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Breakdown of a defensive symbiosis, but not endogenous defences, at elevated temperatures

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Abstract

Environmental factors, including temperature, can have large effects on species interactions, including mutualisms and antagonisms. Most insect species are infected with heritable bacterial symbionts with many protecting their hosts from natural enemies. However, many symbionts or their products are thermally sensitive; hence, their effectiveness may vary across a range of temperatures. In the pea aphid, *Acyrthosiphon pisum*, the bacterial symbiont *Hamiltonella defensa* and its associated APSE bacteriophages confer resistance to this aphid's dominant parasitoid, *Aphidius ervi*. Here, we investigate the effects of temperature on both endogenous and symbiont-based protection against this parasitoid. We also explored the defensive properties of the X-type symbiont, a bacterium hypothesized to shape aphid defence when co-occurring with *H. defensa*. We show that *H. defensa* protection fails at higher temperatures, although some aphid genotype and *H. defensa* strain combinations are more robust than others at moderately warmer temperatures. We also found that a single X-type strain neither defended against parasitism by *A. ervi* nor rescued lost *H. defensa* protection at higher temperatures. In contrast, endogenous aphid resistance was effective across temperatures, revealing that these distinct defensive modes are not equally robust to changing environments. Through a survey of field-collected pea aphids, we found a negative correlation between *H. defensa* frequencies and average daily temperatures across North American locales, fitting expectations for reduced symbiont benefits under warm climates. Based on these findings, we propose that rising global temperatures could promote the widespread breakdown of defensive mutualisms, a prospect with implications for both human and ecosystem health.

KEYWORDS
climate change, heritable symbiont, host–microbe, host–parasitoid, mutualism

1 | INTRODUCTION

Abiotic environmental factors can influence species interactions by affecting the behaviour, development time, phenology and physiology of interacting players (Hegland, Nielsen, Lázaro, Bjerknes, & Totland, 2009; Lazzaro & Little, 2009; Mohan et al., 2014; Romo & Tylianakis, 2013; Six, 2013; Thomas & Blanford, 2003). For example, temperature is often an important factor affecting the outcome of diverse interaction types spanning the continuum from mutualism to antagonism, including specialized host–parasite and host–microbe interactions (Allen & Little, 2011; Corbin, Heyworth, Ferrari, & Hurst, 2017; Garbutt, Scholefield, Vale, & Little, 2014; Hance, van Baaren, Vernon, & Boivin, 2007; Heyworth & Ferrari, 2016; Kiers, Palmer, Ives, Bruno, & Bronstein, 2010; Wernegreen, 2012). While the

effects of temperature have most often been studied on binary associations, a recent appreciation that most plants and animals harbour communities of microbes indicates that symbiotic species interactions involve multiple key players. Given that the performance-breadth and optima for each macroplayer and microplayer may vary across temperatures, the outcome of microbe-mediated species interactions may vary dynamically across a range of conditions. The rapidly changing global climate makes it especially important to study the impacts of temperature on microbe-mediated species interactions, as many important medical and agricultural pests harbour microbial symbionts that contribute to or ameliorate their harmful effects (Cirimotich et al., 2011; Lu, Hulcr, & Sun, 2016; Nakabachi et al., 2013; Newcombe et al., 2009).

In insects, the resident microbiota often provides key contributions required to procure needed resources for growth and reproduction or to protect hosts from natural enemies (Douglas, 2015; Oliver & Martinez, 2014). A majority of insect species harbour maternally transmitted bacterial symbionts, with a growing number shown to contribute to host immunity by conferring protection against pathogens and parasites (Brownlie & Johnson, 2009; Haine, 2008; Hamilton & Perlman, 2013; Moran, McCutcheon, & Nakabachi, 2008; Oliver & Moran, 2009). Given the known impacts of temperature on symbiont performance (Wernegreen, 2012), there is a clear need to understand how temperature shapes microbe-mediated protective services. Such findings will improve our understanding of the efficacies of symbiont defence in the field, which are generally understudied (Jaenike, Unckless, Cockburn, Boelio, & Perlman, 2010; Oliver, Smith, & Russell, 2014; Rothacher, Ferrer-Suay, & Vorburger, 2016; Smith et al., 2015).

Aphids have emerged as important models for studying protective symbioses (Oliver, Degnan, Burke, & Moran, 2010; Oliver et al., 2014; Vorburger, 2014). Pea aphids (*Acyrthosiphon pisum*), for example, are variably infected with eight heritable symbionts (Ferrari, West, Via, & Godfray, 2012; Russell et al., 2013). All individual aphids harbour the obligate nutritional partner, *Buchnera aphidicola*, which provides compensatory nutrients allowing for subsistence on plant phloem (Douglas, 1998). Each may also be infected with one or more of seven heritable facultative symbionts (HFS) with diverse beneficial roles ranging from thermal protection (Heyworth & Ferrari, 2015; Montllor, Maxmen, & Purcell, 2002; Russell & Moran, 2006) and expansion of dietary breadth (Ferrari, Scarborough, & Godfray, 2007; Tsuchida, Koga, & Fukatsu, 2004) to protection against specialized fungal pathogens and parasitic wasps (Heyworth & Ferrari, 2015; Łukasik, van Asch, Guo, Ferrari, & Godfray, 2013; Oliver, Russell, Moran, & Hunter, 2003; Parker, Spragg, Altincicek, & Gerardo, 2013; Scarborough, Ferrari, & Godfray, 2005). Pea aphids reared at warmer temperatures are smaller (Humphreys & Douglas, 1997), and those exposed to short bursts of high heat (i.e., heat shock) often exhibit reduced performance, likely resulting from the deleterious effects of high temperatures on the obligate symbiont *Buchnera* (Ohtaka & Ishikawa, 1991), but little is known about how temperature impacts symbiont-mediated protection against natural enemies (Heyworth & Ferrari, 2016).

The dominant parasitoid of the pea aphid in North America is the braconid wasp *Aphidius ervi*, which develops internally, eventually killing and consuming the contents of the aphid and pupating within its desiccated cuticle (Schellhorn, Kuhman, Olson, & Ives, 2002). In the eastern United States, these parasitoids successfully infect pea aphids from spring through autumn (Smith et al., 2015), subjecting this antagonistic interaction to a variety of environmental conditions within a single locale. Climatic variation across pea aphid and *A. ervi* habitats in North America reveals that these species interactions also experience a range of thermal conditions across a spatial context.

At constant cool temperatures (ca. 20°C), pea aphid clones vary greatly in their susceptibility to parasitism by *A. ervi* (Henter & Via, 1995; Martinez, Ritter, Doremus, Russell, & Oliver, 2014). Much of this variation depends on infection with different strains of the HFS *Hamiltonella defensa* and its associated bacteriophages called APSEs (Oliver, Degnan, Hunter, & Moran, 2009; Oliver, Moran, & Hunter, 2005). Three major APSE variants (APSE2, 3 & 8) have been reported in North American pea aphid populations (Degnan & Moran, 2008a; Doremus & Oliver, 2017; Martinez, Weldon, & Oliver, 2014; Moran, Degnan, Santos, Dunbar, & Ochman, 2005), and in laboratory studies, aphids carrying strains of *H. defensa* with APSE3 receive high to complete protection. In contrast, those with APSE2 and APSE8 bearing *H. defensa* receive moderate protection, while those with phage-free *H. defensa* are highly susceptible (Doremus & Oliver, 2017; Oliver & Moran, 2009; Oliver et al., 2005). Additionally, some aphid clones lacking HFS possess endogenous resistance to *A. ervi* (Martinez, Ritter et al., 2014).

Most laboratory studies examining symbiont-based protection in pea aphids have done so at constant temperatures favourable to aphid rearing (Heyworth & Ferrari, 2016). An intriguing prior study reported that pea aphid clones highly resistant to *A. ervi* at 20°C were increasingly susceptible to parasitism at 25 and 30°C (Bensadia, Boudreault, Guay, Michaud, & Cloutier, 2006). In this same study, resistance at the cooler temperatures was correlated with *H. defensa* infection, indicating that symbiont-based protection likely fails at higher temperatures. A subsequent study found that co-infection with a second HFS, informally called PAXS (Pea Aphid X-type Symbiont) or simply X-type, was present in one *H. defensa*-infected clone that retained defence at higher temperatures (Guay, Boudreault, Michaud, & Cloutier, 2009). This raised the possibility that X-type plays a direct role in defence or that it can rescue lost *H. defensa* protection under warm conditions. However, neither of these studies disentangled effects of host genotype, which was later shown to contribute to resistance to parasitoids (Martinez, Ritter et al., 2014). A recent study using North American pea aphids found that a common X-type strain does not confer protection against another braconid parasitoid, *Praon pequodourm* (or heat shock) either as a single or co-infection with *H. defensa* (Doremus & Oliver, 2017), but this study did not examine interactions with the dominant parasitoid *A. ervi* or incorporate parasitism assays at higher temperatures. Hence, the impacts of temperature on *H. defensa*-mediated defence, and the abilities of co-infecting symbionts to rescue heat-induced detriments, remain uncertain.

Here, we examine the effects of realistic temperature regimens on the outcomes of the pea aphid–parasitoid interaction. To address questions about temperature's impacts on different defensive modes, we use a wide range of experimental pea aphid clonal lines, which vary in source (symbiont vs. aphid encoded) and level (moderate to high) of resistance to the wasp *A. ervi*. Additionally, we created experimental lines to investigate the defensive properties of singly X-type infected aphids, as well as the hypothesized rescue of *H. defensa* failure at higher temperatures when co-occurring with X-type. *H. defensa* (and APSE) densities were examined in the absence of parasitism across rearing temperatures to understand whether lost protection is a simple function of depressed HFS (and phage) population sizes. Finally, *H. defensa* frequencies were estimated across numerous North American populations, helping to address whether laboratory-based findings on temperature and its impacts on defence are consistent with varying symbiont frequencies across climatic gradients.

2 | METHODS

2.1 | Study organisms

The pea aphid, *A. pisum* (Hemiptera: Aphididae), is a phloem-feeding herbivore of leguminous forage crops (Peccoud, Ollivier, Plantegenest, & Simon, 2009). This aphid is a cyclical parthenogen, and clonal lines can be maintained indefinitely in the laboratory by exposure to summerlike photoperiods (Brisson & Stern, 2006). All experimental aphid lines used in this study were established from single parthenogenetic females from alfalfa, *Medicago sativa*. In the laboratory, they were reared on the host plant *Vicia faba* (fava bean) and maintained in biological incubators on a 16L: 8D photoperiod at 20 ± 1°C unless otherwise noted.

