



Still standing: The heat protection delivered by a facultative symbiont to its aphid host is resilient to repeated thermal stress

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ABSTRACT

Insects have evolved diverse strategies to resist extreme high temperatures (EHT). The adaptive value of such strategies has to be evaluated when organisms experience multiple EHT events during their lifetime, as predicted in a changing climate. This is particularly the case for associations with facultative microbial partners involved in insect heat tolerance, the resilience of which to repeated heat stress has never been studied. We compared two artificial lines of the pea aphid (*Acyrtosiphon pisum*) differing by the absence or presence of the heat-protective facultative bacterium *Serratia symbiotica*. We exposed insect nymphs to a varying number of EHT events (between 0 and 3), and recorded fitness parameters. Except survival traits, fitness estimates were affected by the interaction between aphid infection status (absence/presence of *S. symbiotica*) and thermal treatment (number of heat shocks applied). Costs of bacterial infection were detected in the absence of thermal stress: symbiont-hosting aphids incurred longer development, decreased fecundity and body size. However, symbiotic infection turned neutral, and even beneficial for some traits (development and body size), as the number of heat shocks increased, and compared to the aposymbiotic strain. Conversely, symbiotic infection mediated aphid response to heat shock(s): fitness decreased only in the uninfected group. These findings suggest that (i) the facultative symbiont may alternatively act as a pathogen, commensal or mutualist depending on thermal environment, and (ii) the heat protection it delivered to its host persists under frequent EHT. We discuss eco-evolutionary implications and the role of potentially confounding factors (stage-specific effects, genetic polymorphism displayed by the obligate symbiont).

1. Introduction

Extreme high temperatures (EHT) can be defined as temperatures high enough to exceed an organism's physiological limit and trigger a threshold-like biological response (Ma et al., 2021). Such thermal conditions pose a major ecological challenge to insects because of their limited ability to achieve internal thermal homeostasis as surrounding temperatures fluctuate (González-Tokman et al., 2020; Kingsolver et al., 2013; Ma et al., 2021). Indeed, insects usually incur an abrupt fitness drop when thermal conditions approach their critical thermal maximum (CT_{max}), the upper thermal limit an organism can stand before motor function is impaired and death occurs (Angilletta, 2009; Huey and Kingsolver, 1993). Thus, acute or prolonged exposure to EHT usually yields negative fitness outcomes taking the form of increased mortality rates, delayed development, lowered reproductive success, and altered phys-

iological state (Fischer et al., 2014; Iltis et al., 2021; Kingsolver et al., 2021; Sales et al., 2021; Zhang et al., 2015a).

To cope with the recurrent selective pressure exerted by EHT within naturally fluctuating thermal cycles, insects have evolved a series of adaptive mitigation strategies purported to limit the risks of overheating, death and population extinction (González-Tokman et al., 2020; Ma et al., 2021). First, insects can exploit microclimates through behavioral thermoregulation and avoid adverse temperature during EHT (Ma et al., 2018a; Woods et al., 2015). Second, acquisition of heat tolerance may rely on plastic responses (physiological acclimation) occurring over different timescales, from minutes (rapid hardening) to months (transgenerational acclimation) (Sgrò et al., 2016; Terblanche and Hoffmann, 2020). Third, insects can take advantage of ontogenetic variation in heat tolerance to buffer EHT, since their life stages often display contrasting heat tolerance traits (body size, mobility, acclimation capacities) (Bowler and Terblanche, 2008; Kingsolver and Buckley, 2020).

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Fourth, heat tolerance is itself an evolvable trait, but experimental and field reports indicate that CT_{max} displays a low evolutionary potential, and increased thermal tolerance might be an evolutionary traded-off with other fitness-related traits such as plastic responses to temperature (Buckley and Huey, 2016; Hoffmann et al., 2013; van Heerwaarden and Kellermann, 2020). Fifth, insects can mitigate the biological impacts of EHT through the resilience of their ecological networks, involving both trophic and non-trophic interactions (Clissold and Simpson, 2015; Lemoine et al., 2020).

One striking example about the importance of ecological interactions in mediating insect response to EHT is given by mutualistic associations forged with microorganisms (Corbin et al., 2017; Iltis et al., 2022; Lemoine et al., 2020). Some of these microbes are described as obligate because their presence is a prerequisite for host survival and reproductive success, and fulfill nutritive functions in insects feeding upon unbalanced diets (e.g. blood, sap and wood feeders) (Baumann, 2005; Douglas, 2009). The obligate microorganisms often endure rapid population decline under heat stress, eventually leading to a collapse of insect vital functions, sterility and premature death (Kikuchi et al., 2016; Shan et al., 2014; Zhang et al., 2019). Hence, the host reliance upon crucial services ensured by a heat-susceptible partner exacerbates the selective pressures exerted by EHT on insect thermal biology (Renoz et al., 2019; Wernegreen, 2012).

Conceivably, these evolutionary forces drove the spread of heat-protective facultative symbionts: microbes endowing their hosts with capabilities for tolerating heat stress, but acting as commensals or pathogens at permissive temperatures (Clavé et al., 2022; Iltis et al., 2022; Tougeron and Iltis, 2022). In aphids for instance, heat tolerance can be improved by the presence of non-essential bacteria protecting populations of the obligate symbiont *Buchnera aphidicola* from heat-related depletion (Heyworth et al., 2020; Montllor et al., 2002). Similar results were obtained in the whitefly *Bemisia tabaci* hosting the facultative bacterium *Rickettsia* sp., which favors the induction of host genes participating in stress physiological response (Brumin et al., 2011). In other invertebrates, such as *Caenorhabditis elegans* nematodes, associations with the bacterium *Bacillus subtilis* protects from heat shock and increase host fecundity (Hoang et al., 2021).

Even short-lived organisms like insects may be exposed to several bouts of EHT during their lifetime, particularly in a changing climatic context involving an increasing frequency of heat episodes (Kingsolver and Buckley, 2017; Ma et al., 2021). Insect fitness should decrease with the number of EHT events because of the accumulation of physiological damage carried over development (Kingsolver et al., 2021; Ma et al., 2018a; Tian et al., 2022; Zhang et al., 2015b). For instance, larvae of the tobacco hornworm *Manduca sexta* suffer greater fitness losses (extended development, diminished growth, decreased survival) when subjected to multiple bouts of heat stress relative to a single bout or unheated conditions (Kingsolver et al., 2021). However, insects can alleviate the detrimental effects of recurrent exposure to EHT by means of heat-protective strategies mentioned above.

Preliminary exposure to heat conditions can improve subsequent tolerance to EHT as part of acclimation responses underlying adaptive plasticity of upper thermal limits (increase of CT_{max}) (Kingsolver et al., 2016; Sorensen et al., 2019). Furthermore, the biological impacts of multiple episodes of EHT may not always be simply additive and rather depend on the number of heat shocks in a complex stage-dependent fashion (Kingsolver et al., 2021; Zhang et al., 2015a; Zhao et al., 2019). Although this body of knowledge documents the responses of isolated organisms to repeated EHT, the cumulative consequences of multiple heat events for biotic interactions mediating heat tolerance are much less understood, especially in insect-microbe mutualistic systems (Iltis et al., 2022). Such data would be valuable to further the mechanistic understanding of insect buffering strategies against frequent EHT, and shed light on the environmental drivers of insect-microbe associations in a climate-change context.

