the studies, or (iii) figures by using *WebPlotDigitizer* (33). One pair of datapoints corresponded, for a given temperature treatment (either control permissive or stressful heat conditions), to the mean of the trait value (with variation and sample size) for both control and infected individuals. We discarded 19 observations (pairs of datapoints) in our dataset because variation of the mean or sample size could not be accessed. This resulted in a total of 410 pairs of datapoints, including all the data presented in each article. Our dataset included a total of three aphid species developing on Fabaceae and belonging to two genera: *Acyrthosiphon pisum* (318 pairs of datapoints from 14 studies), *Aphis fabae* (76 pairs of datapoints from 3 studies), and *Aphis craccivora* (16 pairs of datapoints from 1 study).

For comparisons among fitness traits and types of heat stress (see the two following subsections below), we excluded data related to the obligate symbiont (20 pairs of datapoints from 3 studies) and only retained those pertaining to the facultative symbionts (390 pairs of datapoints from 15 studies) to restrict the scope of the analyses to a biologically meaningful dataset. Indeed, obligate and facultative symbionts differ in degree of intimacy and coevolution history with their hosts, as well as regarding the source of variation in symbiotic state as defined in this meta-analysis (genetic variation *versus* absence/presence) and the underlying symbiont-related processes that allow a change in host physiology under heat stress. In addition, the fitness impacts of genetic variation displayed by the obligate symbiont has not been consistently studied across the different traits and types of heat stress included in the analyses. Finally, in the case of studies on facultative symbionts, the obligate bacterium is also always present in the host but not studied as such, whereas the reverse is not true. For these reasons, it is not relevant to directly compare or regroup the responses of obligate and facultative bacteria.

Data organization: fitness traits

We focused on aphid phenotypic traits classified into five categories: (i) defense against parasitic wasps (proportion of hosts surviving after exposure to parasitoids, parasitism rates, or emergence rates of the parasitoid), (ii) development (age to adulthood or duration of reproductive period), (iii) fecundity (total or daily number of offspring), (iv) morphology (body mass), and (v) survival (overall lifespan for long-term heat stress, number of days survived or survival rates after treatment for short-term heat stress). Most of the observations were on defense traits (151 pairs of datapoints from 7 studies), followed by fecundity (124 pairs of datapoints from 11 studies), development (54 pairs of datapoints from 5 studies), survival (47 pairs of datapoints from 4 studies) and morphology (14 pairs of datapoints from 2 studies).

Data organization: temperature treatments and types of heat stress

We established a distinction between control temperature treatment (encompassing permissive temperatures, 179 pairs of datapoints) and heat treatment (extending to stressful high temperatures, 211 pairs of datapoints), based on what the authors of each study described in their methods and the thermal biology of studied aphid species, which usually endure a significant decline of performance at temperatures ≥ 25°C (34). The highest mean temperature for a control treatment was 22°C (35) (but more generally it was ≤ 20°C), and the lowest mean temperature for a heat treatment was 21.5°C with maximal daily temperature reaching 25-30°C (36) (but more generally mean temperature was ≥ 25°C). We then classified the heat treatments based on the duration of exposure to stressful high temperatures, either long term (*i.e.*, for at least several days, extending over one or several insect developmental stages, with no return to favorable conditions before measurements of fitness traits) or short term (*i.e.*, for a few hours with return to favorable conditions before measurements of fitness traits). We collected 275 pairs of datapoints (from 7 studies) under long-term heat stress procedures and 115 pairs of datapoints (from 8 studies) under short-term heat stress procedures.

Data organization: symbiotic states and symbiont species

We here included studies focusing on different haplotypes of the obligate bacterial endosymbiont *Buchnera aphidicola* representing a form of "symbiotic plasticity" for the insect (presence/absence of an unfavorable mutation limiting symbiont transcriptional response to heat). To parallel the distinction made for facultative symbionts, aphids likely to be vulnerable to heat stress due to potentially limited symbiont protection (*i.e.*, infected with the mutated heat sensitive genotype of *B. aphidicola*) were considered as negative control, while those potentially benefiting from this protection (*i.e.*, bearing the obligate symbiont genotype without the mutation) were labelled as treatment.

There was a total of five facultative symbiont species identified in our dataset: *Fukatsuia symbiotica*, *Hamiltonella defensa*, *Regiella insecticola*, *Rickettsia* sp. and *Serratia symbiotica*. In case of single infection (*i.e.*, only one facultative symbiont involved), host lines deprived of the facultative symbiont (aposymbiotic individuals) were considered as negative control and infected hosts were considered as treatment. In case of double infection *(F. symbiotica/H. defensa* or *Rickettsia* sp./*S. symbiotica),* individuals infected by only one of the two symbiont species were considered as negative control and compared with individuals co-infected with the two symbionts, thus allowing to specifically dissociate the effects of each microbe involved in the tripartite system. The most studied symbiont species in our dataset was *H. defensa* (214 pairs of datapoints from 10 studies), followed by *S. symbiotica* (71 pairs of datapoints from 4 studies), *Rickettsia* sp. (58 pairs of datapoints from 2 studies), *F. symbiotica* (45 pairs of datapoints from 6 studies), *B. aphidicola* (20 pairs of datapoints from 3 studies) and *R. insecticola* (2 pairs of datapoints from 1 study).

Statistical analyses

The meta-analysis was conducted in R v4.0.1 (37) and was performed using the *metafor* package (38) following, in part, the workflow proposed by Crystal-Ornelas (2020) (39). Data visualization was done using the package *ggplot2* and associated libraries (40, 41).

We used Standardized Mean Differences (SMDs, Hedge's g method) as effect sizes to compare symbiotic treatments (aphids infected with a heat tolerant genotype of obligate symbiont or a particular species of facultative symbiont) with control groups (infection with a heat sensitive genotype of obligate symbiont or absence of facultative symbiont), under either permissive temperatures (control) or heat stress. SMDs are widely used in ecological meta-analyses because they provide standardized and comparable measures of effect size among studies with different experimental procedures and scales for trait measurements. In addition, they are especially useful when sample sizes are small such as in our study, because they include a correction for variance (39, 42).