The parasitic wasp, *A. ervi* (Haliday; Hymenoptera: Braconidae), is the most common parasitoid attacking pea aphids in North America. As a solitary endoparasitoid, the free-living adult female wasp lays an egg in a suitable host, usually second- or third-instar aphid nymphs. The resulting larval wasp develops within a living aphid for approximately 1 week before killing and consuming the aphid and pupating within the desiccated aphid carcass, which is called a mummy. Wasp cultures were established from mummies (>100) collected from pea aphids feeding on alfalfa in North Dakota (USA) and Wisconsin (USA) and maintained on an aphid line (AS3-AB) lacking facultative symbionts at 20 ± 1°C under a 16L: 8D h photoperiod.

2.2 | Experimental aphid lines

To assess the impacts of temperature on an aphid–parasitoid interaction, we conducted parasitism assays across a range of pea aphid experimental lines exposed to several temperature regimens. In total, we used 13 clonal experimental aphid lines (Table 1) varying in biological characteristics, but particular assays each used a subset of these. These lines were comprised of six aphid genotypes, including four that are innately susceptible and two (CJ113 & WA4) that are resistant to *A. ervi* in the absence of HFS at 20°C (Martinez, Ritter et al., 2014). We also examined one strain of the HFS X-type and three *H. defensa* strains carrying different APSE phage types with varying effects on the protective phenotype at 20°C. Specifically, in prior work, strains carrying APSE2 (82B) and APSE8 (5D) confer moderate resistance (Doremus & Oliver, 2017; Oliver et al., 2005), while those associating with APSE3 (A1A) confer high levels of protection (Oliver et al., 2009). Experimental lines were created using microinjection or selective antibiotic curing, establishing genetically identical aphid stocks varying only with respect to their harboured HFS (Douglas, Francois, & Minto, 2006). HFS infection status and APSE type for all lines were confirmed with diagnostic PCR and Sanger sequencing (see Degnan & Moran, 2008b; Henry et al., 2013; Weldon, Strand, & Oliver, 2013 for primers and protocols).

TABLE 1 Experimental pea aphid lines used in this study: aphid genotype, *Hamiltonella defensa* infection status and APSE type, X-type infection status, presence of innate resistance and *ibpA* heat shock promoter mutation: long = wt, short = mutant

Aphid genotype	Experimental lines	Symbiont species and strain	Level of symbiont resistance at 20°C	Level of endogenous Resistance at 20°C?	*Buchnera ibpA* status short = heat sensitive
5A	5A	None	n/a	Low	Short
	82B → 5A	*H. defensa* 82B/APSE2	Moderate	Low	Short
	A1A → 5A	*H. defensa* A1A/APSE3	High	Low	Short
A2A	A2A	None	n/a	Low	Long
	82B → A2A	*H. defensa* 82B/APSE2	Moderate	Low	Long
A2E	A2E	None	n/a	Low	Long
	82B → A2E	*H. defensa* 82B/APSE2	Moderate	Low	Long
5D	5DX + 5DH	*H. defensa* 5D/APSE8 and X-type	Unknown	Low	Long
	5DH	*H. defensa* 5D/APSE8	Moderate	Low	Long
	5DX	X-type	Unknown	Low	Long
	AB	None	n/a	Low	Long
CJ1-13	CJ113ø	None	n/a	High	Short
WA4	WA4ø	None	n/a	High	Long

Denaturing gradient gel electrophoresis (DGGE) was also performed to verify infection with only *H. defensa* or X-type (i.e., no other HFS present) following Russell et al. (2013).

Our chosen aphid clones also varied in a *Buchnera*-encoded gene promoter mutation (*ibpA*) expected to impact aphid and *Buchnera* responses to high temperature (see Table 1; Dunbar, Wilson, Ferguson, & Moran, 2007). We determined *Buchnera ibpA* homopolymer length within the promoter region, giving us expectations on thermal susceptibility that were used in interpreting our results. Promoter length was ascertained by PCR amplification of this region followed by Sanger sequencing, and subsequent classification of promoter genotypes as short (= single bp deletion which makes aphids heat sensitive) or long (wild type); see Dunbar et al. (2007) for primers and reaction conditions.

2.3 | Temperature and *Hamiltonella defensa*-based protection

Prior to parasitism assays, aphids were reared for a minimum of two generations at 20 ± 1°C on 16L: 8D h photoperiod in a Percival environmental chamber on similarly aged (approximately 10 day-old) cohorts of preflowering *V. faba* seedlings. Second- or third-instar pea aphids were then singly parasitized by a mated *A. ervi* female and immediately removed and placed in cohorts of twenty aphids on a single *V. faba* plant in a small cage (as in Oliver et al., 2009). Each cohort of 20 parasitized aphids from each line represented a single replicate. Replicate number varied among assays (details below; minimum of 8) usually depending on wasp availability. In Assay 1, parasitized aphids of each line were reared at a constant 20°C (control) vs. a constant 25°C (heat treatment). For Assay 2, parasitized aphids were reared at one of three temperatures: a constant 20°C (control); a regime of 27°C day (16 hr)/23°C night (8 hr); and a regime of 29°C day (16 hr)/25°C night (8 hr). We used a diurnal temperature for the warmer treatments as holding aphids at constant 27 or 29°C resulted in very high aphid mortality in preliminary trials (data not shown).

Three different outcomes are possible when pea aphids are parasitized by *A. ervi*: (i) wasps successfully complete development through pupation, which results in the formation of mummies (from which eclosion rates are normally very high; Oliver et al., 2012); (ii) aphids survive parasitism, typically going on to reproduce; (iii) parasitized aphids may die in which case neither host nor parasite survives. In past assays (e.g., Oliver et al., 2003, 2005, 2009), all performed at approximately 20°C, dual mortality of parasitized aphids and wasps (i.e., outcome 3) was generally low and not significantly different among treatments. Hence, analyses previously focused on the proportion of aphids successfully parasitized [number of mummies/(number of aphids alive + number of mummies)]. Since then, we have observed that mortality to both aphids and wasps often increases at higher temperatures. Thus, for the assays in this study we considered how higher temperatures affected both aphid survival following parasitism (aphids, *H. defensa* and APSE all benefit when aphids survive parasitism and reproduce) and mortality (to both aphid and wasp).

Even with the diurnal regime (i.e., cooler night-time temperatures), we still found high rates of mortality (see Section 3) of parasitized aphids under warmer conditions. To determine whether mortality was likely due mostly to temperature vs. a combination of temperature and parasitism, we conducted a post hoc control assay of *unparasitized* aphids. We assayed four lines varying in genotype and *H. defensa* strain (AS3, AS3-AB, 82B → 5A, 5A, $N = 8$ for each treatment) reared at either the highest temperature regime (29°C day/25°C night) or the 20°C control temperature and observed whether aphids were alive or dead after 10 days.

2.4 | Effect of X-type infection on protection against parasitoids

To investigate the proposed rescue synergism in which co-infection with X-type prevents the loss of *H. defensa*-based protection at elevated temperatures (Guay et al., 2009), we established a full complement of X-type–*H. defensa* infections in a controlled aphid genotype (Table 1; line 5D; Doremus & Oliver, 2017). Aphids used in the X-type parasitism assays were reared under identical conditions, and parasitism assays were conducted as described in the previous experiments. For this set of experiments, aphids ($N = 8$ replicates of 20 aphids per treatment) were also exposed to a range of temperatures, being reared at either a constant 20°C (control) or at an alternating regime of 30°C (16 hr)/24°C (8 hr) to more closely match conditions reported in Guay et al. (2009). Aphid survival, successful parasitism and dual mortality were recorded 9 days after parasitism.

2.5 | Temperature and endogenous aphid-encoded resistance

We also conducted parasitism assays at two temperature ranges using pea aphid lines with endogenous aphid-based resistance (no HFS) to *A. ervi*, assessing whether temperature impacts this non-symbiont-based mode of defence. These innately resistant lines also differed in the length of their *Buchnera ibpA* promoters, with one (WA4) possessing the wild-type *ibpA* promotor, and the other (CJ113) encoding the "short" promoter associated with heat sensitivity (Table 1). Parasitism assays were conducted as described above, with six replicates of cohorts containing twenty pea aphids placed onto *V. faba* plants postparasitism. Aphids were then held at either a constant 20°C (control) or reared under an alternating regime of 30°C (16 hr)/24°C (8 hr), as in the X-type assays. Nine days after parasitism, aphid survival, successful parasitism and dual mortality were recorded, as described above.

2.6 | Effects of temperature on symbiont titres

To determine whether any alterations in defence correlate with temperature-driven alterations in symbiont density, we used real-time quantitative PCR (qPCR) to compare symbiont (*H. defensa* and *Buchnera*) and phage (APSE) titres for 72-hr-old aphids (the age of attack in our parasitism assays) at three constant temperature regimes of 20, 25

and 27°C. Aphids for each treatment were held on the same cohort of plants in the same Percival incubator at 20°C prior to moving at birth (±3 hr) to the experimental temperature. We conducted the qPCR assays in two clonal aphid backgrounds (5A and A2E) and with two strains of *H. defensa* (82B and A1A), each with its particular APSE bacteriophage (Table 1). To establish cohorts of equally aged aphids, roughly twenty actively reproducing female pea aphids were placed on a single *V. faba* plant and allowed to reproduce for six hours, such that all 72-hr-old aphids were produced within ±3 hr (i.e., 69–75 hr old).

We prepared whole aphid DNA extractions in a lysis buffer (10 mM Tris-Cl, pH8.2; 1 mM EDTA; 25 mM NaCl) with 1% proteinase K (20 mg/ml) (Gloor et al., 1993). "Absolute" standard curves for quantification were produced via serial dilutions (Oliver, Moran, & Hunter, 2006), and efficiencies for all quantification reactions approached 100%. We used established primers and reaction conditions (Martinez, Weldon et al., 2014; Weldon et al., 2013) to amplify fragments of the single-copy genes to estimate microbial titres: *dnaK* for *H. defensa* and *Buchnera* and P28 for APSE. Phage and bacterial abundances were normalized using the aphid gene *EF1α* to account for any differences in aphid size or extraction efficiency. All 10 μl reactions were performed on a Roche LightCycler 480 II using Roche SYBR Green I Master chemistry and 0.5 μM of each primer. Cycling conditions for all reactions were as follows: 95°C for 5 min; amplification (repeated 45 times): 95°C for 10 s, 68 to 55°C touchdown with 1°C steps each at 10 s, 72°C for 10 s; melting curve: 95°C for 5 s, 65°C for 1 min, then ramped to 97°C; hold at 4°C.