This study investigates the response of the host involved with a heat-protective mutualistic bacterium to a varying number of EHT events during insect development: none, one, two, or three exposure(s) to 35 °C for 2 h. We focus on the widely studied interaction between the pea aphid *Acyrtosiphon pisum* and the heat-protective facultative bacterium *Serratia symbiotica* (Montllor et al., 2002; Russell and Moran, 2006; Tougeron and Iltis, 2022), a naturally-occurring symbiosis. In this system, symbiont-mediated protection to heat presumably occurs following lysis of the cells in the population of *S. symbiotica* at EHT, which releases protective metabolites shielding *B. aphidicola* populations from heat-driven decline, resulting in considerable fitness gains for the aphid (Burke et al., 2010a; Montllor et al., 2002). Focusing on several aphid traits (survival, development, reproduction, morphology), we sought to examine the resilience of this association in face of a repeatedly occurring heat stress. Our experiments allowed to test two hypotheses. First, infection by *S. symbiotica* should mostly benefit the aphids when the organisms have been exposed to at least one thermal stress. Insects housing *S. symbiotica* should therefore outperform their uninfected counterparts if they have met at least one heat shock during their development, while the reverse pattern (or no differences) should be observed for individuals being held at non-stressful temperatures. Second, the fitness benefits provided by the symbiont should persist regardless of the number of EHT episodes applied if the system is resilient to multiple heat shocks (i.e. if *S. symbiotica* populations can partially or fully recover from massive cell lysis).

2. Material and methods

2.1. Study model and insect culture

Aphid infection status (absence or presence of *S. symbiotica*) was experimentally manipulated by comparing two clonal lines of *A. pisum* only differing by their facultative symbiont profile. These aphid colonies were reared on broad bean seedlings (*Vicia faba*, cultivar ‘Super-aquadulce’) under long-day abiotic conditions favoring continuous parthenogenetic reproduction (temperature: 19 ± 1 °C, relative humidity: 65 ± 5%, photoperiod: L16:D8 h). Before the experiments, molecular analyses (diagnostic PCRs) were performed to check the infection status of each aphid line, using the primers 16SA1 and PASSmp for the detection of *S. symbiotica*, along with formerly established reaction conditions (Renoz et al., 2015). Initially collected in June 1999 in Madison (WI, USA), the ‘5A’ line was used as an aposymbiotic control group, hosting the obligate symbiont *B. aphidicola* but free from any known facultative symbiont of aphids. As an infected group, the ‘5AR’ line was established in September 2000, following transfection of the ‘5A’ line with hemolymph extracted from a donor line harboring *S. symbiotica* and sampled in 1999 in Tucson (AZ, USA) (Oliver et al., 2003). Since the initial inoculation procedure, the association has evolved towards a stable equilibrium, where the microbe is internalized within specialized host cells dedicated to symbiont multiplication (bacteriocytes), and maternally inherited with perfect fidelity under permissive temperature conditions (Oliver et al., 2003; Russell and Moran, 2006). In addition, *S. symbiotica* retained high levels of protective abilities against heat within this particular combination of host and symbiont genetic lines, making this system an obvious case of heat-protective mutualism (Russell and Moran, 2006).

2.2. Experimental procedure and thermal treatments

For each line, apterous adults were transferred to aphid-free broad bean plants under the rearing conditions described above to produce nymphs used for the experiments (age: 0–24 h). These newly-born nymphs were reared individually using an agar-leaf method developed from Li and Akimoto (2018). Each individual was placed in a Petri dish (100 × 20 mm) with one freshly excised leaf of broad bean (plant age:

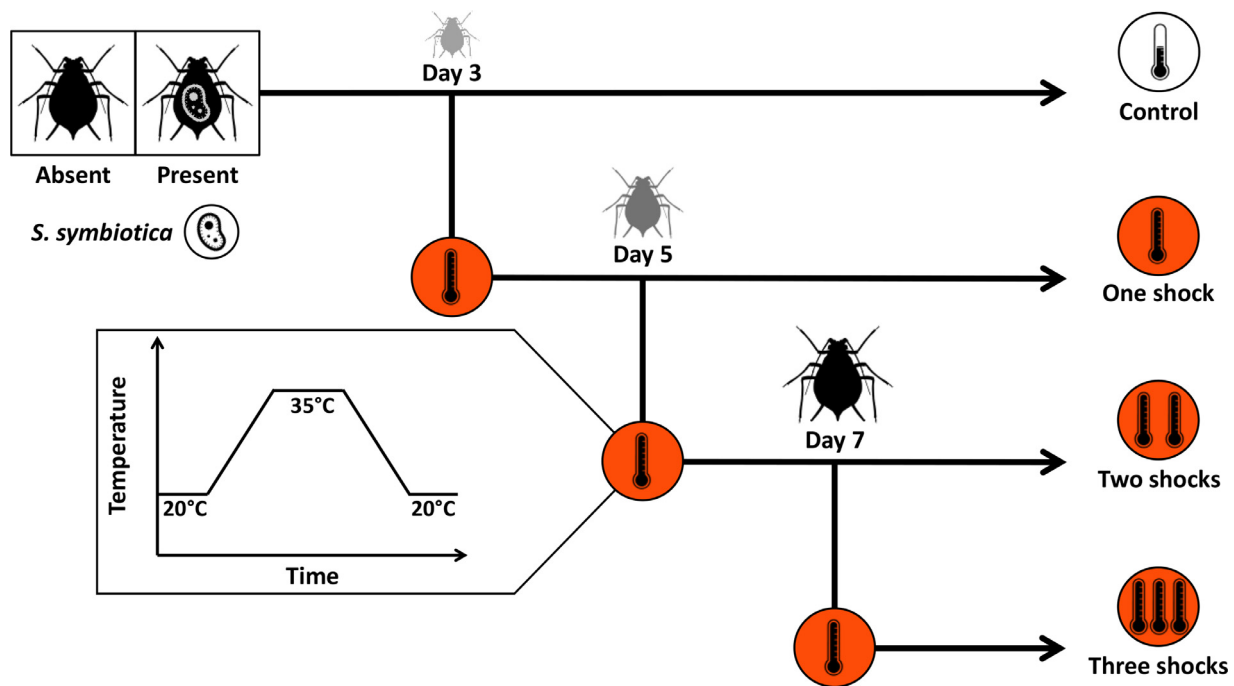


Fig. 1. Aphids from the two laboratory lines (either deprived of *S. symbiotica* or infected by this facultative symbiont) were randomly assigned to four thermal treatments differing by the number of heat shocks applied during insect immature development (red): none (control), one heat shock (applied on day 3), two heat shocks (applied on day 3 and day 5), or three heat shocks (applied on day 3, day 5 and day 7). One bout of heat shock involved three phases of 2 h each: one warming phase (20–35 °C), one plateau (35 °C) and one cooling phase (35–20 °C). Aphids were maintained at 20 °C apart from heat shock periods.

2–4 weeks) placed underside facing on the surface of a thin layer of 2% agar substrate (Intra-Laboratories Ltd., Plymouth, The United Kingdom). This method is space-saving and enable high levels of plant standardization and experimental replication. In addition, it is particularly suitable for the experimental rearing of *A. pisum*, since insects developing on agar-leaf systems display the same performance as those being grown on full plants (Li and Akimoto, 2018). To ensure consistent provisioning with fresh, high-quality food and avoid leaf degradation over time or arising from exposure to EHT, aphids were transferred to new experimental units either (i) every 7 days or (ii) immediately following heat shock application.

Aphids of the two clonal lines were randomly distributed among four different thermal treatments, leading to a total of eight experimental blocks. All thermal treatments were applied within climate chambers (KKS 115, POL-EKO-Aparatura, Wodzisław Śląski, Poland) controlling for temperature (± 0.1 °C) and standardizing the other abiotic variables (relative humidity: $50 \pm 5\%$, photoperiod: L16:D8 h). For the control thermal treatment (no heat shock applied), aphids were held at non-stressful constant thermal conditions (20 °C) during their whole immature development and adult lifetime. The other thermal treatments included one, two or three bout(s) of EHT applied at different aphid ages (nymphal instars). Thus, aphids assigned to the ‘one heat shock’ group were exposed to EHT on day 3 (second-instar nymphs). Those assigned to the ‘two heat shocks’ group were exposed to EHT on day 3 and day 5 (second- and third-instar nymphs). Finally, individuals assigned to the ‘three heat shocks’ group were exposed to EHT on day 3, day 5 and day 7 (second-, third- and fourth-instar nymphs) (Fig. 1). During one heat shock, temperature was gradually increased from 20 °C to 35 °C over 2 h, maintained at 35 °C for an additional 2 h, and gradually decreased from 35 °C to 20 °C over 2 h (Fig. 1). Insects were placed at control conditions (20 °C) apart from heat shock periods. We did not expect acute exposure to 35 °C to be lethal for aphids. Stress intensity (maximal temperature) and duration were calibrated on the basis on former experimental works reporting that similar conditions applied during aphid nymphal life can trigger the lysis mechanisms at the origins of the heat protection de-

livered by *S. symbiotica* to its host (Burke et al., 2010a; Montllor et al., 2002; Russell and Moran, 2006).