Effect sizes significantly different from zero allow to interpret the benefit or disadvantage of carrying a given symbiont genotype or species at different temperatures. For the interpretation to be correct, it is necessary to ensure that a negative effect size represents, biologically speaking, a net fitness cost related with symbiotic infection, and a positive effect size a net fitness benefit. To do this, we multiplied the effect sizes calculated for the developmental traits by -1, because slowly developing phytophagous insects might suffer from increased mortality risks due to an extended window of susceptibility to natural enemies ('slow-growth-high-mortality hypothesis' (43)). We used the same data transformation for defense traits, when what was being measured was the benefit for the parasitoid at the expense of the aphid (*e.g.,* parasitism rates and parasitoid emergence rates). We considered that the summary Hedge's g showed a significant effect of the symbiotic state if the 95% confidence intervals (CI) did not overlap zero.

We ran the meta-analysis via multivariate linear mixed-effects models, using the identity of each independent study as a random effect to account for the nonindependence of observations coming from a same study. We performed five distinct analyses. The first analysis only included the temperature treatment (two levels: permissive temperatures or heat stress) as a moderator to determine if the overall fitness impacts of facultative symbionts on their hosts could be modified by the thermal context (N = 15 studies). All the other analyses incorporated an additional moderator in interaction with the temperature treatment. In the second analysis, we integrated the fitness traits with five levels: defense, development, fecundity, morphology, or survival (N = 15 studies). In the third analysis, we integrated the type of heat stress involving two levels: long term or short term (N = 15 studies). In the fourth analysis, we integrated the symbiont species studied involving five levels: *B. aphidicola*, *F. symbiotica*, *H. defensa*, *Rickettsia* sp., or *S. symbiotica* (N = 18 studies). We removed the datapoints for *R. insecticola* from this analysis because of the limited number of observations available and the lack of replication (one single study involved). In a fifth analysis, we integrated the number of facultative symbionts hosted involving three levels: none, one, or two (N = 18 studies) (**Supplementary Material, Figure S1**).

For each analysis, we tested the linear hypothesis that the estimated Hedge's g values differed from zero (package *multcomp* (44)) and we reported χ^2 statistics together with p-values. In addition, each analysis was followed by a Tukey's post-hoc test with Bonferroni adjustment method (a conservative method to reduce the risk of false-positive) (package *emmeans* (45)) to compare values of effect size for permissive temperatures and heat stress, for each level of the moderator of interest (*i.e.*, each trait, type of heat stress, symbiont species, or number of facultative symbionts). These post-hoc analyses were done on a composite variable merging the levels of the two moderators included in each model, to avoid any issue regarding interactions when running the Tukey's test.

The histogram of the frequency of effect sizes and the funnel plot analysis (**Figure S2**) showed relatively symmetrical data, although slightly skewed toward positive effect-size values, with some points representing a risk of inducing a bias as they fall outside the pseudo 95% CI envelope. Some degree of asymmetry in the dataset was expected given the relatively small number of studies investigating temperature effects on symbiont-mediated aphid traits. However, as pointed out by Sterne et al. (46), there are many possible sources of asymmetry in funnel plots, and it is not necessarily indicating that a publication bias exists. We obtained four extreme effect size values and high corresponding sampling variances (*i.e.*, high imprecision) for fecundity data extracted from one article (47). We decided to retain these outliers in the analyses (yi = 314.1, -162.3, -97.3, -75.6; vi = 1826.8, 598.5, 157.9, 92.3, respectively), assuming that no error was made in the published version of the referenced article, and especially because heterogeneity was already acknowledged by the random effect included in the models (39, 48). In any case, removing these four values did not change the biological conclusions drawn from the analyses.

Results

Global effect of temperature on the fitness outcomes of symbiotic infection

When grouping all traits, types of heat stress and facultative symbiont species, there was a clear global thermal modulation of the fitness consequences of hosting a facultative symbiont. This interaction is indicated by a significant difference in effect sizes (quantifying the fitness impacts of symbiotic infection) between temperature treatments: warming overall increased the adaptive value (fitness gains) of symbiotic infection for the insect (z = 3.75, p < 0.001). This implies that hosting a facultative symbiont was more beneficial under heat stress than under permissive temperatures. Indeed, symbiotic infection had a neutral effect on aphid overall fitness under permissive temperatures (Hedge's g = -0.06, $\chi^2 = 0.11$, p = 0.73), while it became significantly beneficial under heat stress (Hedge's g = 0.65, $\chi^2 = 11.7$, p < 0.001) (**Fig. 1**).

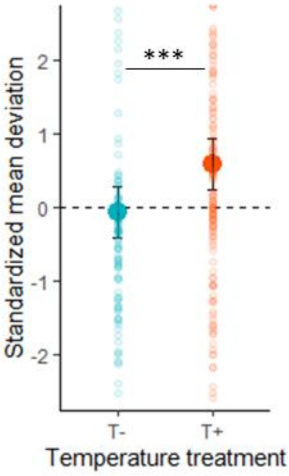

Figure 1: Global effect of temperature treatment on the fitness consequences of symbiotic infection (all traits, types of heat stress, and facultative symbiont species combined). Predicted overall Hedge's g values (standardized mean deviation, large circles) and 95% confidence intervals are shown, for control permissive temperatures (T-, blue) and heat stress (T+, red). A positive effect size value indicates that hosting a given facultative symbiont is favorable for the host, and a negative value indicates that it is costly. Confidence intervals that overlap zero indicate a non-significant effect of symbiotic infection on insect fitness. Note that only 60% of the datapoints are shown in this figure for aesthetic purposes. N = 15 studies, 390 datapoints. Asterisks highlight significant difference in effect sizes between temperature treatments (*** p < 0.001).