2.7 | Field measures of temperature

To gain insight into real-world temperature regimes experienced by pea aphids, Watchdog B100 2K temperature logger probes (Spectrum Technologies, Aurora, IL, USA) were placed into three replicate alfalfa fields in each Cayuga/Tompkins Counties, New York, and in Berks County, Pennsylvania. Probes were positioned approximately one inch from the ground level and were shaded from above by plastic covers and alfalfa plants. Within crop canopy temperatures were recorded every 30 min from 4 May 2011 to 3 October 2011.

2.8 | *Hamiltonella defensa* frequencies across climates

Pea aphids were collected from alfalfa fields across the United States through beat sampling over trays or sweep netting at intervals of approximately 20 m. One or two individuals were taken from each position (i.e., two only if both pink and green colour morphs were available), to minimize resampling of the same clones. Aphids from New York (2011; 2013; 2015) and Pennsylvania (2011–2015) were immediately preserved in 95%–100% ethanol. Those from California (2013), Utah (2011), North Dakota (2012) and Wisconsin (2011–2013) were collected live, and first-generation offspring were screened for symbionts.

For ethanol-collected samples, DNA was extracted from whole, individual aphids and two different diagnostic PCRs were performed on each sample (Russell et al., 2013; Smith et al., 2015). The first targeted the 16S rRNA gene of *Buchnera* (BuchneraF: 5′ CTGTTGCCAGCCAGCGGTTCGG 3′; EcoliR: 5′ CCCCTACG GTAACCTTGTTACG 3′), determining whether extractions were of suitable quality to detect *H. defensa* using diagnostic PCR (*H. defensa* primers: 10F 5′ AGTTTGATCATGGCTCAGATTG 3′ and T419R 5′ AAATGGTATTSGCATTTATCG 3′). The PCR conditions for *H. defensa* screens used a touchdown protocol of: 94°C for 2 min; nine cycles of 94°C for 1 min, 65°C for 1 min decreasing by 1°C per cycle, and 72°C for 2 min; 25 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min; and, finally, 72°C for 6 min. Those for *Buchnera* used an approach of: 94°C for 2 min; 30 cycles of 94°C for 30 s, 62°C for 1 min and 72°C for 1.5 min; and, finally, 72°C for 7 min. Positive and negative control reactions were included for each run. For the live-collected/laboratory-reared aphids, which produce uniformly high-quality extractions, only the *H. defensa* diagnostic PCR was conducted.

In addition to generating new data for the aforementioned collections (totalling 1932 surveyed aphids), we used data from prior publications on collections from nearby regions to enhance our sample sizes in our targeted locales (Pennsylvania, California and Utah) (Leonardo & Muiru, 2003; Oliver et al., 2006; Russell et al., 2013); we also added data from a new locale, southern Quebec (Bilodeau, Simon, Guay, Turgeon, & Cloutier, 2013). Aphids from New York and Pennsylvania had typically been collected across multiple times in each of the surveyed years. In nearly all cases for these locales, each time point was represented by collections from multiple replicate fields. Data were pooled across the various time points and fields within a single year to simplify our attempts to generate regional estimates of *H. defensa* prevalence. Pooling was similarly performed across locales separated by less than 100 miles in California (across two to three counties) within each of the two collection years there, and also across three sites in southern Quebec. For graphing purposes, we computed average *H. defensa* frequencies (and their associated standard errors) across the various years targeted for each locale.

The historical average daily high temperature in the month of July was obtained for each locale via the climatedata.org website (http://en.climate-data.org), with just one exception (Lennoxville, Quebec from http://www.eldoradocountyweather.com). As the collection sites did not have specific weather data, we used temperatures from nearby cities. To obtain a single regional temperature profile for the two locales with multiple collection sites, we averaged temperatures from the cities closest to our separate sampling points in California (Chico, Fairfield and Davis) and Quebec (Lennoxville, Montreal, Saint Augustin de Desmaures).

2.9 | Statistical analyses

Aphid survival, mummification (i.e., *A. ervi* survival) and dual mortality were analysed for each treatment using logistic regression analyses in SAS JMP version 13.0.0. The likelihood of successful parasitism (i.e., mummy production) is provided in Tables S1–S4. Within-host

H. defensa densities in the absence of parasitism were compared across temperature regimens for the 5A and A2A aphid genotypes with ANOVA and Dunnett's test using the 20°C treatment as the control. *H. defensa* frequency was correlated with average temperatures across longitude using linear regression on pooled data (described above) in SAS JMP version 13.0.0.

3 | RESULTS

3.1 | *Hamiltonella defensa*-mediated defence largely fails at warmer temperatures

3.1.1 | Assay 1: Constant 20 vs. 25°C

As expected from previous studies, we found that *H. defensa* infection leads to significant increases in aphid survival following parasitism at the 20°C control temperature. In aphid genotype 5A, we found that two aphid lines infected with different *H. defensa* strains (lines A1A → 5A and 82B → 5A) were more likely to survive parasitism by the wasp *A. ervi* relative to the uninfected control 5A (logistic regression likelihood ratio test (LRT) $\chi^2_{A1A} = 118.1$ $p < .0001$, $\chi^2_{82B} = 34.1$ $p < .0001$, Figure 1; Table S1). At the modestly warmer temperature of 25°C, however, symbiont-mediated defence was substantially compromised. Specifically, we found no difference in aphid survival following parasitism between *H. defensa* line 82B → 5A and the uninfected counterpart 5A (Figure 1, LRT $\chi^2_{82B} = 0.5$ $p = .5$), indicating the complete loss of protective benefits from *H. defensa*. While aphids from line A1A → 5A still received significant protection at 25°C relative to their uninfected counterparts (LRT $\chi^2_{A1A-5A} = 26.9$ $p < .0001$), aphid survival for this line was significantly reduced at 25°C relative to this same line held at 20°C, indicating partial failure in protection (LRT $\chi^2_{25v20C} = 33.7$ $p < .0001$; Figure 1). In contrast, the uninfected 5A line showed similar levels of aphid survival following parasitism at both 20 and 25°C (Figure 1, LRT $\chi^2_{25v20C} = 0.31$ $p = .58$; Figure 1), indicating the altered defence observed in *H. defensa*-infected lines was largely driven by compromised symbiont efficacy. In the second aphid genotype, A2A, the addition of *H. defensa* strain 82B significantly increased aphid survival following parasitism at both 20°C ($\chi^2_{82B} = 52.0$ $p < .0001$; Figure 1) and 25°C (LRT $\chi^2_{82B} = 21.5$ $p < .0001$), relative to uninfected controls (Figure 1). However, postparasitism survival of aphid line 82B → A2A was decreased at 25°C compared to 20°C, indicating protective benefits of *H. defensa* were reduced with warming temperatures (Table S1).

In a full-factorial logistic regression analysis across genotypes, infection status, and temperatures, infection with *H. defensa* (LRT $\chi^2_{ham} = 347.0$ $p < .0001$), temperature ($\chi^2_{temp} = 60.8$ $p < .0001$) and their interaction ($\chi^2_{ham*temp} = 34.1$ $p < .0001$) had major effects on aphid survival following parasitism (Table S1), while only temperature (LRT $\chi^2_{temp} = 24.0$ $p < .0001$) had a significant effect on dual mortality (i.e., the death of both aphid and wasp). In this assay, all lines held at 25°C showed greater dual mortality relative to the control line at 20°C (Figure 1). That the same symbiont strain (82B) protected aphids of one background (A2A) but not a second (5A) at 25°C suggests that the aphid or *Buchnera* genotype may factor into shaping defensive efficacy at warmer temperatures. These *Buchnera* strains vary in *ibpA* promotor length, with line A2A encoding the wild-type promoter associated with better performance under thermal stress (Table 1).

3.1.2 | Assay 2: 20°C control vs. $27^{day}/23^{night}$°C and $29^{day}/25^{night}$

At the control temperature, 20°C, we again found that *H. defensa* infection led to significant increases in aphid survival following parasitism in both aphid genotypes (Figures 2 and 5a: LRT $\chi^2_{A1A} = 104.5$ $p < .0001$, $\chi^2_{82B} = 28.1$ $p < .0001$ and A2E $\chi^2_{82B} = 16.5$ $p < .0001$). However, when reared under the warmer $27^{day}/23^{night}$ or $29^{day}/23^{night}$ treatments, we found no significant increases in aphid survival

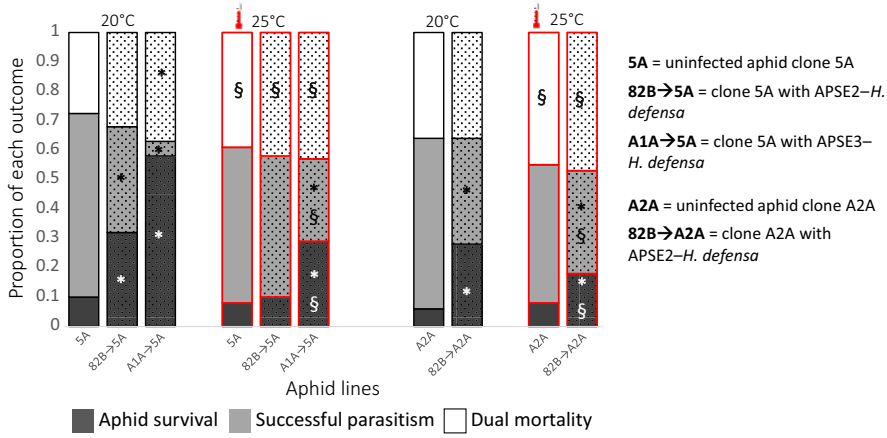

FIGURE 1 Symbiont defence fails at warm temperatures. Aphid survival, successful parasitism and dual mortality postparasitism. Red-bordered bars represent 25°C treatment, and bars lacking border represent 20°C control treatment. Stippled bars show lines bearing *Hamiltonella defensa*. * represent significant differences in outcome (e.g., survival) relative to uninfected control at same temperature. § represent significant differences in outcome relative to control line of same genotype at 20°C. $N \geq 8$ replicates of 20 aphids for each treatment [Colour figure can be viewed at wileyonlinelibrary.com]