2.3. Aphids fitness traits

Measurements of aphid fitness parameters were performed following a daily monitoring of experimental units. Aphid traits included a set of survival, developmental, reproductive and morphological correlates of fitness, reflecting the lethal and sublethal consequences of exposure to heat. As survival traits, we recorded immature survival rate (fraction of nymphs that reached adult reproductive period) and aphid total longevity, from birth to death. As developmental trait, we considered immature development time, expressed as the time elapsed before first larviposition (in days). As reproductive trait, we quantified lifetime fecundity: the cumulative number of offspring produced by each individual throughout its reproductive period. During each day of survey, freshly laid nymphs were removed to minimize plant degradation caused by herbivory pressure. As morphological trait, we estimated adult body size, approximated by measuring hind tibia length *post-mortem* with a stereomicroscope (S APO Stereo Zoom Microscope, 215 Leica Microsystems, Wetzlar, Germany) and a 12.6 x magnification (precision: ± 0.01 mm). Alate (winged) aphids (7.5% of the dataset) were discarded for fitness assays, because wing polymorphism occurs alongside profound physiological and morphological changes, resulting in contrasting life histories (Braendle et al., 2006; Zera and Denno, 1997). With the exception of immature survival rates (lethal effects of heat stress), all traits were measured for individuals successfully surviving until the adult stage and reproduction.

2.4. Statistical analyses

All statistical models included the effect of aphid infection status (absence or presence of *S. symbiotica*), thermal treatment (number of heat shocks applied) and their interaction on the array of aphid fitness traits listed above. We chose to model thermal treatment as a factor with four

Table 1

Summary of the statistical analyses investigating the effects of aphid infection status (absence (A) or presence (P) of *S. symbiotica*), thermal treatment (number of heat shocks (HS) applied: none (control), one, two, or three) and their interaction on aphid fitness traits. Post-hoc differences underlying interactive effects are shown. Subscripts provided with statistics indicate the degrees of freedom of the corresponding tests. Bold values outline significant effects ($P < 0.05$).

	Aphid infection status		Thermal treatment		Interaction		Post-hoc differences
	Test value	P	Test value	P	Test value	P	
Immature survival rate ^a	$\chi^2_1 = 12.7$	< 0.001	$\chi^2_3 = 2.44$	0.49	$\chi^2_3 = 0.84$	0.84	NA
Total longevity ^b	$\chi^2_1 = 2.95$	0.08	$\chi^2_3 = 3.19$	0.36	$\chi^2_3 = 0.51$	0.92	NA
Immature development time ^c	$\chi^2_1 = 0.07$	0.04	$\chi^2_3 = 0.35$	< 0.001	$\chi^2_3 = 0.29$	< 0.001	$P < A$ (control) $P > A$ (3 HS) $P < A$ (control or 1 HS)
Lifetime fecundity ^d	$F_{1,237} = 5.15$	0.02	$F_{3,237} = 1.54$	0.20	$F_{3,237} = 5.07$	< 0.001	
Hind tibia length ^c	$\chi^2_1 = 10^{-5}$	0.97	$\chi^2_3 = 0.46$	< 0.001	$\chi^2_3 = 0.16$	< 0.001	$P < A$ (control) $P > A$ (2 or 3 HS)

^a GLM binomial error structure.

^b Cox proportional hazard regression.

^c GLM Gamma error structure.

^d ANOVA.

modalities instead of a covariate to take into account potential ontogenetic thermal effects in our experimental design (Kingsolver et al., 2021). The underlying assumption is that the fitness effect of temperature treatment should not be solely expressed as a function of the number of heat shocks applied, because of (i) differences in heat susceptibility between insect life stages and (ii) interactive effects between the thermal conditions successively encountered by these different life stages (Kingsolver et al., 2021; Zhang et al., 2015a; Zhao et al., 2019). Linear models (LMs) or generalized linear models (GLMs) were fitted depending on the nature of the data, their residual variance and distribution. Immature survival rate was compared between the different groups with a GLM assuming a binomial error structure and a logit link function. Total longevity was analyzed as a survival probability function of time since birth within a Cox proportional hazard regression (package 'survival', Therneau, 2021). Both immature development time and hind tibia length displayed heteroskedastic variances and were processed through GLMs assuming a Gamma error structure and an inverse link function. Lifetime fecundity data met both the homoskedasticity and normality criteria, allowing to run a linear model with analysis of variance (ANOVA). Differences among groups underlying interactive effects were teased out with post hoc tests based on contrasts among estimated marginal means (package 'lsmeans', Lenth and Lenth, 2018). A Bonferroni correction was applied to account for the multiplication of type I error risks. Assumptions for LMs (homoskedasticity and normality) were evaluated through visual inspection of the residuals of the fitted models. All statistical analyses were conducted with R software version 4.2.1.

2.5. Data availability

Raw data of this study has been made publicly available on Zenodo at DOI: 10.5281/zenodo.6778025

3. Results

3.1. Survival traits

Immature survival rate was affected by the simple effect of aphid infection status, but neither by the thermal treatment nor by the interaction between these two factors (Table 1). Regardless of temperature, aphids hosting *S. symbiotica* were more likely to survive until adulthood than their uninfected counterparts. Mean values of immature survival rate and their bootstrapped 95% confidence intervals (CI_{95%}) were 82.8% for uninfected aphids (CI_{95%} = [76.3; 89.3], $n = 82$) and 95.7% for infected aphids (CI_{95%} = [92.3; 99.1], $n = 95$). Total longevity was not affected by any of the predictor variables included in the model (Table 1).

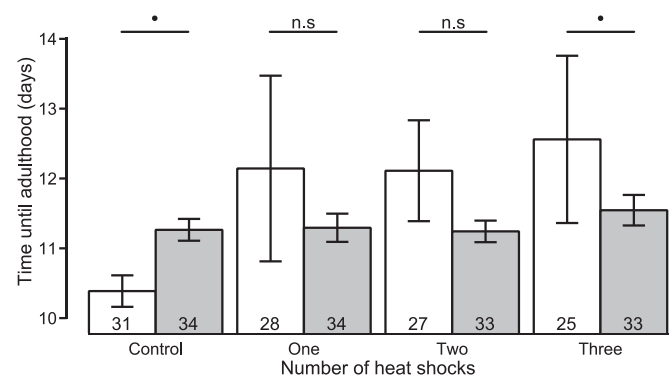


Fig. 2. Effects of infection status (white: absence of *S. symbiotica*, gray: presence of *S. symbiotica*) and thermal treatment (defined by the number of heat shocks applied during insect nymphal stages) on aphid immature development time. Represented values are means and their 95% confidence intervals. Numbers indicate sample sizes and symbols highlight marginally significant differences (* $P < 0.1$, n.s. non-significant).