Fitness traits

The impacts of temperature on the fitness outcomes of symbiotic infection were trait-specific, with three different patterns of response. For defense against parasitoids, warming lowered the fitness benefits (protection) provided by some facultative symbionts (z = -9.85, p < 0.001). Still, in a parasitism context, a high fitness advantage remained of carrying the studied protective symbionts (*H. defensa* and *F. symbiotica*) under both permissive temperatures (Hedge's g = 3.20, $\chi^2 = 78.0$, p < 0.001) and heat stress (Hedge's g = 2.13, $\chi^2 = 45.3$, p < 0.001). This suggests that the symbiont-mediated protection towards parasitoids persisted under different temperature treatments. For development and fecundity, warming shifted the fitness consequences of symbiotic infection from a costly to a neutral outcome (development: z = 9.93, p < 0.001; fecundity: z = 16.3, p < 0.001). Under permissive temperatures, aphids infected by facultative symbionts incurred a prolonged development time (Hedge's g = -1.31, $\chi^2 = 15.9$, p < 0.001) and a reduced fecundity (Hedge's g = -1.56, $\chi^2 = 22.7$, p < 0.001) relative to uninfected individuals. These infection costs were no longer apparent under heat stress (development: Hedge's g = -0.24, $\chi^2 = 0.53$, p = 0.47; fecundity: Hedge's g = -0.22, $\chi^2 = 0.48$, p = 0.49). For morphology (body size) and survival, temperature did not significantly modulate the fitness consequences of symbiotic infection (morphology: z = 1.54, p = 1.00;

survival: z = 3.10, p = 0.09). Aphids infected by facultative symbionts grew lighter (Hedge's g = -1.17, χ^2 = 9.41, p < 0.01 and Hedge's g = -0.85, χ^2 = 5.35, p < 0.05, for permissive and heat stress temperatures, respectively) and died faster (Hedge's g = -1.25, χ^2 = 14.3, p < 0.001 and Hedge's g = -0.90, χ^2 = 7.16, p < 0.01, for permissive and heat stress temperatures, respectively) compared with uninfected individuals (**Fig. 2**).

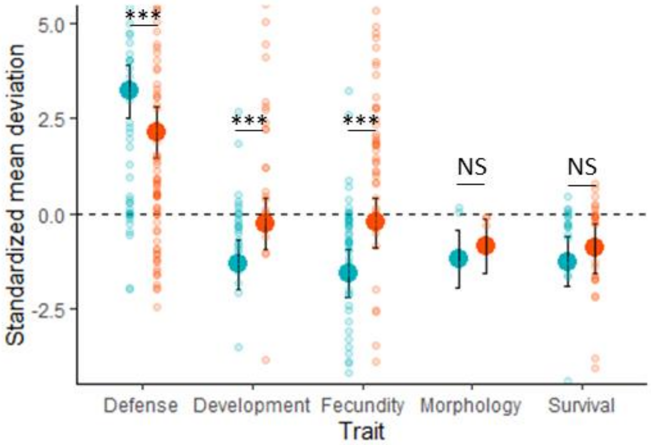

Figure 2: Effect of temperature treatment on the fitness consequences of symbiotic infection for different aphid traits (all types of heat stress and facultative symbiont species combined). Predicted overall Hedges'g values (standardized mean deviation, large circles) and 95% confidence intervals are shown, for control permissive temperatures (blue) and heat stress (red). A positive effect size value indicates that hosting a given facultative symbiont is favorable for the host, and a negative value indicates that it is costly. Confidence intervals that overlap zero indicate a non-significant effect of symbiotic infection on insect fitness. Note that only 75% of the datapoints are shown in this figure for aesthetic purposes. N = 7, 5, 11, 2 and 4 studies, for defense, development, fecundity, morphology and survival, respectively. Asterisks highlight significant difference in effect sizes between temperature treatments for a given trait, according to Tukey's post-hoc comparisons (*** p < 0.001, NS = not significant).

Types of heat stress

The impacts of temperature on the fitness consequences of symbiotic infection varied according to the type of heat stress applied. Long-term heat treatment did not modulate these outcomes (z = 2.02, p = 0.26), while short-term heat treatment did (z = 8.14, p < 0.001). Under permissive temperatures, symbiotic infection had neutral effects on aphid overall fitness indicators in both long-term and short-term heat stress experimental procedures (long term: Hedge's g = 0.05, χ^2 = 0.04, p = 0.84; short term: Hedge's g = -0.24, χ^2 = 0.89, p = 0.34). Under heat stress, aphids infected by facultative symbionts benefited from a fitness advantage relative to their uninfected counterparts, irrespective of the duration of exposure to the heat treatment (long term: Hedge's g = 0.65, χ^2 = 6.42, p < 0.05; short term: Hedge's g = 0.58, χ^2 = 5.30, p < 0.05). However, the interaction mentioned above indicates that warming significantly increased the adaptive value of symbiotic infection only if applied through short-term heat stress (sporadic bout of heat shock) (**Fig. 3**).

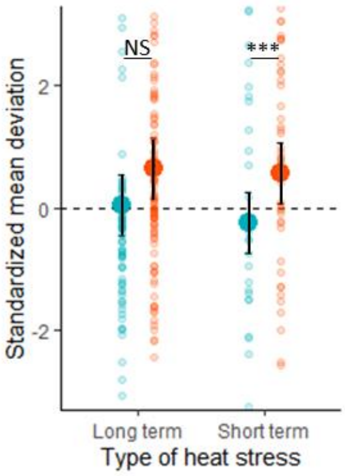

Figure 3: Effect of temperature treatment on the fitness consequences of symbiotic infection under different types of heat stress (all traits and facultative symbiont species combined). Predicted overall Hedges'g values (standardized mean deviation, large circles) and 95% confidence intervals are shown, for control permissive temperatures (blue) and heat stress (red). A positive effect size value indicates that hosting a given facultative symbiont is favorable for the host, and a negative value indicates that it is costly. Confidence intervals that overlap zero indicate a non-significant effect of symbiotic infection on insect fitness. Note that only 63% of the datapoints are shown in this figure for aesthetic purposes. N = 7 and 8 studies, for long-term and short-term heat stress treatments, respectively. Asterisks highlight significant difference in effect sizes between temperature treatments for a given type of heat stress, according to Tukey's post-hoc comparisons (*** $p < 0.001$, NS = not significant).

Symbiont species

The impacts of temperature on the fitness consequences of symbiotic infection were dependent on the microbial species involved. For the obligate symbiont, warming significantly enhanced the adaptive value of hosting the heat tolerant genotype of *B. aphidicola* ($z = 3.73$, $p < 0.01$). Insects infected with a heat tolerant genotype of *B. aphidicola* performed as well as those infected with a heat sensitive genotype under permissive temperatures (Hedge's $g = -0.31$, $\chi^2 = 0.28$, $p = 0.59$), and greatly better under heat stress (Hedge's $g = 2.17$, $\chi^2 = 12.7$, $p < 0.001$).