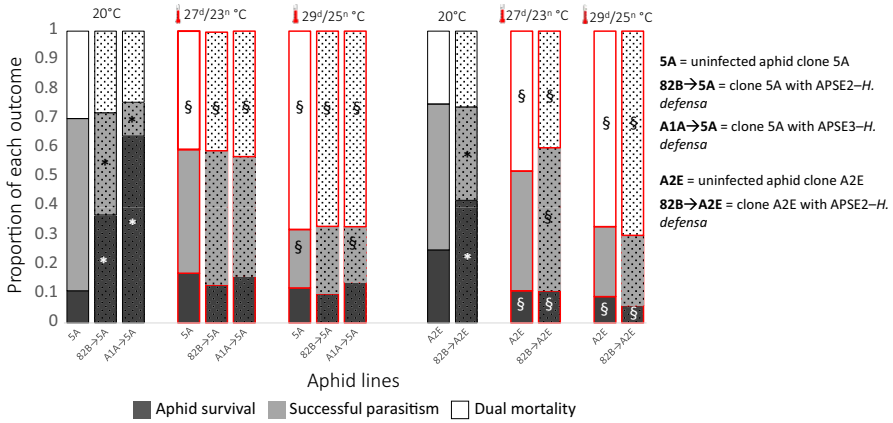

FIGURE 2 Symbiont defence fails, with increased dual mortality at warmer temperatures. Aphid survival, successful parasitism and dual mortality postparasitism. Red-bordered bars represent $27^{day}/23^{night}$°C or $29^{day}/25^{night}$°C treatment; bars lacking border represent 20°C control treatment. Stippled bars show lines bearing *Hamiltonella defensa*. * represent significant differences in outcome (e.g., survival) relative to uninfected control at same temperature. § represent significant differences in outcome relative to control line of same genotype at 20°C. $N \geq 8$ replicates of 20 aphids for each treatment [Colour figure can be viewed at wileyonlinelibrary.com]

following parasitism in *H. defensa*-infected sublines compared to their uninfected counterparts; this was true in both the 5A (Figure 2, Table S2; LRT $27^{day}/23^{night}$ $\chi^2_{A1A} = 0.02$ $p = .88$, $\chi^2_{82B} = 1.22$ $p = .27$; $29^{day}/25^{night}$ $\chi^2_{A1A} = 0.3$ $p = .62$, $\chi^2_{82B} = 0.3$ $p = .62$) and A2E genetic backgrounds ($27^{day}/23^{night}$ $\chi^2_{82B} = 1.71$ $p = .19$; $29^{day}/25^{night}$ $\chi^2_{82B} = 0.7$ $p = .40$). This indicates that symbiont-based protection effective at 20°C is completely lost at only moderately elevated temperatures. Unlike the 20 vs. 25°C assay, we did not see retention of partial protection for particular strain x genotype combinations.

In our full-factorial logistic regression analysis, infection with *H. defensa* (LRT $\chi^2_{Ham} = 78.6$ $p < .0001$) and temperature ($\chi^2_{temp} = 81.7$ $p < .0001$) were the most important factors associated with aphid survival. Temperature was the only significant factor contributing to dual mortality, with higher values resulting in greater mortality (LRT $29^{day}/25^{night}$ $\chi^2 = 91.2$ $p < .0001$; $27^{day}/23^{night}$ $\chi^2 = 44.1$ $p < .0001$). As in the previous assay, the increase in dual mortality was consistent across all lines held at the warmer temperature relative to their respective control lines at 20°C (Figure 2).

To determine whether the substantial increase in dual mortality observed in these assays was a result of heat alone or the combination of heat and parasitism, we conducted survival assays in the absence of parasitism using our highest temperature regime ($29^{day}/25^{night}$) to establish baseline expectations. The high temperature treatment resulted in significant (20%–25%) increases (ANOVA $p < .001$) in aphid mortality for all four aphid lines (varying in genotype and symbiont status) relative to the 20°C control. The four aphid lines also exhibited similar survival rates at control ($\bar{x} = 18.6/20$; ANOVA $p = .47$; Tukey's HSD = NSD) and elevated temperatures ($\bar{x} = 14.4/20$; ANOVA $p = .1$; Tukey's HSD = NSD). While not directly comparable, dual mortality in *parasitized* aphids at the $29^{day}/25^{night}$ temperature regime was typically at least 50% greater than mortality of 20°C unparasitized controls. Thus, in addition to suppressing symbiont-based protection, high temperatures can increase aphid mortality in ways that are exacerbated by wasp parasitism.

3.2 | X-type does not rescue the loss of *Hamiltonella defensa*-based protection at higher temperatures

In a separate experiment using a different aphid genotype (5D) and *H. defensa* strain (5DH, harbouring phage APSE8), we again found moderately increased survival rates for *H. defensa*-bearing pea aphids when challenged by *A. ervi* at 20°C (Figure 3, LRT $\chi^2_{ham} = 22.8$ $p = .0002$). Pea aphids co-infected with *H. defensa* + X-type also showed a significant increase in aphid survival compared to uninfected controls at 20°C (LRT $\chi^2_{ham} = 22.8$, $p < .0001$) and protection levels did not differ significantly from *H. defensa*-only infected aphids (LRT $\chi^2_{h+x-vs-ham} = 1.0$, $p = .31$). In contrast, pea aphids infected with only X-type did not exhibit increased survival after *A. ervi* parasitism at 20°C (LRT $\chi^2_x = 0.02$ $p = .89$).

At the higher temperature (30°/24°C), the protection conferred by *H. defensa* at 20°C was again completely lost (Figure 3, Table S3; LRT $\chi^2_{ham} = 0.33$, $p = 57$). X-type infection also did not increase aphid survival at 30°/24°C (LRT $\chi^2_x = 3.1$ $p = .08$), nor did it reduce parasitism success (LRT $\chi^2_x = 0.56$ $p = .45$). Importantly, and contrary to our expectations, X-type co-infection did not rescue failed *H. defensa* protection in the higher temperature treatment (LRT $\chi^2_{x+ham} = 0.08$ $p = .77$). While temperature did have a small impact on likelihood of aphid survival following parasitism (LRT $\chi^2_{temp} = 9.0$ $p = .002$), we did not find significant increases in dual mortality at the increased temperature in this experiment (Figure 3, LRT $\chi^2_{temp} = 0.01$ $p = .94$) unlike our previous assays.

3.3 | Warmer temperatures do not negatively impact endogenous aphid resistance

Contrary to our results showing repeated failure of symbiont-based protection, aphid survival following parasitism did not differ between temperature treatments in the endogenously resistant CJ1-13 (Figure 4, Table S4; LRT $\chi^2_{temp} = 0.02$ $p = .61$) or WA4 genotypes

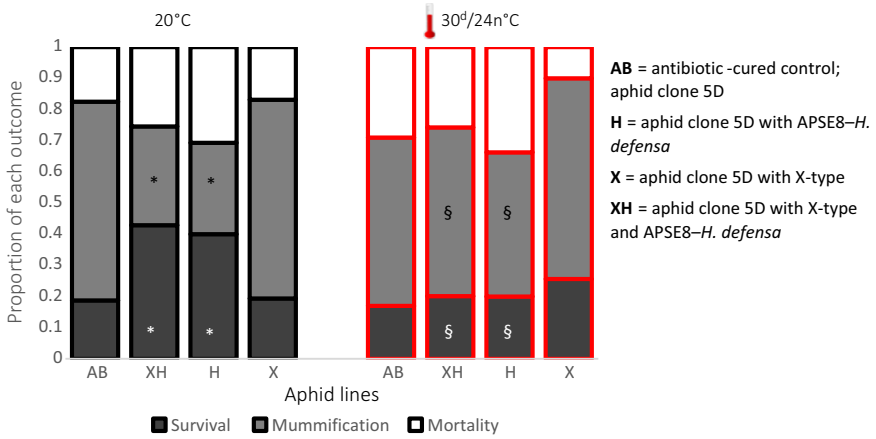

FIGURE 3 The X-type symbiont does not rescue failed *Hamiltonella defensa* protection at warmer temperatures. Aphid survival, mummification and mortality postparasitism. Red-bordered bars represent 30°C/24°C treatment, and bars lacking border represent 20°C treatment. * represent significant differences in outcome (e.g., survival) relative to uninfected control at same temperature. § represent significant differences in outcome relative to same genotype at control 20°C. N = 8 replicates of 20 aphids for each treatment [Colour figure can be viewed at wileyonlinelibrary.com]

(Figure 4, LRT χ^2_{temp} = 1.0 p = .33). We also found that temperature did not significantly alter dual mortality in either genotype.

3.4 | Effects of temperature on pea aphid heritable symbiont densities

Across all lines and treatments neither temperature (20 vs. 25 or 27°C, GLM effects test F_{temp} = 0.52, p = .48), infection with *H. defensa* (GLM ET F_{inf} = 0.77 p = .38), nor aphid clone (A2E vs. 5A, GLM ET F_{clone} = 0.52 p = .47) contributed to variability in *Buchnera* abundance after 72-hr exposure. Using ANOVA to compare *Buchnera* abundances in each aphid line reared at 25°C or 27°C relative to the same aphid line at 20°C, we also saw no consistent effects of the higher temperature on abundance (Fig. S1), although there were significant decreases in *Buchnera* in line 82B → 5A at 27°C and in line A2E at 25°C (Table S5).