3.2. Developmental trait

Immature development time was affected by the interaction between aphid infection status and thermal treatment, implying that the fitness outcomes of symbiotic infection hinged on temperatures experienced by aphids as nymphs, or conversely that infection status influenced aphid response to temperature shock(s) (Table 1, Fig. 2). Regarding infection effect, aphids hosting *S. symbiotica* tended to have a longer development time than uninfected individuals under control temperature conditions (+9%) ($z = 2.39$, $P = 0.07$). However, these infection costs were no longer apparent when insects had been exposed to one ($z = -2.09$, $P = 0.15$) or two heat shock(s) ($z = -2.11$, $P = 0.14$). Most interesting, the developmental difference between the two aphid lines tended to reverse under the most stressful conditions (three heat shocks): aphids infected by *S. symbiotica* tended to develop faster than uninfected individuals (-9%) ($z = -2.34$, $P = 0.08$). Regarding temperature effect, uninfected aphids had delayed development when exposed to at least one thermal shock relative to control conditions, regardless of the number of heat shocks applied (between +16% and +21%) (one heat shock: $z = 4.39$, $P < 0.001$, two heat shocks: $z = 4.28$, $P < 0.001$, three heat shocks: $z = 5.19$, $P < 0.001$). By contrast, development remained fairly constant across all thermal treatments within the infected aphid group, as no significant difference was spotted by post hoc tests.

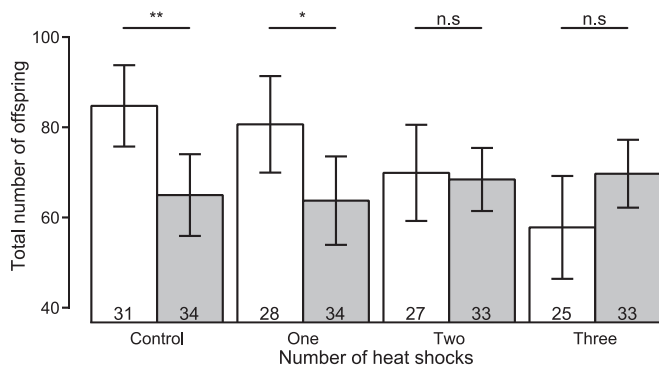


Fig. 3. Effects of infection status (white: absence of *S. symbiotica*, gray: presence of *S. symbiotica*) and thermal treatment (defined by the number of heat shocks applied during insect nymphal stages) on aphid lifetime fecundity. Represented values are means and their 95% confidence intervals. Numbers indicate sample sizes and symbols highlight significant differences (** $P < 0.01$, * $P < 0.05$, n.s. non-significant).

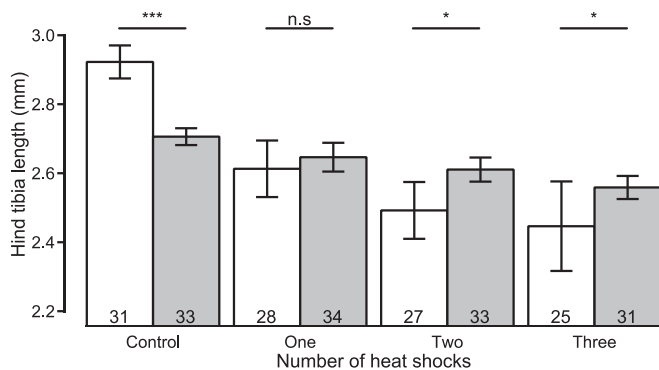


Fig. 4. Effects of infection status (white: absence of *S. symbiotica*, gray: presence of *S. symbiotica*) and thermal treatment (defined by the number of heat shocks applied during insect nymphal stages) on aphid body size. Represented values are means and their 95% confidence intervals. Numbers indicate sample sizes and symbols highlight significant differences (*** $P < 0.001$, * $P < 0.05$, n.s. non-significant).

3.3. Reproductive trait

Lifetime fecundity was affected by the interaction between aphid infection status and thermal treatment (Table 1, Fig. 3). Regarding infection effect, aphids hosting *S. symbiotica* produced less nymphs than uninfected individuals under control conditions (−23%) ($t = -3.15$, $P = 0.01$) and when exposed to one single heat shock (−21%) ($t = -2.62$, $P = 0.04$). However, these infection costs did not persist when insects were subjected to multiple heat shocks (two heat shocks: $t = -0.22$, $P = 1.00$, three heat shocks: $t = 1.78$, $P = 0.31$). Regarding temperature effect, uninfected aphids suffered from decreased reproductive output when exposed to three heat shocks relative to control conditions (−32%) ($t = -3.97$, $P < 0.001$) and one heat shock (−28%) ($t = -3.29$, $P = 0.01$). By contrast, reproduction of infected aphids was not affected by thermal treatment.

3.4. Morphological traits

Body size (hind tibia length) was affected by the interaction between aphid infection status and thermal treatment (Table 1, Fig. 4). Regarding infection effect, infected aphids grew smaller than their uninfected counterparts under control conditions (−7%) ($z = -4.74$, $P < 0.001$). However, this size difference disappeared when insects had been exposed to one heat shock ($z = 0.77$, $P = 1.00$), and infected aphids grew eventually

larger than uninfected individuals in the event of multiple heat shocks (between +4% and +5%) (two heat shocks: $z = 2.75$, $P = 0.02$, three heat shocks: $z = 2.57$, $P = 0.04$). Regarding temperature effect, uninfected aphids grew smaller when exposed to at least one heat shock relative to control conditions (between −11% and −16%) (one heat shock: $z = -6.59$, $P < 0.001$, two heat shocks: $z = -9.23$, $P < 0.001$, three heat shocks: $z = -10.0$, $P < 0.001$). Moreover, uninfected aphids exposed to three heat shocks grew smaller than those exposed to one heat shock (−6%) ($z = -3.68$, $P < 0.001$). By contrast, for infected aphids, the only difference in body size was found between individuals exposed to three heat shocks and those assigned to the control group: the former grew smaller than the latter (−5%) ($z = -3.44$, $P = 0.01$).

4. Discussion

How insects can keep pace with increasingly frequent heat spells through diverse adaptive strategies is particularly pressing to tackle given the ongoing evolution of worldwide climate and escalating temperatures (Kingsolver et al., 2021; Ma et al., 2018b, 2021). This work focused on a relatively understudied yet widespread strategy employed by many insect species to resist thermal fluctuations leading to EHT: to associate with facultative symbionts bestowing heat protection (Corbin et al., 2017; Hector et al., 2022; Itlis et al., 2022; Lemoine et al., 2020). The objective was to evaluate how infection by a heat-protective facultative symbiont (the bacterium *S. symbiotica*) may affect the performance of its host (the pea aphid *A. pisum*) when exposed to an experimentally manipulated number of heat shocks during nymphal life.

Except survival traits, the different fitness estimates (development, reproduction, morphology) were affected by the interplay between symbiotic infection and temperature. This means that the number of heat shocks influenced the fitness outcomes of infection (costs and benefits for the insect), and that symbiotic infection in turn shaped aphid fitness responses to (repeated) EHT. More specifically, various infection costs were observed under conducive control conditions (delayed development, reduced reproductive output, decreased body size). However, most of these infection costs were no longer apparent when insect nymphs had been exposed to at least one heat shock. Most interesting, symbiotic infection yielded either neutral or beneficial fitness outcomes in the event of multiple heat shocks, depending on the trait considered, and compared to the aposymbiotic aphid strain. This occurred because fitness generally decreased with the number of heat shocks in the uninfected aphid group, while it remained fairly constant in the infected aphid group. In this sense, we consider that the beneficial (protective) effect is mainly to be interpreted as the comparison between the two strains for each temperature regime tested, not only as the response of the infected strain which, for some traits, does not vary much between treatments. Thus, far from fading, the heat protection granted by this strain of *S. symbiotica* turned out to be resilient to several heat shocks. This could be either because *S. symbiotica* population is resilient to heat shocks, and/or that the protective effect on *B. aphidicola* is long lasting.