For the facultative symbionts, warming significantly enhanced the adaptive value of hosting the bacteria *Rickettsia* sp. ($z = 9.88$, $p < 0.001$) and *S. symbiotica* ($z = 15.7$, $p < 0.001$). Harboring *Rickettsia* sp. was slightly detrimental to aphids under permissive temperatures, since the CIs of effect size marginally overlap zero (Hedge's $g = -0.76$, $\chi^2 = 2.81$, $p = 0.09$), while no fitness consequences related with this infection were seen under heat stress (Hedge's $g = 0.17$, $\chi^2 = 0.13$, $p = 0.72$). Symbiotic association with *S. symbiotica* switched from a slightly detrimental to a slightly ameliorative impact on aphid fitness from permissive temperatures (Hedge's $g = -0.69$, $\chi^2 = 2.34$, $p = 0.13$) to heat stress (Hedge's $g = 0.65$, $\chi^2 = 2.07$, $p = 0.15$), respectively, although effect sizes and CIs only indicate trends. Warming did not affect the fitness outcomes of infection with *F. symbiotica* ($z = 0.42$, $p = 1.00$) and *H. defensa* ($z = -3.19$, $p = 0.06$). Hosting *F. symbiotica* remained neutral in terms of aphid fitness under both permissive temperatures (Hedge's $g = -0.21$, $\chi^2 = 0.50$, $p = 0.48$) and heat stress (Hedge's $g = -0.14$, $\chi^2 = 0.22$, $p = 0.64$). Individuals infected with *H. defensa* exhibited a net fitness advantage relative to their uninfected counterparts under both permissive temperatures (Hedge's $g = 0.97$, $\chi^2 = 12.0$, $p < 0.001$) and heat stress (Hedge's $g = 0.68$, $\chi^2 = 6.10$, $p < 0.05$) (**Fig. 4**).

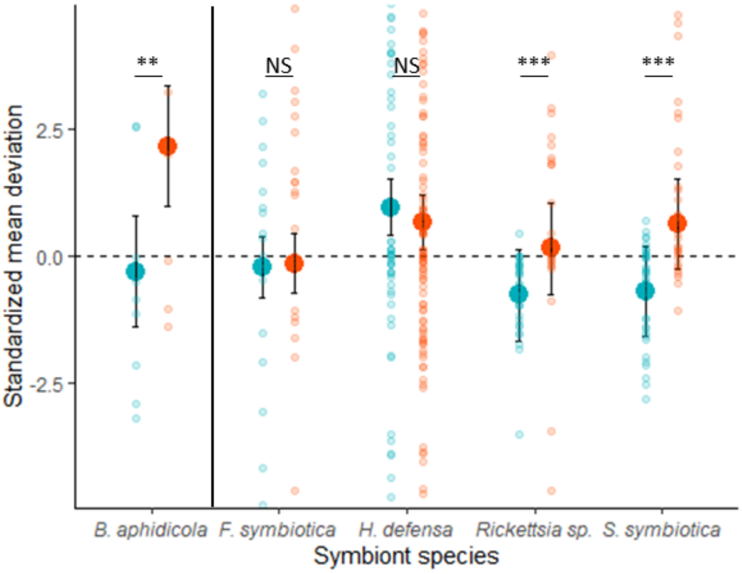

Figure 4: Effect of temperature treatment on the fitness consequences of symbiotic infection involving different symbiont species, engaged in either obligate or facultative relationship with their aphid hosts (all traits and types of heat stress combined). Predicted overall Hedges'g values (standardized mean deviation, large circles) and 95% confidence intervals are shown, for control permissive temperatures (blue) and heat stress (red). Note that it is not relevant to directly compare the responses of obligate and facultative symbionts (separated here by a vertical solid line), as they differ in the processes involved in modulation of host physiology under heat stress and regarding the source of variation in symbiotic state as defined in this meta-analysis (genetic variation of obligate symbiont *versus* absence/presence of facultative symbionts). A positive effect size value indicates that hosting a heat tolerant genotype of the obligate symbiont or a given facultative symbiont is favorable for the host, and a negative value indicates that it is costly. Confidence intervals that overlap zero indicate a non-significant effect of symbiotic infection on insect fitness. Note that only 74% of the datapoints are shown in this figure for aesthetic purposes. N = 3, 6, 10, 2 and 4 studies, for *Buchnera aphidicola*, *Fukatsuia symbiotica*, *Hamiltonella defensa*, *Rickettsia* sp. and *Serratia symbiotica*, respectively. Asterisks highlight significant difference in effect sizes between temperature treatments for a given symbiont species, according to Tukey's post-hoc comparisons (*** $p < 0.001$, ** $p < 0.01$, NS = not significant).

Discussion

This meta-analysis sought to quantify the fitness impacts (phenotypic effects) of symbiotic infection on aphid hosts under permissive temperatures and heat stress. We found that infection by facultative symbionts benefited the aphids in terms of overall fitness under heat stress or, conversely, that warming increased the adaptive value of such infection for the insects. However, this general pattern was far from being generalizable across fitness traits, types of heat stress and symbiont species. Warming lowered the benefits provided by some facultative symbionts (defense against parasitoids), but limited the costs of symbiotic infection on insect development and fecundity. Infection by facultative symbionts was mostly beneficial to the aphids in case of short-term exposure to high temperature spikes rather than prolonged warming. Heat tolerant genotypes of the obligate symbiont *B. aphidicola* on the one hand, and the presence of the facultative symbionts *Rickettsia* sp. and *S. symbiotica* on the other, specifically contributed to improve (or at least maintain) aphid fitness under heat stress. These different findings support the important role played by some microbes in the response of their hosts to high temperatures, but also highlight subtle trait- and environment-specific responses that may underlie complex ecological and evolutionary consequences for insect-microbe associations in a gradually warming climate, which also entails an increased risk of exposure to sudden thermal shocks.