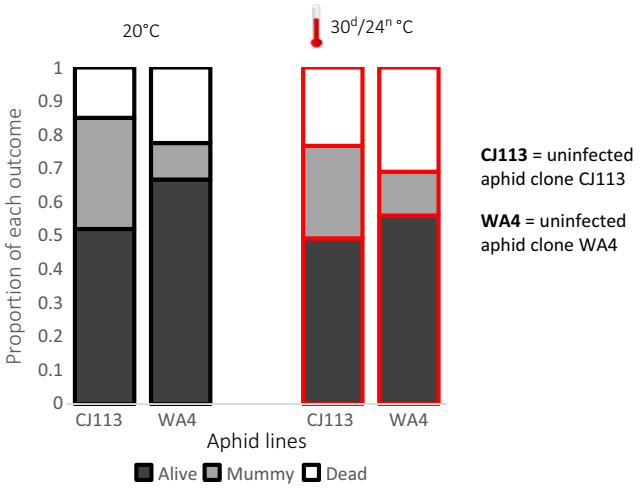

FIGURE 4 Endogenous aphid defences are robust to warmer temperatures. Mean survival, mummification and mortality postparasitism in innately resistant aphid genotypes. Red-bordered bars with thermostats represent 30°C/24°C treatment, and bars lacking border represent 20°C treatment. No significant differences were detected between treatments for either genotype (CJ113, WA4) using logistic regression (α = 0.05). N = 6 replicates of 20 aphids for each treatment [Colour figure can be viewed at wileyonlinelibrary.com]

In 72-hr-old nymphs, aphid clone (A2E vs. 5A) had a significant effect on *H. defensa* abundance (GLM ET F_{clone} = 11.2, p = .002), but exposure to higher temperatures (20 vs. 25 or 27°C) did not shape the density of this symbiont in a significant or consistent manner (Figure 5, GLM ET F_{temp} = 1.69, p = .20). Examining effects of temperature on *H. defensa* titres within lines, we found a significant increase in *H. defensa* at 25°C (but not 27°C) only in line 82B-A2E (Table S6). Across all lines, we detected no significant changes in APSE abundance after 72-hr exposure owing to temperature (GLM ET F_{temp} = 2.72, p = .71). The ratio of APSE genomic copy number to *H. defensa* cell number varied among experimental lines and APSE strains, ranging from 16.0 in 82B → 5A to 50.0 in 82B → A2E (both APSE2) to 129.4 in A1A → 5A (APSE3). Within experimental lines, these ratios did not vary across temperatures (Figure 5).

3.5 | In-field temperature measures

Daily high temperatures regularly exceeded 30°C in New York and Pennsylvania alfalfa fields from June through August (Fig. S2). In Pennsylvania, average daily temperatures from late June to early August approached or exceeded 25°C with many days showing temperatures similar to our 27°C/23°C day/night treatment, which eliminated *H. defensa* protection (Figure 2). In contrast, New York alfalfa fields exhibited average daily temperatures rarely exceeding 25°C with night-time temperatures usually lower than 20°C, suggesting conditions more favourable to *H. defensa*-driven protection.

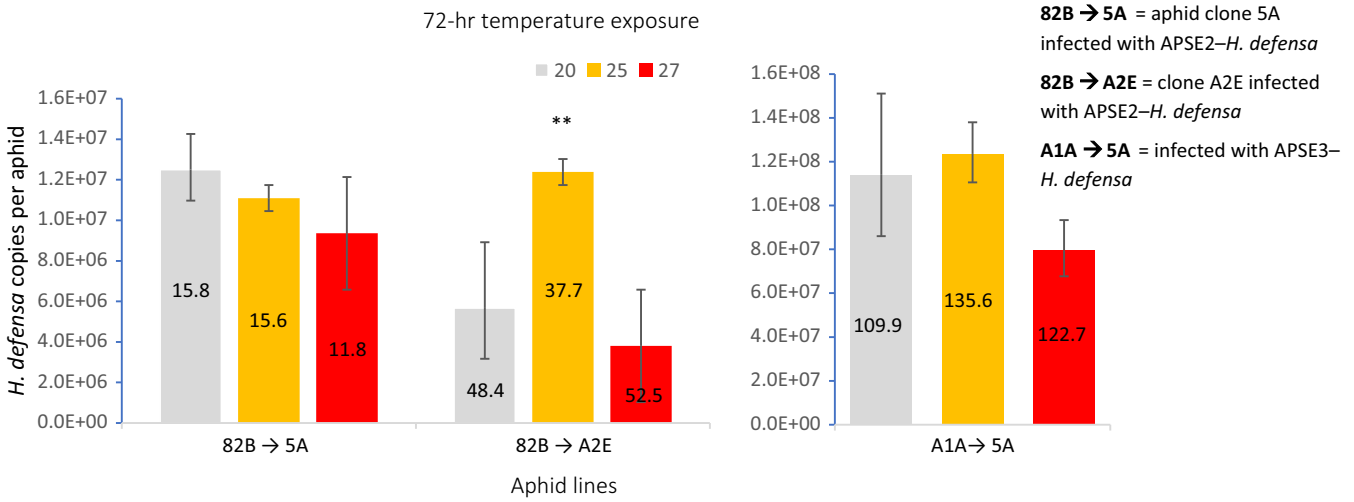

FIGURE 5 Estimates of *Hamiltonella defensa* and APSE phage densities at different temperatures. Within-aphid *H. defensa* abundance after 72-hr exposure to one of three temperatures (20, 25 & 27). The numbers within bars represent the ratio of APSE genomic copy per *H. defensa* cell. Bars represent *SE* and ** indicates $p < .01$ [Colour figure can be viewed at wileyonlinelibrary.com]

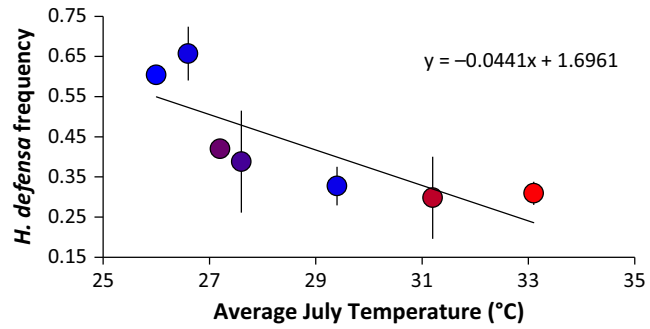

FIGURE 6 *Hamiltonella defensa* frequencies vary across North American pea aphid populations exposed to varying summer temperatures. Depicted here are average high temperatures in July. Colours represent approximate longitude, with blue = east, purple = mid-longitude and red = west. $R^2 = 0.64$, $p = .0318$ [Colour figure can be viewed at wileyonlinelibrary.com]

3.6 | *Hamiltonella defensa* frequencies across climates

Widespread collections of pea aphids from across North America revealed variability in *H. defensa* frequencies across locales (Table S7). While some locations showed year-to-year variation in frequencies, we saw, overall, that the historical average daily high temperature in July (a measure of the hottest temperatures during the year's hottest month) was negatively correlated with *H. defensa* frequencies ($R^2 = 0.64$; $F_{1,5} = 8.9$; $p = .03$) (Figure 6; Table S8).

4 | DISCUSSION

We found that temperature significantly alters pea aphid susceptibility to its major parasitoid, *A. ervi*, depending on the mode of protection. At cooler temperatures (20°C), infection with the HFS *H. defensa* resulted in increased rates of aphid survival (and fewer parasitoid mummies) following parasitism in every instance relative to uninfected controls (Figures 1–3). Levels of symbiont-based protection varied across strains, with our APSE3-bearing *H. defensa* strain (A1A) showing stronger defence than those harbouring APSE2 or APSE8 (strains 82B and 5D, respectively), consistent with earlier assays (Doremus & Oliver, 2017; Martinez, Doremus, Kraft, Kim, & Oliver, 2017; Oliver et al., 2005, 2009). At a range of warmer daytime temperatures (25, 27, 29 & 30°C), however, the effectiveness of *H. defensa*-based protection was significantly reduced, and no strain proved capable of defence at the highest temperature regimes in our study (Figures 1–3). In many cases, aphids infected with *H. defensa* were as susceptible to parasitism at the warmer temperatures as genetically identical aphids without defensive symbionts, indicating the complete failure of some strains to protect at temperatures experienced seasonally by these aphids across their North American range.

Our findings confirm predictions from prior work (Bensadia et al., 2006; Guay et al., 2009) that *H. defensa* protection largely fails under warmer conditions. But in contrast to prior expectations (Guay et al., 2009), co-infection with the X-type HFS did not rescue the temperature-induced failure of *H. defensa*-based protection, nor did this X-type symbiont itself confer protection against *A. ervi* (Figure 3).

While *H. defensa*-conferred protection consistently failed under moderately warmer temperatures, endogenous protection, assayed in the absence of HFS, remained robust (Figure 4). Collectively, these results indicate that symbiont-based protection may be less effective in the field than predicted by laboratory assays conducted at favourable temperatures. Under such conditions, endogenous defences may become more important. Our findings indicate the potential for widespread temperature-driven impacts on animal immunity across diverse systems, given that protective symbionts are common in insects and other poikilothermic animals (Florez, Biedermann, Engl, & Kaltenpoth, 2015; Oliver & Moran, 2009) and that symbionts (Johanowicz & Hoy, 1998; Pintureau & Bolland, 2001), or their

products (e.g., Johnson & Lior, 1988), are often sensitive to temperature changes.

4.1 | Are the impacts of temperature on defensive symbiont efficacy likely to shape natural symbiont dynamics?

If *H. defensa* frequencies in pea aphid populations are significantly governed by their defensive benefits (Oliver, Campos, Moran, & Hunter, 2008; Oliver et al., 2014; Rothacher et al., 2016; Smith et al., 2015), then we expect lower proportions of *H. defensa*-bearing aphids under warmer temperatures, where protective efficacies are diminished. Field recordings indicate that pea aphids often experience temperatures (mid-season 2011 PA alfalfa) sufficient to reduce *H. defensa* protection and our findings of lower *H. defensa* frequencies in regions with hotter summers are consistent with this expectation (Figure 6). Transect studies specifically designed to estimate associations between temperature and infection frequencies are needed to confirm robust patterns. We also note that not all reports show temperature variation correlating with shifting *H. defensa* dynamics. For example, while we recently reported diminished prevalence of *H. defensa* during hotter summer months in a warm region of the mid-Atlantic (Southeastern Pennsylvania), similar seasonal shifts were not found under a cooler climate (Central New York) (Smith et al., 2015); nor were the same trends recapitulated in the warmer mid-Atlantic region in a second year of field sampling (Smith, 2015). Thus, while ineffective HFS-mediated protection may limit *H. defensa* infection frequencies in field populations, other factors, including context-dependent rates of maternal transfer and other selective forces, are likely to shape *H. defensa* dynamics (Rock, 2017), potentially yielding lower *H. defensa* frequencies at higher temperatures.