Why facultative symbioses fail to reach fixation within natural insect populations and what ecological forces may drive symbiont prevalence dynamics are two questions spurring massive research interest until recently (Oliver et al., 2014; Smith et al., 2021, 2015; Zytynska et al., 2021). Our study provides new experimental proof that a facultative endosymbiont may alternatively act as a mutualist, a commensal or a pathogen towards its insect host. In addition, we found that temperatures were a key determinant of such symbiotic effects, matching the conclusions established by former experimental works (Clavé et al., 2022; Heyworth et al., 2020; Montllor et al., 2002; Russell and Moran, 2006) and a recent meta-analysis building on these empirical knowledge (Tougeron and Itlis, 2022). This variability of biological effects can be understood as the output of a cost-benefit fitness balance arising from symbiotic infection, and tipping in a costly or a beneficial state depending on ecological contingency (Bronstein, 1994; Zytynska et al., 2021). Sublethal fitness costs caused by symbiont

maintenance are inherent to many forms of insect-microbe mutualism (Ankrah et al., 2018). However, these injuries can be outweighed by benefits provided through symbiont-ensured services (here heat protection) under specific ecological contexts. One could argue that the pathogenic effect of *S. symbiotica* had reduced host fitness to such an extent that sub-lethal heat stresses had no further impact. However, this hypothesis is quite unlikely because of known heavy effects of heat stress in host-symbiont systems (Ittis et al., 2022) and because for most traits in our study, performance was negatively affected when not carrying symbiont. In any case, the net fitness benefits observed under frequent heat stress corroborate field investigations showing that *S. symbiotica* tends to spread within aphid populations as local temperatures rise, leading to increased infection frequency in time or space (Henry et al., 2013; Montllor et al., 2002; Pons et al., 2022). This body of knowledge indicates that heat waves might constitute a major driver of the prevalence dynamics of this heat-protective symbiont in natural environments. However, further field and experimental data (pertaining to other aphid and symbiont genotypes) should be required to validate this statement. Indeed, many covarying factors such as local coevolutionary dynamics between host and symbiont, host plant diversity or parasitoid abundance might affect symbiont cost-benefit balance and prevalence trajectories in the field (Rock et al., 2018; Smith et al., 2021; Zytynska et al., 2016).

The results showing that the performance of infected aphids did not decline with the number of heat shocks are instructive for the understanding of insect-symbiont interactions at EHT. It adds extra information to the literature exploring the response of these associations to one single heat burst, in various systems (Brumin et al., 2011; Burdina et al., 2021; Heyworth et al., 2020). The aphid-*Serratia* model is particularly relevant to study in the context of chronic heat stress, because the heat protection relies on metabolites released upon symbiont cell lysis (Burke et al., 2010a). This raises the question about the potential for microbial population surviving and rebuilding from massive cell lysis between successive heat shocks. Populations of aphid endosymbiotic bacteria can fluctuate over days, underlying high responsiveness to temperature variations (Burke et al., 2010a; Doremus et al., 2018; Heyworth et al., 2020). One could speculate that symbiont populations (and subsequent heat protection) are not fully eliminated by several heat shocks and there is total or partial population replenishment between two hot events, or that heat-protective metabolites persist over time. These hypotheses now require quantifications of symbiont abundances during aphid development and their relation with EHT.

Through an eco-evolutionary lens, this study suggests that infection by a facultative symbiont can improve an insect ability to withstand multiple heat shocks, with interesting implications for host persistence in face of climate change. This matches evolutionary scenarios predicting that heat-protective symbionts could become an increasingly prevalent component of future insect populations, by propagating through positive directional selection of infected individuals (Renoz et al., 2019; Tougeron and Ittis, 2022). Again, this evolutionary scenario is based on the performance comparison between aposymbiotic and symbiont-bearing aphid strains, under various thermal contexts (i.e., symbiont infection does not increase aphid performance in absolute terms at stressful temperatures, but the negative impact of infection is relatively smaller or the ratio to aposymbiotics reverses with increasing EHT exposure). Despite the fitness costs of carrying bacterial symbionts in benign temperature conditions, the advantages that the association provides under heat stress may have allowed—and will likely allow—the maintenance of such relationships through selection (Tougeron and Ittis, 2022). However, the evolutionary impacts of facultative symbionts on their host will also depend on their ability to associate durably with the insect through vertical transmission, which can be impeded at high temperatures (Chang et al., 2022; Corbin et al., 2017; Dykstra et al., 2014). We also emphasize that infection status should be a major parameter to consider when making eco-evo projections. For instance, if development time is reduced in infected aphid lines after multiple heat shocks,

this could result in increasing voltinism to account for in phenological models in the context of climate change. Long-term experimental studies examining the evolution of symbiont prevalence and phenotypic effects throughout insect generations should be valuable to examine the stability of the interaction and account for the evolutionary potential of insect or other hosts and microbial protagonists. In *C. elegans* nematodes evolved under heat stress for 20 host generations, selection favored strains bearing the bacterium *B. subtilis* because hosts exhibited the greatest fitness increase (Hoang et al., 2021). Such findings strengthen the importance of the context-dependency of beneficial host-bacteria evolution, potentially leading to mutualism over the long term.

Our experimental design does not completely rule out stage-specific effects (because insects were exposed to heat at different nymphal instars depending on thermal treatment), even though this study was not purported to precisely identify such effects from a mechanistic viewpoint. Variation in basal heat tolerance between aphid life stages can affect the heat response of the aphid-*Serratia* system (Montllor et al., 2002; Russell and Moran, 2006). An accumulating number of experimental studies have shown that nymphal heat tolerance increases as the aphids get closer to their sexual maturity (Chen et al., 2021; Zhao et al., 2019, 2017). Similar conclusions were obtained for individuals hosting *S. symbiotica* (Montllor et al., 2002), which can be caused by a progressive increase in densities of the heat-protective symbiont with aphid age (Laughton et al., 2014). When connecting these observations to our study, it appears that aphids in the different heat treatments received a significant dose of stress as they were all exposed to EHT during one early heat-susceptible stage (second-instar nymphs). This is in line with our results showing that aphid fitness decreased after one heat shock, especially in the uninfected group. However, ontogenic variation in aphid heat tolerance poorly explains why the exposure to several heat shocks during later nymphal instars (presumably the most tolerant ones) resulted in the lowest fecundity and body size among the different thermal treatments. In an elegant study controlling for both the number of heat shocks and the developmental timing of these hot events, Kingsolver et al. (2021) found that the fitness effects of heat stress occurring at different stages were either additive or interactive depending on the aphid trait. Thus, successive EHT episodes may sometimes produce complex, counterintuitive biological responses compared with predictions based on ontogenic heat tolerance. A promising research lead would lie in examining how developmental effects could alter responses to heat in aphids infected by *S. symbiotica*. Future works should consider the relation between heat tolerance and the different steps of symbiogenesis (establishment of microbial populations) during aphid development, as recently done in coral-based mutualistic systems (Ruggeri et al., 2022).

Another key player influencing aphid responses to EHT is the obligate nutrient-provisioning symbiont *B. aphidicola*. Repetitive heat shocks may progressively decrease *B. aphidicola* populations and cause aphid sterility or impact the next insect generation, since neonates are endowed with fewer symbionts from maternal origin (Parven et al., 2021). Our results could thus be explained by variation in *B. aphidicola* abundances and provision of vital functions, for both uninfected and infected aphids. Indeed, *S. symbiotica* prevents *B. aphidicola* populations and aphid fitness from collapsing under heat conditions (Burke et al., 2010a; Montllor et al., 2002). In addition, *B. aphidicola* displays genetic variability affecting the promoter of the *ibpA* gene encoding molecules involved in heat stress response, which may ultimately affect aphid fitness under heat conditions (Tougeron and Ittis, 2022). After a genetic screening of insect cultures, we noticed that the *Serratia*-uninfected stock fixed the mutated, less functional allelic version of the promoter in *B. aphidicola* genotype, which attenuates symbiont transcriptional response and exacerbates aphid susceptibility to heat stress ($\geq 35^\circ\text{C}$) (Dunbar et al., 2007; Zhang et al., 2019). By contrast, this mutation did not arise in the aphid stock hosting *S. symbiotica*. This is congruent with other studies showing that the mutation (i) tends to occur repeatedly during the laboratory maintenance of aposymbiotic aphid clones;