Several mechanistic explanations can be proposed to account for the trait-specific conclusions about the interactive effects of temperature and symbiotic infection on host fitness. First, variability could arise from energetic trade-offs shaping resource allocation between traits (*e.g.,* defense and fecundity), particularly under physiologically constraining conditions such as infection by facultative symbionts and stress-inducing temperatures (15, 49). Second, life-history traits frequently differ in their thermal requirement and response to warming (50, 51). Heat sensitive traits are thus likely to be the most responsive to temperature rise and its potential interaction with symbiotic infection. Third, trait expression can be modulated by different symbiotic processes in a temperature-dependent fashion. On the one hand, the direct involvement of protective facultative endosymbionts (especially toxin-producing *H. defensa*) in aphid defense against parasitoids should explain the considerable fitness gains provided by symbiotic infection and their decline under heat stress, since protective symbiosis is weakened at high temperatures (25–27). However, we stress that the widespread idea that protection against parasitoids fails under heat stress could be largely overstated, as it remained overall beneficial to the insect even under warmer temperature. On the other hand, some aphid vital functions (especially development and reproduction) are closely linked to titres of the obligate symbiont *B. aphidicola* (24, 52, 53). Our study confirms the costly nature of symbiotic infection for several aphid fitness traits under permissive temperatures, thereby corroborating the results of a recent analysis (15). However, under heat stress, symbiotic infection turned neutral for aphid development and fecundity, which may be explained by the protective role played by some facultative symbionts (*F. symbiotica*, *S. symbiotica*) in shielding populations of *B. aphidicola* from heat-related depletion (24, 54), or because heat stress can trigger greater physiological costs relative to infection by facultative symbionts. Whatever their mechanistic basis, these trait-specific conclusions illustrate the concept of an environmentally contingent cost-benefit balance of symbiotic infection and highlight the relevance of multi-trait approaches to get an integrative picture about how facultative symbionts can shape their host life histories under different thermal conditions.

The adaptive value of symbiotic infection was also contingent on the nature of the heat stress applied. Why symbiotic infection turned more advantageous under short-term heat stress can be explained by the underlying mechanisms of symbiont-mediated protection to heat. Specifically, the heat protection delivered by facultative symbionts is effective on the short term as it apparently consists of either maintaining populations of *B. aphidicola* or facilitating their recovery if aphids readily return to benign temperatures (24, 54, 55). It might thus be speculated that long-term heat stress treatments (where insects are held in warm conditions for a prolonged duration without return to cooler environments) could hamper the efficiency of such protective mechanisms. This could occur if these mechanisms operate once temperatures shift from stressful to favorable conditions, or if they are triggered by threshold effects related with temperature fluctuations, as evidenced in the event of repeated exposure to heat shocks leading *B. aphidicola* abundances to decline below a value causing aphid sterility (56). The distinction here made between long- and short-term heat stress is artificial, but it could shed light on the thermal sensitivity of mechanisms underpinning symbiont-mediated tolerance to high temperatures. The results suggesting how impactful the temporal dynamic of temperatures is for aphid-symbiont associations should prompt further efforts to probe into the responses of these systems to patterns of temperature fluctuations hitherto unexplored, both along temporal (*e.g.,* diurnal and seasonal temperature cycles) and spatial axes (*e.g.,* (micro)climatic gradients) (17, 19, 36). This should be of great relevance for general predictions about the evolutionary fate of insect-microbe partnerships facing climate change, which will alter both mean temperature and variability around that mean, this latter thermal parameter having already documented impacts on the outcomes of other kinds of biotic interactions (57, 58).

When it comes to species-specific conclusions, the important contribution of different genotypes of *B. aphidicola* to aphid response to heat stress is not a surprising result, considering the high levels of mutual dependence between insect fitness and microbe abundance (as explained above). Moreover, studies compared *B. aphidicola* genotypes purposely chosen for differing *a priori* in their thermal sensitivity, because of a naturally recurring mutation that affects the symbiont transcriptional pathways involved in heat response (21, 22, 59). Therefore, the prevalence and levels of heat protection mediated by the obligate symbiont could be overestimated relative to ecologically more relevant contexts (24, 59). The contribution of obligate endosymbionts to the evolvability of their carriers facing rising temperatures is thought to be rather minor across different phyla of sap-feeding insects, because of their genomic features limiting their evolutionary potential, making them the potential "Achilles' heel" of the interaction (7, 60, 61). Future studies should consider how natural genetic variability in populations of hosts and their obligate symbionts structured by temperature variations (along geographical gradients for instance) could modulate the ability of the partnership to withstand heat stress, through additive or interactive pathways.

Outside of obligate symbiosis, four species of facultative symbionts have been reported to increase aphid heat tolerance: *F. symbiotica*, *H. defensa*, *Rickettsia* sp. and *S. symbiotica* (62). From our quantitative study, it appears that only infection with *Rickettsia* sp. and *S. symbiotica* yielded different fitness impacts under permissive temperatures and heat stress (although CIs of effect sizes only indicated modest fitness consequences of symbiotic infection). This could alternatively indicate a direct symbiont involvement in heat protection, or a temperature-driven reduction of fitness costs incurred with infection. This does not necessarily imply that our study contradicts the literature, but rather that the net fitness gains of symbiotic infection under heat stress could not be apparent through the lens of a meta-analysis conducted at the species level. Plausible explanations to this could involve an insufficient set of independent observations from the literature, the influence of mechanisms acting at finer levels of biological entity (*i.e.,* interactions between host and symbiont genotypes) or other environmental factors (whereby levels of heat protection depend on the intensity of parasitism pressure exerted by parasitoids or the plant species upon which aphids fed) (35, 63, 64).

Conclusion

Understanding how temperature can modulate the fitness outcomes of symbiotic infection is crucial for predicting the eco-evolutionary dynamics of mutualism in the era of climate change. It raises the issue of the condition-dependence of the evolutionary interest for an insect to host a facultative symbiont (cost-benefit balance of symbiotic infection in various thermal contexts). Our quantitative study confirms the net global increase in the adaptive value of infection by facultative symbionts under heat stress, thereby pointing towards a general role of these microorganisms in facilitating their host adaptation to a warming world. Relationships forged with microbial partners could thus become an additional evolutionary opportunity exploited by insects to persist under climate change, not only through dynamic of symbiont prevalence within host populations, but also through genetic innovations generated by rapid symbiont evolution (7, 65, 66). Conclusions will likely depend on the biological model investigated and the coevolutionary history between the different organisms at play, the aspects of life history measured, and the way we experimentally mimic climate change through the different thermal treatments applied. In that prospect, combining multi-trait approaches with realistic projections associated with different scenarios of climate change (*e.g.,* 36, 61) should constitute a step towards a more integrative picture on symbiont-mediated insect responses to ongoing climate disturbance. This broadened focus should also expand to other herbivorous insects for which the thermal sensitivity of the interactions with obligate (heteropterans, psyllids, mealybugs, planthoppers) and facultative symbionts (whiteflies) is only beginning to be elucidated, while being a cornerstone of the structure and the functioning of many terrestrial ecosystems.