4.2 | Variation in the effects of temperature on the loss of symbiont-mediated protection

While most *H. defensa* protection failed at higher temperatures, we did observe variation in the effectiveness of defence at the milder temperatures owing to aphid (or *Buchnera*) and *H. defensa* genotype. For example, in the 5A aphid genotype at a constant 25°C, protection by *H. defensa* strain 82B (APSE2) was completely lost, while strain A1A (APSE3) continued to confer significant, albeit diminished protection (Figure 1). Yet the same *H. defensa* strain 82B that failed to protected aphid clone 5A continued to confer significant, albeit reduced, protection in aphid genotype A2A (Figure 1). Our experimental aphid genotypes (Table 1) harbour *Buchnera* strains that differ in their *ibpA* promotors with known impacts on aphid thermotolerance (Dunbar et al., 2007). Thus, *Buchnera*-driven thermal sensitivity could not only impact aphid thermotolerance in the field (Harmon, Moran, & Ives, 2009), but also have a role in exposing thermally sensitive symbiont defence. With the caveat that we only examined one aphid genotype (5A) encoding the thermally sensitive "mutant" promotor (*ibpA*^{short}), we did not find that heat-induced loss of *H. defensa*-driven protection was generally different in this genotype (*ibpA*^{short}) compared to those (clones 5D, A2A or A2E) encoding the wild-type (*ibpA*^{long}) promoter. Instead, defence was lost at high temperatures in each of these backgrounds. The other thermally sensitive (*ibpA*^{short}) clone in our study, CJ1-13, is endogenously resistant to *A. ervi* and was studied without the addition of *H. defensa*. However, this genotype was defended similarly at high and low temperatures, further suggesting that *ibpA* variability is not having a large impact on interactions with parasitoids.

While warmer temperatures strongly affected symbiont-based defences, our assays examined only those effects resulting from increased temperature exposure occurring after parasitism. Of course, symbiont-defended aphids may experience stressful temperatures before parasitism, even in prior generations. The impacts of short- vs. long-term exposure await exploration, as does the question of how temperature mediates aphid–wasp interactions under natural field conditions.

4.3 | Does co-infection with X-type shape the thermal sensitivity of *Hamiltonella defensa*-encoded defence?

Co-infection with multiple HFS is a common phenomenon in the pea aphid and X-type is most commonly found co-infecting aphids, specifically those with *H. defensa* (Doremus & Oliver, 2017; Ferrari et al., 2012; Henry et al., 2013; Rock et al., 2017; Russell et al., 2013; Smith, 2015). That single X-type infections are uncommon, and co-infections with *H. defensa* are enriched in the field, suggests that effects on aphids likely vary between single and co-infection contexts. A previous report proposed that X-type rescues the loss of *H. defensa*-conferred protection at warmer temperatures (Guay et al., 2009), offering a potential co-infection-specific benefit under warmer conditions with parasitism pressure. As symbionts and host genotypes were not experimentally manipulated in this earlier work, it remained unclear whether host clone, *H. defensa* strain, X-type or an interaction among these parties was responsible for the observed association.

Here, we repeated similar experiments, fully controlling for aphid and symbiont genotypes using four sublines created from a single aphid genotype (5D) containing all HFS combinations (No HFS, *H. defensa* only, X-type only and both X-type and *H. defensa*). Consistent with Martinez et al. (2017), we found, that our APSE8 phage-bearing *H. defensa*-conferred moderate protection against the wasp *A. ervi* at 20°C. Yet X-type conferred no protection against this parasitoid as a single infection, nor did it enhance or degrade *H. defensa*-mediated protection at the control temperature under co-infection (Figure 3). At the higher temperature, APSE8-harbouring *H. defensa* protection was lost, indicating that all three *H. defensa* associated APSE types from North America (2, 3 & 8) examined to date lose some or all protection at elevated temperatures. Most notably, X-type did not rescue failed *H. defensa* protection (Figure 4) as previously hypothesized (Guay et al., 2009).

Unfortunately, we were unable to examine multiple strains of X-type, or the same X-type strain infecting additional aphid genotypes,

limiting efforts to generalize our findings as we have done for *H. defensa*. Multiple attempts were made to create the requisite experimental lines, but these efforts did not result in stable infections, most often due to high costs associated with X-only lines (Doremus & Oliver, 2017). While we only examined a single X-type strain, extensive sampling and strain typing indicate very low strain diversity of X-type (perhaps only a single strain) in the Wisconsin (USA) populations where this clone was collected (Doremus & Oliver, 2017). Also, in this region, X-type almost always occurs with the strain of APSE8 *H. defensa* used in this study. Hence, the limited strain diversity of X-type, coupled with its common co-occurrence with this particular strain of *H. defensa*, suggests that X-type may generally fail to rescue *H. defensa* protection unless there are unexpectedly strong symbiont x genotype interactions. Intriguingly, a recent survey of field-collected pea aphids revealed substantially lower X-type frequencies in the warmer Pennsylvania regions vs. cooler regions from New York; locales represented in our field temperature survey assays (Fig. S2) (Smith et al., 2015). This pattern is consistent with our finding that X-type does not rescue failed *H. defensa* protection as this would favour higher X-type frequencies under warm climates.

While we find no evidence for a substantial role of this common X-type strain in defence of pea aphids against *A. ervi*, several studies using European pea aphids suggest that some X-type strains infecting pea aphids may influence protection against parasitoids under co-infection. One study found that co-infection with X-type and *H. defensa* correlated with complete resistance to parasitoids, although host genotype or *H. defensa* may provide all the protection (Leclair et al., 2016). A second study found that X-type, in association with the HFS *Spiroplasma*, contributed resistance towards *A. ervi*; it remains unclear, however, whether such European X-type strains confer resistance to *A. ervi* in the absence of *Spiroplasma* (Heyworth & Ferrari, 2015). The same authors conducted a follow-up study investigating the effects of brief "heat shocks" prior to, and after, parasitism by *A. ervi* (Heyworth & Ferrari, 2016). While co-infection with X-type and *Spiroplasma* provided only marginally non-significant protection at the control temperature (20°C), heat shock prior to parasitism resulted in *decreased* protection in co-infected aphids compared to *Spiroplasma*-only infected aphids. This finding does not support a role for X-type in enhancing symbiont-based resistance at higher temperatures. Yet it does further indicate that heat stress can impact symbiont-modulated protection to parasitoids.

4.4 | Lose–lose or win–lose: propensities for dual mortality vary across aphid genotypes

Studies using the black bean aphid, *Aphis fabae*, also reported effects of temperature on aphid susceptibility, with lines exhibiting effective *H. defensa*-based protection under permissive temperatures becoming more susceptible to *Lysiphlebus fabarum* parasitoids at 29°C (Cayetano & Vorburger, 2013b). In this same system, however, exposure to a 39°C heat shock was strongly detrimental to aphid survival, rendering aphids unsuitable hosts for wasps that had overcome their defences under more permissive temperatures (Cayetano & Vorburger, 2013a). We report a similar phenomenon of high mortality for both aphids and wasps at higher temperatures in two aphid backgrounds: while wasps experienced heightened success in these clones (5A and A2E) in the $27^{day}/23^{night}$°C treatment, both aphids and wasps suffered heightened mortality at $29^{day}/25^{night}$°C. Thus, while *H. defensa* infection effectively suppressed developing wasps under cooler conditions, symbiont defence was largely eliminated at moderate temperatures, tipping the balance in favour of wasps, while hot temperatures caused mortality of both aphids and wasps after parasitism (Figure 2). In certain aphid genotypes, the odds of success for each participant may, thus, be greatest under a narrow range of environmental conditions. In contrast to these results, two aphid clones with endogenous resistance (CJ1-13, WA4) and one susceptible, symbiont-free aphid subclone (5D) did not experience higher dual mortality at the warmest temperatures ($30^{day}/24^{night}$°C). Hence, genetic variability for the mode and strength of defence may shape the viability of *A. ervi* populations across varying climates.

4.5 | Do symbiont densities predict defensive levels?

Temperature is known to influence titres of HFS, which in turn may influence the strength of phenotype expression (e.g., Bourtzis, Nirgianaki, Markakis, & Savakis, 1996; Breeuwer & Werren, 1993; Hurst, Johnson, vd Schulenburg, & Fuyama, 2000). Surprisingly, however, our qPCR estimates across aphids exposed to moderately warmer temperatures (25 or 27°C) for 72 hr (i.e., second- to third-instar nymphs and the age when aphids were parasitized in our assays) revealed few consistent effects on abundances of nutritional (Fig. S1) or protective symbionts (Figure 5). This idiosyncratic response suggests that *H. defensa* and APSE titre may not be strong indicators of defensive capability and that loss of protection is not simply a function of symbiont mortality under elevated temperatures (Martinez, Weldon et al., 2014). One plausible explanation is that warmer temperatures influence symbiont or phage products involved in defence, such as heat-labile toxins, rendering them less effective. Alternatively, increasing temperatures could accelerate wasp development rates, shortening the period of time during which young wasps are susceptible to *H. defensa*-APSE and allowing the parasitoid to "bypass" exposure (Martinez, Weldon et al., 2014).

5 | CONCLUSIONS AND IMPLICATIONS

Effects of temperature on symbiont-mediated defence may have important implications for biological control programmes as parasitoids potentially vary in their effectiveness across seasons and locales. In natural populations, predicted increases in average and extreme temperatures (Schar et al., 2004) may disrupt long-standing defensive mutualisms and threaten the long-term viability of the antagonistic host–parasite interactions, with effects potentially

extending to the community of organisms interacting with the focal species. While symbiont roles in host–enemy co-evolution remain largely underexplored (Oliver et al., 2014), the varying thermal sensitivities of distinct defensive modalities may shape patterns of antagonistic co-evolution. As a result, we might predict a geographic mosaic, whereby thermosensitive symbionts largely govern host–enemy co-evolution in cooler regions, while thermally robust modes of resistance (i.e., host-driven defences) are the target of virulence/counter-virulence dynamics where it is warm. This intriguing possibility awaits further testing within the tractable pea aphid system.

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DATA ACCESSIBILITY

Data from the bioassays are available at Dryad https://doi.org/10.5061/dryad.jk88v.

AUTHOR CONTRIBUTIONS

M.R.D. and K.M.O. designed experiments; M.R.D., A.H.S., K.L.K. and A.J.H. performed experiments; M.R.D., A.H.S. and K.M.O. analysed data; M.R.D., J.A.R. and K.M.O. wrote the manuscript.