and (ii) associates poorly with facultative symbionts, as witnessed by rare events of allelic substitution in infected laboratory and wild populations (Burke et al., 2010b; Dunbar et al., 2007). This genetic polymorphism could generate confounding effects in our study, by further increasing the heat vulnerability of aphids not hosting the facultative symbiont. However, the magnitude of the fitness effect of heat stress we observed was to no point comparable to the literature dealing with the *ibpA* mutation, which might cause dramatic mortality (40%) and sterility rates (80%) among aphids after nymphal exposition to EHT (Dunbar et al., 2007; Zhang et al., 2019). Even in the most stressful conditions (three exposures to 35 °C), we noticed that mortality rates remained relatively low in the uninfected group (<25%), and no individual failed to reproduce. The fitness consequences of *ibpA* genetic polymorphism could depend on various factors (e.g. aphid genetic background, heat stress intensity and temporal pattern) (Dunbar et al., 2007; Moran and Yun, 2015; Tougeron and Iltis, 2022; Zhang et al., 2019). It is also plausible that the progressive decrease of *B. aphidicola* populations with the number of heat shocks applied could minimize the importance of these symbiont genetic features for aphid fitness (Parven et al., 2021). Understanding the fitness implications of the *ibpA* mutation in diverse ecological and genetic contexts would definitely deserve increased recognition to thoroughly elucidate the ability of the tripartite system (aphid, obligate and facultative symbionts) to cope with frequent EHT.

To conclude, our study suggests that (i) fluctuating temperatures extending to EHT can reshape the cost-benefit balance of hosting a facultative symbiont, (ii) the heat protection granted by the symbiont is resilient to repeated EHT, and (iii) our results open up interesting research perspectives about factors susceptible to mediate the response of the symbiotic system to EHT.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Kévin Tougeron: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Corentin Iltis:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Eliott Rampnoux:** Conceptualization, Methodology, Formal analysis, Investigation, Visualization. **Alexandre Goerlinger:** Methodology, Investigation. **Linda Dhondt:** Methodology, Investigation. **Thierry Hance:** Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability

Raw data of this study have been made publicly available on Zenodo at DOI: 10.5281/zenodo.6778025

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References

- Angilletta, M.J., 2009. Thermal Adaptation: a Theoretical and Empirical Synthesis. Oxford University Press, Oxford doi:[10.1093/acprof:oso/9780198570875.001.1](https://doi.org/10.1093/acprof:oso/9780198570875.001.1).
- Ankrah, N.Y.D., Chouaia, B., Douglas, A.E., 2018. The cost of metabolic interactions in symbioses between insects and bacteria with reduced genomes. *MBio* 9, doi:[10.1128/mBio.01433-18](https://doi.org/10.1128/mBio.01433-18), e01433-18.
- Baumann, P., 2005. Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annu. Rev. Microbiol.* 59, 155–189. doi:[10.1146/annurev.micro.59.030804.121041](https://doi.org/10.1146/annurev.micro.59.030804.121041).
- Bowler, K., Terblanche, J.S., 2008. Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? *Biol. Rev.* 83, 339–355. doi:[10.1111/j.1469-185X.2008.00046.x](https://doi.org/10.1111/j.1469-185X.2008.00046.x).
- Braendle, C., Davis, G.K., Brisson, J.A., Stern, D.L., 2006. Wing dimorphism in aphids. *Heredity (Edinb)* 97, 192–199. doi:[10.1038/sj.hdy.6800863](https://doi.org/10.1038/sj.hdy.6800863).
- Bronstein, J.L., 1994. Conditional outcomes in mutualistic interactions. *Trends Ecol. Evol.* 9, 214–217. doi:[10.1016/0169-5347\(94\)90246-1](https://doi.org/10.1016/0169-5347(94)90246-1).
- Brumin, M., Kotsedavlov, S., Ghanim, M., 2011. *Rickettsia* influences thermotolerance in the whitefly *Bemisia tabaci* B biotype. *Insect Sci.* 18, 57–66. doi:[10.1111/j.1744-7917.2010.01396.x](https://doi.org/10.1111/j.1744-7917.2010.01396.x).
- Buckley, L.B., Huey, R.B., 2016. How extreme temperatures impact organisms and the evolution of their thermal tolerance. *Integr. Comp. Biol.* 56, 98–109. doi:[10.1093/ICB/ICW004](https://doi.org/10.1093/ICB/ICW004).
- Burdina, E.V., Bykov, R.A., Menshanov, P.N., Ilinsky, Y.Y., Gruntenko, N., 2021. Unique *Wolbachia* strain wMelPlus increases heat stress resistance in *Drosophila melanogaster*. *Arch. Insect Biochem. Physiol.* 106, e21776. doi:[10.1002/arch.21776](https://doi.org/10.1002/arch.21776).
- Burke, G.R., Fiehn, O., Moran, N.A., 2010a. Effects of facultative symbionts and heat stress on the metabolome of pea aphids. *ISME J.* 4, 242–252. doi:[10.1038/ismej.2009.114](https://doi.org/10.1038/ismej.2009.114).
- Burke, G.R., McLaughlin, H.J., Simon, J.C., Moran, N.A., 2010b. Dynamics of a recurrent *Buchnera* mutation that affects thermal tolerance of pea aphid hosts. *Genetics* 186, 367–372. doi:[10.1534/genetics.110.117440](https://doi.org/10.1534/genetics.110.117440).
- Chang, C.Y., Sun, X.W., Tian, P.P., Miao, N.H., Zhang, Y.L., Liu, X.D., 2022. Plant secondary metabolite and temperature determine the prevalence of *Arsephenophus* endosymbionts in aphid populations. *Environ. Microbiol.* 24, 3764–3776. doi:[10.1111/1462-2920.15929](https://doi.org/10.1111/1462-2920.15929).
- Chen, Y., Quan, Y., Verheggen, F., Wang, Z., Francis, F., He, K., 2021. Differential thermal tolerance across life stages under extreme high temperatures crossed with feeding status in corn leaf aphid. *Ecol. Entomol.* 46, 533–540. doi:[10.1111/EEN.12998](https://doi.org/10.1111/EEN.12998).
- Clavé, C., Sugio, A., Morlière, S., Pincebourde, S., Simon, J.C., Foray, V., 2022. Physiological costs of facultative endosymbionts in aphids assessed from energy metabolism. *Funct. Ecol.* 36, 2580–2592. doi:[10.1111/1365-2435.14157](https://doi.org/10.1111/1365-2435.14157).
- Clissold, F.J., Simpson, S.J., 2015. Temperature, food quality and life history traits of herbivorous insects. *Curr. Opin. Insect Sci.* 11, 63–70. doi:[10.1016/J.COIS.2015.10.011](https://doi.org/10.1016/J.COIS.2015.10.011).
- Corbin, C., Heyworth, E.R., Ferrari, J., Hurst, G.D.D., 2017. Heritable symbionts in a world of varying temperature. *Heredity (Edinb)* 118, 10–20. doi:[10.1038/hdy.2016.71](https://doi.org/10.1038/hdy.2016.71).
- Doremus, M.R., Smith, A.H., Kim, K.L., Holder, A.J., Russell, J.A., Oliver, K.M., 2018. Breakdown of a defensive symbiosis, but not endogenous defences, at elevated temperatures. *Mol. Ecol.* 27, 2138–2151. doi:[10.1111/mec.14399](https://doi.org/10.1111/mec.14399).
- Douglas, A.E., 2009. The microbial dimension in insect nutritional ecology. *Funct. Ecol.* 23, 38–47. doi:[10.1111/j.1365-2435.2008.01442.x](https://doi.org/10.1111/j.1365-2435.2008.01442.x).
- Dunbar, H.E., Wilson, A.C.C., Ferguson, N.R., Moran, N.A., 2007. Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. *PLoS Biol.* 5, e96. doi:[10.1371/journal.pbio.0050096](https://doi.org/10.1371/journal.pbio.0050096).
- Dykstra, H.R., Weldon, S.R., Martinez, A.J., White, J.A., Hopper, K.R., Heimpel, G.E., Asplen, M.K., Oliver, K.M., 2014. Factors limiting the spread of the protective symbiont *Hamiltonella defensa* in *Aphis craccivora* aphids. *Appl. Environ. Microbiol.* 80, 5818–5827. doi:[10.1128/AEM.01775-14](https://doi.org/10.1128/AEM.01775-14).
- Fischer, K., Klockmann, M., Reim, E., 2014. Strong negative effects of simulated heat waves in a tropical butterfly. *J. Exp. Biol.* 217, 2892–2898. doi:[10.1242/jeb.106245](https://doi.org/10.1242/jeb.106245).
- González-Tokman, D., Córdoba-Aguilar, A., Dáttilo, W., Lira-Noriega, A., Sánchez-Guillén, R.A., Villalobos, F., 2020. Insect responses to heat: physiological mechanisms, evolution and ecological implications in a warming world. *Biol. Rev.* 95, 802–821. doi:[10.1111/brv.12588](https://doi.org/10.1111/brv.12588).
- Hector, T.E., Hoang, K.L., Li, J., King, K.C., 2022. Symbiosis and host responses to heating. *Trends Ecol. Evol.* 37, 611–624. doi:[10.1016/J.TREE.2022.03.011](https://doi.org/10.1016/J.TREE.2022.03.011).
- Henry, L.M., Peccoud, J., Simon, J.C., Hadfield, J.D., Maiden, M.J.C., Ferrari, J., Godfray, H.C.J., 2013. Horizontally transmitted symbionts and host colonization of ecological niches. *Curr. Biol.* 23, 1713–1717. doi:[10.1016/j.cub.2013.07.029](https://doi.org/10.1016/j.cub.2013.07.029).
- Heyworth, E.R., Smee, M.R., Ferrari, J., 2020. Aphid facultative symbionts aid recovery of their obligate symbiont and their host after heat stress. *Front. Ecol. Evol.* 8, 56. doi:[10.3389/fevo.2020.00056](https://doi.org/10.3389/fevo.2020.00056).
- Hoang, K.L., Gerardo, N.M., Morran, L.T., 2021. Association with a novel protective microbe facilitates host adaptation to a stressful environment. *Evol. Lett.* 5, 118–129. doi:[10.1002/evl3.223](https://doi.org/10.1002/evl3.223).
- Hoffmann, A.A., Chown, S.L., Clusella-Trullas, S., 2013. Upper thermal limits in terrestrial ectotherms: how constrained are they? *Funct. Ecol.* 27, 934–949. doi:[10.1111/J.1365-2435.2012.02036.X](https://doi.org/10.1111/J.1365-2435.2012.02036.X).
- Huey, R.B., Kingsolver, J.G., 1993. Evolution of resistance to high temperature in ectotherms. *Am. Nat.* 142, S21–S46. doi:[10.1086/285521](https://doi.org/10.1086/285521).