Acknowledgments

We thank the members of the Hance's lab for stimulating discussions. We thank C. Bugli for her help with data analysis. We are grateful to the authors who made their dataset readily available online, and to those who responded promptly to our request. KT was supported by the F.R.S.-FNRS. CI was a beneficiary of a FSR incoming Post-doctoral Fellowship of the UCLouvain.

Data availability statement

Our dataset has been made publicly available at: DOI: 10.5281/zenodo.5714555.

Supplementary Material

We subset the dataset by the number of facultative symbionts involved, making a distinction between cases of non-infection by any known facultative symbiont (20 pairs of datapoints from 3 studies), single infection (318 pairs of datapoints from 15 studies) or double infection (72 pairs of datapoints from 5 studies).

The impacts of temperature on the fitness consequences of symbiotic infection differed according to the number of facultative symbionts being involved. In cases where no or one facultative symbiont was involved in the interaction, warming increased the adaptive value of symbiotic infection (no facultative symbiont: $z = 3.86$, $p < 0.01$; single infection: $z = 16.25$, $p < 0.001$). Under permissive temperatures, harboring a heat tolerant genotype of the obligate bacterium *B. aphidicola* or one species of facultative symbiont did not impact aphid fitness (no facultative symbiont: Hedge's g = -0.31, $\chi^2 = 0.34$, $p = 0.56$; one facultative symbiont: Hedge's g = -0.25, $\chi^2 = 1.30$, $p = 0.25$). Under heat stress, these two infection patterns significantly benefited the aphids relative to individuals infected with heat sensitive genotype of *B. aphidicola* or free from any facultative symbiont, respectively (no facultative symbiont: Hedge's g = 2.15, $\chi^2 = 13.9$, $p < 0.001$; one facultative symbiont: Hedge's g = 0.74, $\chi^2 = 11.7$, $p < 0.001$). In cases where two facultative symbionts were involved, warming did not mediate the fitness consequences of symbiotic infection ($z = -0.49$, $p = 1.00$), which remained neutral under both permissive temperatures (Hedge's g = 0.15, $\chi^2 = 0.47$, $p = 0.49$) and heat stress (Hedge's g = 0.11, $\chi^2 = 0.24$, $p = 0.62$) (**Fig. S1**).

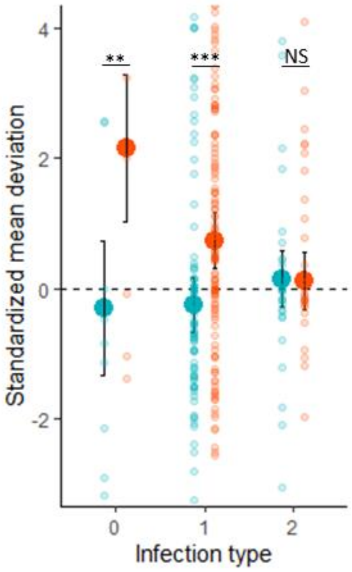

Figure S1: Effect of temperature treatment on the fitness consequences of symbiotic infection depending on the number of facultative symbionts being involved in the interaction (infection type: 0 = none, 1 = single infection, 2 = double infection), all traits, types of heat stress and symbiont species combined. Predicted overall Hedges'g values (standardized mean deviation, large circles) and 95% confidence intervals are shown, for control permissive temperatures (blue) and heat stress (red). A positive effect size value indicates that hosting a heat tolerant genotype of the obligate symbiont (for individuals free from any facultative symbiont) or a given facultative symbiont is favorable for the host, and a negative value indicates that it is costly. Confidence intervals that overlap zero indicate a non-significant effect of symbiotic infection on insect fitness. Note that only 68% of the datapoints are shown in this figure for aesthetic purposes. N = 3, 15, and 5 studies, for no facultative symbiont, single infection and double infection, respectively. Asterisks highlight significant difference in effect sizes between temperature treatments for a given infection type, according to Tukey's post-hoc comparisons (*** $p < 0.001$, ** $p < 0.01$, NS = not significant).

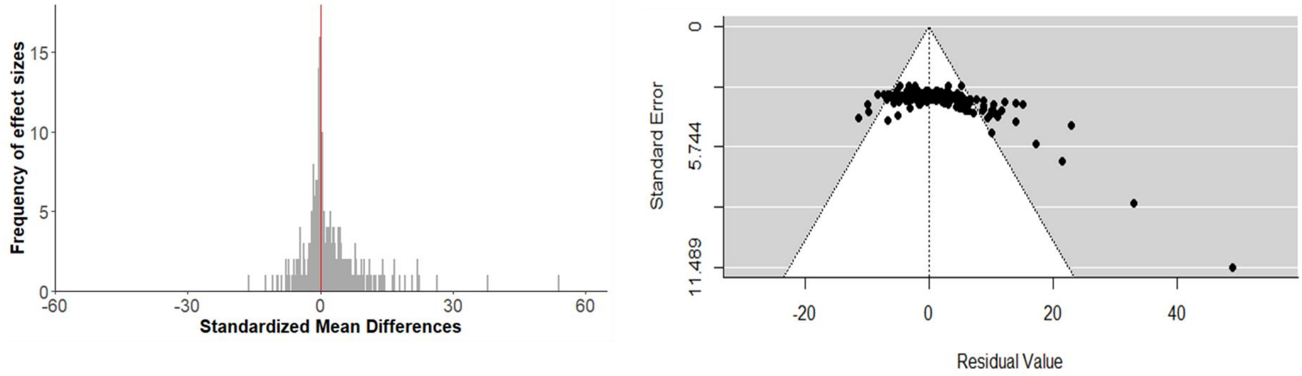

Figure S2: Histogram of the frequency of effect sizes (left) and funnel plot analysis with pseudo 95% CIs (right) when removing four outliers (from Dykstra et al., 2014). The dataset is relatively symmetric, but some points are out of the 95% CI on the funnel plot, which means they are at higher risk of inducing a bias. Here, the calculated effect sizes spread over a large range, with some degrees of asymmetry especially in favor to positive values, but they have very close associated standard errors (the calculated SMDs have relatively little imprecision, and a high uniformity of imprecisions). When there are few studies, the power of tests for funnel plot asymmetry such as Eggers' test is too low to distinguish chance from real asymmetry, so it was impossible to run such tests.