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REFERENCES

Allen, D. E., & Little, T. J. (2011). Dissecting the effect of a heterogeneous environment on the interaction between host and parasite fitness traits. *Evolutionary Ecology*, *25*, 499–508. https://doi.org/10.1007/s10682-010-9413-2

Bensadia, F., Boudreault, S., Guay, J.-F., Michaud, D., & Cloutier, C. (2006). Aphid clonal resistance to a parasitoid fails under heat stress. *Journal of Insect Physiology*, *52*, 146–157. https://doi.org/10.1016/j.jinsphys.2005.09.011

Bilodeau, E., Simon, J. C., Guay, J. F., Turgeon, J., & Cloutier, C. (2013). Does variation in host plant association and symbiont infection of pea aphid populations induce genetic and behaviour differentiation of its main parasitoid, *Aphidius ervi*? *Evolutionary Ecology*, *27*, 165–184. https://doi.org/10.1007/s10682-012-9577-z

Bourtzis, K., Nirgianaki, A., Markakis, G., & Savakis, C. (1996). Wolbachia infection and cytoplasmic incompatibility in *Drosophila* species. *Genetics*, *144*, 1063–1073.

Breeuwer, J. A. J., & Werren, J. H. (1993). Cytoplasmic incompatibility and bacterial density in *Nasonia vitripennis*. *Genetics*, *135*, 565–574.

Brisson, J. A., & Stern, D. L. (2006). The pea aphid, *Acyrthosiphon pisum*: An emerging genomic model system for ecological, developmental and evolutionary studies. *BioEssays*, *28*, 747–755. https://doi.org/10.1002/(ISSN)1521-1878

Brownlie, J. C., & Johnson, K. N. (2009). Symbiont-mediated protection in insect hosts. *Trends in Microbiology*, *17*, 348–354. https://doi.org/10.1016/j.tim.2009.05.005

Cayetano, L., & Vorburger, C. (2013a). Effects of heat shock on resistance to parasitoids and on life history traits in an aphid/endosymbiont system. *PLoS One*, *8*, e75966. https://doi.org/10.1371/journal.pone.0075966

Cayetano, L., & Vorburger, C. (2013b). Genotype-by-genotype specificity remains robust to average temperature variation in an aphid/endosymbiont/parasitoid system. *Journal of Evolutionary Biology*, *26*, 1603–1610. https://doi.org/10.1111/jeb.2013.26.issue-7

Cirimotich, C. M., Dong, Y. M., Clayton, A. M., Sandiford, S. L., Souza-Neto, J. A., Mulenga, M., & Dimopoulos, G. (2011). Natural microbe-mediated refractoriness to *Plasmodium* infection in *Anopheles gambiae*. *Science*, *332*, 855–858. https://doi.org/10.1126/science.1201618

Corbin, C., Heyworth, E. R., Ferrari, J., & Hurst, G. D. D. (2017). Heritable symbionts in a world of varying temperature. *Heredity*, *118*, 10–20. https://doi.org/10.1038/hdy.2016.71

Degnan, P. H., & Moran, N. A. (2008a). Diverse phage-encoded toxins in a protective insect endosymbiont. *Applied and Environmental Microbiology*, *74*, 6782–6791. https://doi.org/10.1128/AEM.01285-08

Degnan, P. H., & Moran, N. A. (2008b). Evolutionary genetics of a defensive facultative symbiont of insects: Exchange of toxin-encoding bacteriophage. *Molecular Ecology*, *17*, 916–929. https://doi.org/10.1111/j.1365-294x.2007.03616.x

Doremus, M. R., & Oliver, K. M. (2017). Aphid heritable symbiont exploits defensive mutualism. *Applied and Environmental Microbiology*, *83*, e03276-16. https://doi.org/10.1128/aem.03276-16

Douglas, A. (1998). Nutritional interactions in insect-microbial symbioses: Aphids and their symbiotic bacteria *Buchnera*. *Annual Review of Entomology*, *43*, 17–37. https://doi.org/10.1146/annurev.ento.43.1.17

Douglas, A. E. (2015). Multiorganismal insects: Diversity and function of resident microorganisms. *Annual Review of Entomology*, *60*, 17–34. https://doi.org/10.1146/annurev-ento-010814-020822

Douglas, A., Francois, C., & Minto, L. (2006). Facultative 'secondary' bacterial symbionts and the nutrition of the pea aphid, *Acyrthosiphon pisum*. *Physiological Entomology*, *31*, 262–269. https://doi.org/10.1111/pen.2006.31.issue-3

Dunbar, H. E., Wilson, A. C., Ferguson, N. R., & Moran, N. A. (2007). Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. *PLoS Biology*, *5*, e96. https://doi.org/10.1371/journal.pbio.0050096

Ferrari, J., Scarborough, C. L., & Godfray, H. C. J. (2007). Genetic variation in the effect of a facultative symbiont on host-plant use by pea aphids. *Oecologia*, *153*, 323–329. https://doi.org/10.1007/s00442-007-0730-2

Ferrari, J., West, J. A., Via, S., & Godfray, H. C. J. (2012). Population genetic structure and secondary symbionts in host-associated populations of the pea aphid complex. *Evolution*, *66*, 375–390. https://doi.org/10.1111/evo.2012.66.issue-2

Florez, L. V., Biedermann, P. H. W., Engl, T., & Kaltenpoth, M. (2015). Defensive symbioses of animals with prokaryotic and eukaryotic microorganisms. *Natural Product Reports*, *32*, 904–936. https://doi.org/10.1039/C5NP00010F

Garbutt, J. S., Scholefield, J. A., Vale, P. F., & Little, T. J. (2014). Elevated maternal temperature enhances offspring disease resistance in *Daphnia magna*. *Functional Ecology*, *28*, 424–431. https://doi.org/10.1111/fec.2014.28.issue-2

Gloor, G. B., Preston, C. R., Johnsonschlitz, D. M., Nassif, N. A., Phillis, R. W., Benz, W. K., … Engels, W. R. (1993). Type-I repressors of p-element mobility. *Genetics*, *135*, 81–95.

Guay, J.-F., Boudreault, S., Michaud, D., & Cloutier, C. (2009). 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. https://doi.org/10.1016/j.jinsphys.2009.06.006

Haine, E. R. (2008). Symbiont-mediated protection. *Proceedings of the Royal Society B-Biological Sciences*, *275*, 353–361. https://doi.org/10.1098/rspb.2007.1211

Hamilton, P. T., & Perlman, S. J. (2013). Host defense via symbiosis in *Drosophila*. *PLoS Pathogens*, *9*, e1003808. https://doi.org/10.1371/journal.ppat.1003808

Hance, T., van Baaren, J., Vernon, P., & Boivin, G. (2007). Impact of extreme temperatures on parasitoids in a climate change perspective. *Annual Review of Entomology*, *52*, 107–126. https://doi.org/10.1146/annurev.ento.52.110405.091333

Harmon, J. P., Moran, N. A., & Ives, A. R. (2009). Species response to environmental change: Impacts of food web interactions and evolution. *Science*, *323*, 1347–1350. https://doi.org/10.1126/science.1167396

Hegland, S. J., Nielsen, A., Lázaro, A., Bjerknes, A. L., & Totland, Ø. (2009). How does climate warming affect plant-pollinator interactions? *Ecology Letters*, *12*, 184–195. https://doi.org/10.1111/ele.2009.12.issue-2

Henry, L. M., Peccoud, J., Simon, J. C., Hadfield, J. D., Maiden, M. J., Ferrari, J., & Godfray, H. C. (2013). Horizontally transmitted symbionts and host colonization of ecological niches. *Current Biology*, *23*, 1713–1717. https://doi.org/10.1016/j.cub.2013.07.029

Henter, H. J., & Via, S. (1995). The potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. *Evolution*, *49*, 427–438. https://doi.org/10.1111/evo.1995.49.issue-3

Heyworth, E., & Ferrari, J. (2015). A facultative endosymbiont in aphids can provide diverse ecological benefits. *Journal of Evolutionary Biology*, *28*, 1753–1760. https://doi.org/10.1111/jeb.12705

Heyworth, E. R., & Ferrari, J. (2016). Heat stress affects facultative symbiont-mediated protection from a parasitoid wasp. *PLoS One*, *11*, e0167180. https://doi.org/10.1371/journal.pone.0167180

Humphreys, N. J., & Douglas, A. E. (1997). Partitioning of symbiotic bacteria between generations of insect: A quantitative study of a *Buchnera* sp. in the pea aphid (*Acyrthosiphon pisum*) reared at different temperatures. *Applied and Environmental Microbiology*, *63*, 3294–3296.

Hurst, G. D., Johnson, A. P., vd Schulenburg, J. H. G., & Fuyama, Y. (2000). Male-killing *Wolbachia* in *Drosophila*: A temperature-sensitive trait with a threshold bacterial density. *Genetics*, *156*, 699–709.

Jaenike, J., Unckless, R., Cockburn, S. N., Boelio, L. M., & Perlman, S. J. (2010). Adaptation via symbiosis: Recent spread of a *Drosophila* defensive symbiont. *Science*, *329*, 212–215. https://doi.org/10.1126/science.1188235

Johanowicz, D. L., & Hoy, M. A. (1998). Experimental induction and termination of non-reciprocal reproductive incompatibilities in a parahaploid mite. *Entomologia Experimentalis Et Applicata*, *87*, 51–58. https://doi.org/10.1046/j.1570-7458.1998.00303.x

Johnson, W. M., & Lior, H. (1988). A new heat-labile cytolethal distending toxin (CLDT) produced by *Escherichia coli* isolates from clinical material. *Microbial Pathogenesis*, *4*, 103–113. https://doi.org/10.1016/0882-4010(88)90052-6

Kiers, E. T., Palmer, T. M., Ives, A. R., Bruno, J. F., & Bronstein, J. L. (2010). Mutualisms in a changing world: An evolutionary perspective. *Ecology Letters*, *13*, 1459–1474. https://doi.org/10.1111/ele.2010.13.issue-12

Lazzaro, B. P., & Little, T. J. (2009). Immunity in a variable world. *Philosophical Transactions of the Royal Society B-Biological Sciences*, *364*, 15–26. https://doi.org/10.1098/rstb.2008.0141

Leclair, M., Pons, I., Maheo, F., Morlière, S., Simon, J. C., & Outreman, Y. (2016). Diversity in symbiont consortia in the pea aphid complex is associated with large phenotypic variation in the insect host. *Evolutionary Ecology*, *30*, 925–941. https://doi.org/10.1007/s10682-016-9856-1