- Ittis, C., Louâpre, P., Vogelweith, F., Thiéry, D., Moreau, J., 2021. How to stand the heat? Post-stress nutrition and developmental stage determine insect response to a heat wave. *J. Insect Physiol.* 131, 104214. doi:10.1016/J.JINSPHYS.2021.104214.
- Ittis, C., Tougeron, K., Hance, T., Louâpre, P., Foray, V., 2022. A perspective on insect-microbe holobionts facing thermal fluctuations in a climate-change context. *Environ. Microbiol.* 24, 18–29. doi:10.1111/1462-2920.15826.
- Kikuchi, Y., Tada, A., Musolin, D.L., Hari, N., Hosokawa, T., Fujisaki, K., Fukatsu, T., 2016. Collapse of insect gut symbiosis under simulated climate change. *MBio* 7. doi:10.1128/mBio.01578-16. e01578-16.
- Kingsolver, J.G., Buckley, L.B., 2020. Ontogenetic variation in thermal sensitivity shapes insect ecological responses to climate change. *Curr. Opin. Insect Sci.* 41, 17–24. doi:10.1016/J.COIS.2020.05.005.
- Kingsolver, J.G., Buckley, L.B., 2017. Quantifying thermal extremes and biological variation to predict evolutionary responses to changing climate. *Philos. Trans. R. Soc. B Biol. Sci.* 372, 20160147. doi:10.1098/RSTB.2016.0147.
- Kingsolver, J.G., Diamond, S.E., Buckley, L.B., 2013. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Funct. Ecol.* 27, 1415–1423. doi:10.1111/1365-2435.12145.
- Kingsolver, J.G., MacLean, H.J., Goddin, S.B., Augustine, K.E., 2016. Plasticity of upper thermal limits to acute and chronic temperature variation in *Manduca sexta* larvae. *J. Exp. Biol.* 219, 1290–1294. doi:10.1242/jeb.138321.
- Kingsolver, J.G., Moore, M.E., Augustine, K.E., Hill, C.A., 2021. Responses of *Manduca sexta* larvae to heat waves. *J. Exp. Biol.* 224, jeb236505. doi:10.1242/JEB.236505/238099.
- Laughton, A.M., Fan, M.H., Gerardo, N.M., 2014. The combined effects of bacterial symbionts and aging on life history traits in the pea aphid, *Acyrtosiphon pisum*. *Appl. Environ. Microbiol.* 80, 470–477. doi:10.1128/AEM.02657-13.
- Lemoine, M.M., Engl, T., Kaltenpoth, M., 2020. Microbial symbionts expanding or constraining abiotic niche space in insects. *Curr. Opin. Insect Sci.* 39, 14–20. doi:10.1016/j.cois.2020.01.003.
- Lenth, R., Lenth, M.R., 2018. Package 'lme4'. *Am. Stat.* 34 (4), 216–221.
- Li, Y., Akimoto, S.I., 2018. Evaluation of an aphid-rearing method using excised leaves and agar medium. *Entomol. Sci.* 21, 210–215. doi:10.1111/ens.12296.
- Ma, G., Bai, C.M., Wang, X.J., Majeed, M.Z., Ma, C.S., 2018a. Behavioural thermoregulation alters microhabitat utilization and demographic rates in ectothermic invertebrates. *Anim. Behav.* 142, 49–57. doi:10.1016/J.ANBEHAV.2018.06.003.
- Ma, C.S., Ma, G., Pincebourde, S., 2021. Survive a warming climate: insect responses to extreme high temperatures. *Annu. Rev. Entomol.* 66, 163–184. doi:10.1146/annurev-ento-041520-074454.
- Ma, C.S., Wang, L., Zhang, W., Rudolf, V.H.W., 2018b. Resolving biological impacts of multiple heat waves: interaction of hot and recovery days. *Oikos* 127, 622–633. doi:10.1111/oik.04699.
- Montllor, C.B., Maxmen, A., Purcell, A.H., 2002. Facultative bacterial endosymbionts benefit pea aphids *Acyrtosiphon pisum* under heat stress. *Ecol. Entomol.* 27, 189–195. doi:10.1046/j.1365-2311.2002.00393.x.
- Moran, N.A., Yun, Y., 2015. Experimental replacement of an obligate insect symbiont. *Proc. Natl. Acad. Sci. U.S.A.* 112, 2093–2096. doi:10.1073/pnas.1420037112.
- Oliver, K.M., Russell, J.A., Moran, N.A., Hunter, M.S., 2003. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proc. Natl. Acad. Sci. U.S.A.* 100, 1803–1807. doi:10.1073/pnas.0335320100.
- Oliver, K.M., Smith, A.H., Russell, J.A., 2014. Defensive symbiosis in the real world – advancing ecological studies of heritable, protective bacteria in aphids and beyond. *Funct. Ecol.* 28, 341–355. doi:10.1111/1365-2435.12133.
- Parven, N., Yao, I., Kanbe, T., Akimoto, S.I., 2021. Heat shock alters pea aphid–*Buchnera* interactions: negative allometry of gene densities. *Entomol. Exp. Appl.* 169, 462–471. doi:10.1111/EEA.13039.
- Pons, I., Scieur, N., Dhondt, L., Renard, M.E., Renoz, F., Hance, T., 2022. Pervasiveness of the symbiont *Serratia symbiotica* in the aphid natural environment: distribution, diversity and evolution at a multitrophic level. *FEMS Microbiol. Ecol.* 98. doi:10.1093/FEMSEC/FIAC012, fiac012.
- Renoz, F., Noël, C., Errachid, A., Foray, V., Hance, T., 2015. Infection dynamic of symbiotic bacteria in the pea aphid *Acyrtosiphon pisum* gut and host immune response at the early steps in the infection process. *PLoS One* 10, e0122099. doi:10.1371/journal.pone.0122099.
- Renoz, F., Pons, I., Hance, T., 2019. Evolutionary responses of mutualistic insect–bacterial symbioses in a world of fluctuating temperatures. *Curr. Opin. Insect Sci.* 35, 20–26. doi:10.1016/j.cois.2019.06.006.
- Rock, D.I., Smith, A.H., Joffe, J., Albertus, A., Wong, N., O'Connor, M., Oliver, K.M., Russell, J.A., 2018. Context-dependent vertical transmission shapes strong endosymbiont community structure in the pea aphid, *Acyrtosiphon pisum*. *Mol. Ecol.* 27, 2039–2056. doi:10.1111/mec.14449.