References

1. J. Ferrari, F. Vavre, Bacterial symbionts in insects or the story of communities affecting communities. *Philosophical Transactions of the Royal Society B: Biological Sciences* **366**, 1389–1400 (2011).
2. S. Sudakaran, C. Kost, M. Kaltenpoth, Symbiont acquisition and replacement as a source of ecological innovation. *Trends in Microbiology* **25**, 375–390 (2017).
3. P. H. Biedermann, F. E. Vega, Ecology and evolution of insect–fungus mutualisms. *Annual review of entomology* **65**, 431–455 (2020).
4. A. E. Douglas, The microbial dimension in insect nutritional ecology. *Functional Ecology* **23**, 38–47 (2009).
5. A. K. Hansen, N. A. Moran, The impact of microbial symbionts on host plant utilization by herbivorous insects. *Molecular ecology* **23**, 1473–1496 (2014).
6. O. Duron, Y. Gottlieb, Convergence of nutritional symbioses in obligate blood feeders. *Trends in Parasitology* (2020).
7. F. Renoz, I. Pons, T. Hance, Evolutionary responses of mutualistic insect–bacterial symbioses in a world of fluctuating temperatures. *Current opinion in insect science* **35**, 20–26 (2019).
8. J. J. Wernegreen, In it for the long haul: evolutionary consequences of persistent endosymbiosis. *Current opinion in genetics & development* **47**, 83–90 (2017).
9. G. M. Bennett, N. A. Moran, Heritable symbiosis: the advantages and perils of an evolutionary rabbit hole. *Proceedings of the National Academy of Sciences* **112**, 10169–10176 (2015).
10. K. M. Oliver, P. H. Degnan, G. R. Burke, N. A. Moran, Facultative Symbionts in Aphids and the Horizontal Transfer of Ecologically Important Traits. *Annual Review of Entomology* **55**, 247–266 (2010).
11. H. Feldhaar, Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecological Entomology* **36**, 533–543 (2011).
12. S. E. Zytynska, W. W. Weisser, The natural occurrence of secondary bacterial symbionts in aphids. *Ecological Entomology* **41**, 13–26 (2016).
13. C. Vorburger, A. Gouskov, Only helpful when required: a longevity cost of harbouring defensive symbionts. *Journal of evolutionary biology* **24**, 1611–1617 (2011).
14. H. Mathé-Hubert, H. Kaech, P. Ganesanandamoorthy, C. Vorburger, Evolutionary costs and benefits of infection with diverse strains of Spiroplasma in pea aphids. *Evolution* **73**, 1466–1481 (2019).
15. S. E. Zytynska, K. Tighiouart, E. Frago, The benefits and costs of hosting facultative symbionts in plant-sucking insects: a meta-analysis. *Mol Ecol*, mec.15897 (2021).
16. A. H. Smith, *et al.*, Does getting defensive get you anywhere?—Seasonal balancing selection, temperature, and parasitoids shape real-world, protective endosymbiont dynamics in the pea aphid. *Molecular Ecology* **30**, 2449–2472 (2021).
17. C. Corbin, E. R. Heyworth, J. Ferrari, G. D. D. Hurst, Heritable symbionts in a world of varying temperature. *Heredity* **118**, 10–20 (2017).
18. M. M. Lemoine, T. Engl, M. Kaltenpoth, Microbial symbionts expanding or constraining abiotic niche space in insects. *Current opinion in insect science* **39**, 14–20 (2020).
19. C. Iltis, K. Tougeron, T. Hance, P. Louapre, V. Foray, A perspective on insect-microbe holobionts facing thermal fluctuations in a climate-change context. *Environmental Microbiology* (2021) https:/doi.org/10.1111/1462-2920.15826.
20. J. J. Wernegreen, Mutualism meltdown in insects: bacteria constrain thermal adaptation. *Current opinion in microbiology* **15**, 255–262 (2012).
21. H. E. Dunbar, A. C. Wilson, N. R. Ferguson, N. A. Moran, Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. *PLoS Biology* **5**, e96 (2007).
22. N. A. Moran, Y. Yun, Experimental replacement of an obligate insect symbiont. *Proc Natl Acad Sci USA* **112**, 2093–2096 (2015).
23. M. Brumin, S. Kontsedalov, M. Ghanim, Rickettsia influences thermotolerance in the whitefly Bemisia tabaci B biotype. *Insect Science* **18**, 57–66 (2011).
24. E. R. Heyworth, M. R. Smee, J. Ferrari, Aphid Facultative Symbionts Aid Recovery of Their Obligate Symbiont and Their Host After Heat Stress. *Front. Ecol. Evol.* **8**, 56 (2020).
25. F. Bensadia, S. Boudreault, J.-F. Guay, D. Michaud, C. Cloutier, Aphid clonal resistance to a parasitoid fails under heat stress. *Journal of Insect Physiology* **52**, 146–157 (2006).
26. J.-F. Guay, S. Boudreault, D. Michaud, C. Cloutier, Impact of environmental stress on aphid clonal resistance to parasitoids: Role of Hamiltonella defensa bacterial symbiosis in association with a new facultative symbiont of the pea aphid. *Journal of Insect Physiology* **55**, 919–926 (2009).
27. M. R. Doremus, *et al.*, Breakdown of a defensive symbiosis, but not endogenous defences, at elevated temperatures. *Molecular ecology* **27**, 2138–2151 (2018).
28. IPCC, *Climate change 2013 the physical science basis: final draft underlying scientific-technical assessment : Working Group I contribution to the IPCC fifth assessment report. Stocker, T.F., D. Qin, G.-*

K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.). 1535p., Cambridge University Press, Cambridge (2014).