Leonardo, T. E., & Muiru, G. T. (2003). Facultative symbionts are associated with host plant specialization in pea aphid populations. *Proceedings of the Royal Society B-Biological Sciences*, *270*, S209–S212. https://doi.org/10.1098/rsbl.2003.0064

Lu, M., Hulcr, J., & Sun, J. H. (2016). The role of symbiotic microbes in insect invasions. *Annual Review of Ecology, Evolution, and Systematics*, *47*, 487–505. https://doi.org/10.1146/annurev-ecolsys-121415-032050

Łukasik, P., van Asch, M., Guo, H., Ferrari, J., & Godfray, C. J. (2013). Unrelated facultative endosymbionts protect aphids against a fungal pathogen. *Ecology Letters*, *16*, 214–218. https://doi.org/10.1111/ele.2012.16.issue-2

Martinez, A. J., Doremus, M. R., Kraft, L. J., Kim, K. L., & Oliver, K. M. (2017). Multi-modal defences in aphids offer redundant protection and increased costs likely impeding a protective mutualism. *Journal of Animal Ecology*, *00*, 1–14. https://doi.org/10.1111/1365-2656.12675

Martinez, A. J., Ritter, S. G., Doremus, M. R., Russell, J. A., & Oliver, K. M. (2014). Aphid-encoded variability in susceptibility to a parasitoid. *BMC Evolutionary Biology*, *14*, 127. https://doi.org/10.1186/1471-2148-14-127

Martinez, A. J., Weldon, S. R., & Oliver, K. M. (2014). Effects of parasitism on aphid nutritional and protective symbioses. *Molecular Ecology*, *23*, 1594–1607. https://doi.org/10.1111/mec.12550

Mohan, J. E., Cowden, C. C., Baas, P., Dawadi, A., Frankson, P. T., Helmick, K., … Taylor, M. (2014). Mycorrhizal fungi mediation of terrestrial ecosystem responses to global change: Mini-review. *Fungal Ecology*, *10*, 3–19. https://doi.org/10.1016/j.funeco.2014.01.005

Montllor, C. B., Maxmen, A., & Purcell, A. H. (2002). Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. *Ecological Entomology*, *27*, 189–195. https://doi.org/10.1046/j.1365-2311.2002.00393.x

Moran, N. A., Degnan, P. H., Santos, S. R., Dunbar, H. E., & Ochman, H. (2005). The players in a mutualistic symbiosis: Insects, bacteria, viruses, and virulence genes. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 16919–16926. https://doi.org/10.1073/pnas.0507029102

Moran, N. A., McCutcheon, J. P., & Nakabachi, A. (2008). Genomics and evolution of heritable bacterial symbionts. *Annual Review of Genetics*, *42*, 165–190. https://doi.org/10.1146/annurev.genet.41.110306.130119

Nakabachi, A., Ueoka, R., Oshima, K., Teta, R., Mangoni, A., Gurgui, M., … Inoue, H. (2013). Defensive bacteriome symbiont with a drastically reduced genome. *Current Biology*, *23*, 1478–1484. https://doi.org/10.1016/j.cub.2013.06.027

Newcombe, G., Shipunov, A., Eigenbrode, S., Raghavendra, A. K., Ding, H., Anderson, C. L., … Schwarzländer, M. (2009). Endophytes influence protection and growth of an invasive plant. *Communicative & Integrative Biology*, *2*, 29–31. https://doi.org/10.4161/cib.2.1.7393

Ohtaka, C., & Ishikawa, H. (1991). Effects of heat-treatment on the symbiotic system of an aphid mycetocyte. *Symbiosis*, *11*, 19–30.

Oliver, K. M., Campos, J., Moran, N. A., & Hunter, M. S. (2008). Population dynamics of defensive symbionts in aphids. *Proceedings of the Royal Society of London B: Biological Sciences*, *275*, 293–299. https://doi.org/10.1098/rspb.2007.1192

Oliver, K. M., Degnan, P. H., Burke, G. R., & Moran, N. A. (2010). Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. *Annual Review of Entomology*, *55*, 247–266. https://doi.org/10.1146/annurev-ento-112408-085305

Oliver, K. M., Degnan, P. H., Hunter, M. S., & Moran, N. A. (2009). Bacteriophages encode factors required for protection in a symbiotic mutualism. *Science*, *325*, 992–994. https://doi.org/10.1126/science.1174463

Oliver, K. M., & Martinez, A. J. (2014). How resident microbes modulate ecologically-important traits of insects. *Current Opinion in Insect Science*, *4*, 1–7. https://doi.org/10.1016/j.cois.2014.08.001

Oliver, K. M., & Moran, N. A. (2009). Defensive symbionts in aphids and other insects. In J. F. White & M. S. Torres (Eds.), *Defensive mutualism in microbial symbiosis* (pp. 129–147). Boca Raton, FL: CRC Press.

Oliver, K. M., Moran, N. A., & Hunter, M. S. (2005). Variation in resistance to parasitism in aphids is due to symbionts not host genotype. *Proceedings of the National Academy of Sciences*, *102*, 12795–12800. https://doi.org/10.1073/pnas.0506131102

Oliver, K. M., Moran, N. A., & Hunter, M. S. (2006). Costs and benefits of a superinfection of facultative symbionts in aphids. *Proceedings of the Royal Society of London B: Biological Sciences*, *273*, 1273–1280. https://doi.org/10.1098/rspb.2005.3436

Oliver, K. M., Noge, K., Huang, E. M., Campos, J. M., Becerra, J. X., & Hunter, M. S. (2012). Parasitic wasp responses to symbiont-based defense in aphids. *BMC Biology*, *10*, 11. https://doi.org/10.1186/1741-7007-10-11

Oliver, K. M., Russell, J. A., Moran, N. A., & Hunter, M. S. (2003). Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 1803–1807. https://doi.org/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. *Functional Ecology*, *28*, 341–355. https://doi.org/10.1111/fec.2014.28.issue-2

Parker, B. J., Spragg, C. J., Altincicek, B., & Gerardo, N. M. (2013). Symbiont-mediated protection against fungal pathogens in pea aphids: A role for pathogen specificity? *Applied and Environmental Microbiology*, *79*, 2455–2458. https://doi.org/10.1128/AEM.03193-12

Peccoud, J., Ollivier, A., Plantegenest, M., & Simon, J. C. (2009). A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. *Proceedings of the National Academy of Sciences*, *106*, 7495–7500. https://doi.org/10.1073/pnas.0811117106

Pintureau, B., & Bolland, P. (2001). Response to selection for a high or a low sex ratio in *Wolbachia*-infected lines of *Trichogramma cordubensis* subjected to high temperature. *Vestnik Zoology*, *35*, 83–86.

Rock, D. (2017). *Transmission Dynamics of Symbionts in Pea Aphids*, Drexel University, MS Thesis.

Romo, C. M., & Tylianakis, J. M. (2013). Elevated temperature and drought interact to reduce parasitoid effectiveness in suppressing hosts. *PLoS One*, *8*, e58136. https://doi.org/10.1371/journal.pone.0058136

Rothacher, L., Ferrer-Suay, M., & Vorburger, C. (2016). Bacterial endosymbionts protect aphids in the field and alter parasitoid community composition. *Ecology*, *97*, 1712–1723. https://doi.org/10.1890/15-2022.1

Russell, J. A., & Moran, N. A. (2006). Costs and benefits of symbiont infection in aphids: Variation among symbionts and across temperatures. *Proceedings of the Royal Society of London B: Biological Sciences*, *273*, 603–610. https://doi.org/10.1098/rspb.2005.3348

Russell, J. A., Weldon, S., Smith, A. H., Kim, K. L., Hu, Y., Łukasik, P., … Oliver, K. M. (2013). Uncovering symbiont-driven genetic diversity across North American pea aphids. *Molecular Ecology*, *22*, 2045–2059. https://doi.org/10.1111/mec.12211

Scarborough, C. L., Ferrari, J., & Godfray, H. (2005). Aphid protected from pathogen by endosymbiont. *Science*, *310*, 1781. https://doi.org/10.1126/science.1120180

Schar, C., Vidale, P. L., Luthi, D., Frei, C., Häberli, C., Liniger, M. A., & Appenzeller, C. (2004). The role of increasing temperature variability in European summer heatwaves. *Nature*, *427*, 332–336. https://doi.org/10.1038/nature02300

Schellhorn, N. A., Kuhman, T. R., Olson, A. C., & Ives, A. R. (2002). Competition between native and introduced parasitoids of aphids: Nontarget effects and biological control. *Ecology*, *83*, 2745–2757. https://doi.org/10.1890/0012-9658(2002)083[2745:CBNAIP]2.0.CO;2

Six, D. L. (2013). The bark beetle holobiont: Why microbes matter. *Journal of Chemical Ecology*, *39*, 989–1002. https://doi.org/10.1007/s10886-013-0318-8

Smith, A. H. (2015). *The causes and consequences of a diverse and dynamic microbiome*, Drexel University. Ph.D. Dissertation.

Smith, A. H., Łukasik, P., O'Connor, M. P., Lee, A., Mayo, G., Drott, M. T., … Oliver, K. M. (2015). Patterns, causes and consequences of defensive microbiome dynamics across multiple scales. *Molecular Ecology*, *24*, 1135–1149. https://doi.org/10.1111/mec.13095

Thomas, M. B., & Blanford, S. (2003). Thermal biology in insect-parasite interactions. *Trends in Ecology & Evolution*, *18*, 344–350. https://doi.org/10.1016/S0169-5347(03)00069-7

Tsuchida, T., Koga, R., & Fukatsu, T. (2004). Host plant specialization governed by facultative symbiont. *Science*, *303*, 1989. https://doi.org/10.1126/science.1094611

Vorburger, C. (2014). The evolutionary ecology of symbiont-conferred resistance to parasitoids in aphids. *Insect Science*, *21*, 251–264. https://doi.org/10.1111/1744-7917.12067

Weldon, S., Strand, M., & Oliver, K. (2013). Phage loss and the breakdown of a defensive symbiosis in aphids. *Proceedings of the Royal Society of London B: Biological Sciences*, *280*, 20122103. https://doi.org/10.1098/rspb.2012.2103

Wernegreen, J. J. (2012). Mutualism meltdown in insects: Bacteria constrain thermal adaptation. *Current Opinion in Microbiology*, *15*, 255–262. https://doi.org/10.1016/j.mib.2012.02.001

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