- Ruggeri, M., Zhang, Y., Aglyamova, G.V., Kenkel, C.D., 2022. Divergent transcriptional response to thermal stress among life stages could constrain coral adaptation to climate change. *bioRxiv*, 490056 doi:10.1101/2022.04.29.490056.
- Russell, J.A., Moran, N.A., 2006. Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. *Proc. R. Soc. B Biol. Sci.* 273, 603–610. doi:10.1098/rspb.2005.3348.
- Sales, K., Vasudeva, R., Gage, M.J.G., 2021. Fertility and mortality impacts of thermal stress from experimental heatwaves on different life stages and their recovery in a model insect. *R. Soc. Open Sci.* 8, 201717. doi:10.1098/RSOS.201717.
- Sgrò, C.M., Terblanche, J.S., Hoffmann, A.A., 2016. What can plasticity contribute to insect responses to climate change? *Annu. Rev. Entomol.* 61, 433–451. doi:10.1146/annurev-ento-010715-023859.
- Shan, H.W., Lu, Y.H., Bing, X.L., Liu, S.S., Liu, Y.Q., 2014. Differential responses of the whitefly *Bemisia tabaci* symbionts to unfavorable low and high temperatures. *Microb. Ecol.* 68, 472–482. doi:10.1007/s00248-014-0424-3.
- Smith, A.H., Łukasik, P., O'Connor, M.P., Lee, A., Mayo, G., Drott, M.T., Doll, S., Tuttle, R., Disciullo, R.A., Messina, A., Oliver, K.M., Russell, J.A., 2015. Patterns, causes and consequences of defensive microbiome dynamics across multiple scales. *Mol. Ecol.* 24, 1135–1149. doi:10.1111/MEC.13095.
- Smith, A.H., O'Connor, M.P., Deal, B., Kotzer, C., Lee, A., Wagner, B., Joffe, J., Woloszynek, S., Oliver, K.M., Russell, J.A., 2021. Does getting defensive get you anywhere?—Seasonal balancing selection, temperature, and parasitoids shape real-world, protective endosymbiont dynamics in the pea aphid. *Mol. Ecol.* 30, 2449–2472. doi:10.1111/mec.15906.
- Sørensen, M.H., Kristensen, T.N., Lauritzen, J.M.S., Noer, N.K., Høye, T.T., Bahrndorff, S., 2019. Rapid induction of the heat hardening response in an Arctic insect. *Biol. Lett.* 15, 20190613. doi:10.1098/RSBL.2019.0613.
- Terblanche, J.S., Hoffmann, A.A., 2020. Validating measurements of acclimation for climate change adaptation. *Curr. Opin. Insect Sci.* 41, 7–16. doi:10.1016/j.cois.2020.04.005.
- Therneau, T., 2021. A Package for Survival Analysis in R. R package version 3.5.
- Tian, M., Zhang, Y., Liu, T.X., Zhang, S.Z., 2022. Effects of periodically repeated high-temperature exposure on the immediate and subsequent fitness of different developmental stages of *Propylaea japonica*. *Pest Manag. Sci.* 78, 1649–1656. doi:10.1002/PS.6784.
- Tougeron, K., Ittis, C., 2022. Impact of heat stress on the fitness outcomes of symbiotic infection in aphids: a meta-analysis. *Proc. R. Soc. B Biol. Sci.* 289, 20212660. doi:10.1098/rspb.2021.2660.
- van Heerwaarden, B., Kellermann, V., 2020. Does plasticity trade off with basal heat tolerance? *Trends Ecol. Evol.* 35, 874–885. doi:10.1016/J.TREE.2020.05.006.
- Wernegreen, J.J., 2012. Mutualism meltdown in insects: bacteria constrain thermal adaptation. *Curr. Opin. Microbiol.* 15, 255–262. doi:10.1016/j.mib.2012.02.001.
- Woods, H.A., Dillon, M.E., Pincebourde, S., 2015. The roles of microclimatic diversity and of behavior in mediating the responses of ectotherms to climate change. *J. Therm. Biol.* 54, 86–97. doi:10.1016/j.jtherbio.2014.10.002.
- Zera, A.J., Denno, R.F., 1997. Physiology and ecology of dispersal polymorphism in insects. *Annu. Rev. Entomol.* 42, 207–230. doi:10.1146/annurev.ento.42.1.207.
- Zhang, B., Leonard, S.P., Li, Y., Moran, N.A., 2019. Obligate bacterial endosymbionts limit thermal tolerance of insect host species. *Proc. Natl. Acad. Sci. U.S.A.* 116, 24712–24718. doi:10.1073/pnas.1915307116.
- Zhang, W., Chang, X.Q., Hoffmann, A., Zhang, S., Ma, C.S., 2015a. Impact of hot events at different developmental stages of a moth: the closer to adult stage, the less reproductive output. *Sci. Rep.* 5, 10436. doi:10.1038/srep10436.
- Zhang, W., Rudolf, V.H.W., Ma, C.S., 2015b. Stage-specific heat effects: timing and duration of heat waves alter demographic rates of a global insect pest. *Oecologia* 179, 947–957. doi:10.1007/s00442-015-3409-0.
- Zhao, F., Hoffmann, A.A., Xing, K., Ma, C.S., 2017. Life stages of an aphid living under similar thermal conditions differ in thermal performance. *J. Insect Physiol.* 99, 1–7. doi:10.1016/J.JINSPHYS.2017.03.003.
- Zhao, F., Xing, K., Hoffmann, A.A., Ma, C.S., 2019. The importance of timing of heat events for predicting the dynamics of aphid pest populations. *Pest Manag. Sci.* 75, 1866–1874. doi:10.1002/ps.5344.
- Zytynska, S.E., Meyer, S.T., Sturm, S., Ullmann, W., Mehrparvar, M., Weisser, W.W., 2016. Secondary bacterial symbiont community in aphids responds to plant diversity. *Oecologia* 180, 735–747. doi:10.1007/s00442-015-3488-y.
- Zytynska, S.E., Tighiouart, K., Frago, E., 2021. Benefits and costs of hosting facultative symbionts in plant-sucking insects: a meta-analysis. *Mol. Ecol.* 30, 2483–2494. doi:10.1111/mec.15897.