29. K. Oliver, C. Higashi, Symbiosis in a Rapidly Changing World. *Microbes: The Foundation Stone of the Biosphere*, 263–296 (2021).
30. Y. Kikuchi, T. Hosokawa, T. Fukatsu, Insect-microbe mutualism without vertical transmission: a stinkbug acquires a beneficial gut symbiont from the environment every generation. *Applied and environmental microbiology* **73**, 4308–4316 (2007).
31. P. Baumann, Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annu. Rev. Microbiol.* **59**, 155–189 (2005).
32. C. OHTAKA, H. ISHIKAWA, Effects of heat treatment on the symbiotic system of an aphid mycetocyte. *Symbiosis* (1991).
33. A. Rohatgi, WebPlotDigitizer: HTLM5 Based Online Took to Extract Numerical Data from Plot Images. Version 3.9. *https://automeris.io/WebPlotDigitizer/* (2015).
34. D. Morgan, K. Walters, J. Aegerter, Effect of temperature and cultivar on pea aphid, Acyrthosiphon pisum (Hemiptera: Aphididae) life history. *Bulletin of entomological research* **91**, 47–52 (2001).
35. L. Cayetano, C. Vorburger, Genotype-by-genotype specificity remains robust to average temperature variation in an aphid/endosymbiont/parasitoid system. *Journal of Evolutionary Biology* **26**, 1603–1610 (2013).
36. C. H. V. Higashi, B. T. Barton, K. M. Oliver, Warmer nights offer no respite for a defensive mutualism. *J Anim Ecol* **89**, 1895–1905 (2020).
37. R Core Team, *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.* (2020).
38. W. Viechtbauer, Conducting meta-analyses in R with the metafor package. *Journal of statistical software* **36**, 1–48 (2010).
39. R. Crystal-Ornelas, Meta-analysis of Ecological Data in R. First release (Version v0.1.0). *DOI: 10.5281/zenodo.4320107* (2020).
40. E. Campitelli, ggnewscale: Multiple Fill and Color Scales in ‘ggplot2 ‘. *R package version* **2** (2019).
41. H. Wickham, *ggplot2: Elegant Graphics for Data Analysis*. (2016).
42. L. V. Hedges, Distribution theory for Glass’s estimator of effect size and related estimators. *journal of Educational Statistics* **6**, 107–128 (1981).
43. K. Chen, Y. Chen, Slow-growth high-mortality: A meta-analysis for insects. *Insect science* **25**, 337–351 (2018).
44. T. Hothorn, F. Bretz, P. Westfall, Simultaneous inference in general parametric models. *Biometrical journal* **50**, 346–363 (2008).
45. R. Lenth, H. Singmann, J. Love, P. Buerkner, M. Herve, Emmeans: Estimated marginal means, aka least-squares means. *R package version* **1**, 3 (2018).
46. J. A. Sterne, *et al.*, Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *Bmj* **343** (2011).
47. H. R. Dykstra, *et al.*, Factors limiting the spread of the protective symbiont Hamiltonella defensa in Aphis craccivora aphids. *Applied and Environmental Microbiology* **80**, 5818–5827 (2014).
48. J. Koricheva, J. Gurevitch, K. Mengersen, Eds., *Handbook of meta-analysis in ecology and evolution* (Princeton University Press, 2013).
49. C. Luo, *et al.*, Comparison of life-history traits and resistance for Sitobion avenae (Fabricius) harboring a facultative symbiont. *Entomologia Generalis* **40**, 39–47 (2020).
50. V. Kellermann, B. van Heerwaarden, Terrestrial insects and climate change: adaptive responses in key traits. *Physiological Entomology* **44**, 99–115 (2019).
51. C. Iltis, *et al.*, Are life-history traits equally affected by global warming? A case study combining a multi-trait approach with fine-grain climate modeling. *Journal of insect physiology* **117**, 103916 (2019).
52. C.-Y. Chen, C.-Y. Lai, M.-H. Kuo, Temperature effect on the growth of Buchnera endosymbiont in Aphis craccivora (Hemiptera: Aphididae). *Symbiosis* **49**, 53–59 (2009).
53. R. A. Chong, N. A. Moran, Intraspecific genetic variation in hosts affects regulation of obligate heritable symbionts. *Proceedings of the National Academy of Sciences* **113**, 13114–13119 (2016).
54. C. B. Montllor, A. Maxmen, A. H. Purcell, Facultative bacterial endosymbionts benefit pea aphids Acyrthosiphon pisum under heat stress. *Ecol Entomol* **27**, 189–195 (2002).
55. G. Burke, O. Fiehn, N. Moran, Effects of facultative symbionts and heat stress on the metabolome of pea aphids. *The ISME journal* **4**, 242 (2010).
56. N. Parven, I. Yao, T. Kanbe, S. Akimoto, Heat shock alters pea aphid–Buchnera interactions: negative allometry of gene densities. *Entomologia Experimentalis et Applicata* **169**, 462–471 (2021).
57. C. J. Speights, J. P. Harmon, B. T. Barton, Contrasting the potential effects of daytime versus nighttime warming on insects. *Current opinion in insect science* **23**, 1–6 (2017).

58. R. Stoks, J. Verheyen, M. Van Dievel, N. Tüzün, Daily temperature variation and extreme high temperatures drive performance and biotic interactions in a warming world. *Current Opinion in Insect Science* **23**, 35–42 (2017).
59. B. Zhang, S. P. Leonard, Y. Li, N. A. Moran, Obligate bacterial endosymbionts limit thermal tolerance of insect host species. *Proc Natl Acad Sci USA* **116**, 24712–24718 (2019).
60. N. A. Moran, When obligate partners melt down. *MBio* **7**, e01904-16 (2016).
61. Y. Kikuchi, *et al.*, Collapse of insect gut symbiosis under simulated climate change. *MBio* **7**, e01578-16 (2016).
62. J. Guo, *et al.*, Nine facultative endosymbionts in aphids. A review. *Journal of Asia-Pacific Entomology* **20**, 794–801 (2017).
63. D.-Q. Chen, C. B. Montllor, A. H. Purcell, Fitness effects of two facultative endosymbiotic bacteria on the pea aphid, Acyrthosiphon pisum, and the blue alfalfa aphid, A. kondoi. *Entomologia Experimentalis et Applicata* **95**, 315–323 (2000).
64. J. A. Russell, N. A. Moran, Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. *Proc. R. Soc. B* **273**, 603–610 (2006).
65. J. Perreau, N. A. Moran, Genetic innovations in animal–microbe symbioses. *Nature Reviews Genetics*, 1–17 (2021).
66. E. A. Mueller, N. I. Wisnoski, A. L. Peralta, J. T. Lennon, Microbial rescue effects: How microbiomes can save hosts from extinction. *Functional Ecology* **34**, 2055–2064 (2020).