Obligate bacterial endosymbionts limit thermal tolerance of insect host species

Bo Zhang[a,b], Sean P. Leonard[b], Yiyuan Li[b], and Nancy A. Moran[b,1]

[a]Laboratory of Predatory Mites, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, 100193 Beijing, People's Republic of China; and [b]Department of Integrative Biology, University of Texas, Austin, TX 78712

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The thermal tolerance of an organism limits its ecological and geographic ranges and is potentially affected by dependence on temperature-sensitive symbiotic partners. Aphid species vary widely in heat sensitivity, but almost all aphids are dependent on the nutrient-provisioning intracellular bacterium *Buchnera*, which has evolved with aphids for 100 million years and which has a reduced genome potentially limiting heat tolerance. We addressed whether heat sensitivity of *Buchnera* underlies variation in thermal tolerance among 5 aphid species. We measured how heat exposure of juvenile aphids affects later survival, maturation time, and fecundity. At one extreme, heat exposure of *Aphis gossypii* enhanced fecundity and had no effect on the *Buchnera* titer. In contrast, heat suppressed *Buchnera* populations in *Aphis fabae*, which suffered elevated mortality, delayed development and reduced fecundity. Likewise, in *Acyrthosiphon kondoi* and *Acyrthosiphon pisum*, heat caused rapid declines in *Buchnera* numbers, as well as reduced survivorship, development rate, and fecundity. Fecundity following heat exposure is severely decreased by a *Buchnera* mutation that suppresses the transcriptional response of a gene encoding a small heat shock protein. Similarly, absence of this *Buchnera* heat shock gene may explain the heat sensitivity of *Ap. fabae*. Fluorescent in situ hybridization revealed heat-induced deformation and shrinkage of bacteriocytes in heat-sensitive species but not in heat-tolerant species. Sensitive and tolerant species also differed in numbers and transcriptional responses of heat shock genes. These results show that shifts in *Buchnera* heat sensitivity contribute to host variation in heat tolerance.

symbiosis | aphididae | thermal adaptation | RNA-Seq | heat shock proteins

Thermal tolerance is a basic determinant of an organism's ecology: The ability to survive and to reproduce in the face of variation in temperature affects geographic range, competitive ability, and population dynamics. Many animals and plants engage in intimate symbiotic interactions with microorganisms, and these symbionts can impact thermal tolerance. When hosts are dependent on symbionts that are themselves heat sensitive or cold sensitive, the host's temperature range and geographic distribution may be curtailed by the symbiosis. For example, the leaf-cutter ant *Atta texana* extends its geographic range into colder regions by adopting cold-tolerant mutualistic fungi (1). Often, symbionts appear to be less heat tolerant than hosts. Thus, corals are subject to heat-induced elimination of their obligate algal symbionts, and if symbionts are killed by warming, the corals then bleach and die (2). Similarly, gut symbionts of stinkbugs are heat sensitive: Warming suppresses these symbionts causing stinkbug fitness to drop (3).

Many insect groups maintain obligate symbioses with maternally transmitted bacteria that provide essential nutrients to their hosts (4, 5), raising the question of whether this dependence on heritable symbionts curtails temperature tolerances and geographic ranges (6, 7). Heritable symbionts may be particularly heat sensitive due to long-term accumulation of deleterious mutations that cause rapid evolution of proteins and loss of many genes (5, 8). The resulting extreme genome reduction reflects clonality and limited genetic population sizes over millions of years of vertical transmission within hosts (5). Among the consequences are deleterious amino acid replacements, including replacements that affect translational machinery itself, leading to decline in protein quality (9).

Aphids and their intracellular bacterial associates *Buchnera aphidicola* are a widely studied model of obligate symbiosis. *Buchnera* has diversified with aphids through maternal transmission for >100 million years; their tiny genomes encode only 354–587 proteins (10) but retain genes underlying production of amino acids needed for host nutrition (11). Several observations suggest that *Buchnera* is heat sensitive. First, *Buchnera* proteins show reduced thermal stability compared to homologous proteins of related free-living bacteria (12). Second, as in other insect endosymbionts (6), *Buchnera* shows constitutively elevated expression of heat shock proteins including GroEL and DnaK; high expression of these chaperones rescues misfolded proteins in a destabilized proteome (13). Functionality of *Buchnera* enzymes expressed in *Escherichia coli* requires overexpression of GroEL (14) or growth at lower temperature (15). Third, in the pea aphid, *Ac. pisum*, some *Buchnera* genotypes are especially susceptible to heat due to a recurring mutation in the heat shock promoter of *ibpA*, which encodes a universal small heat shock protein (16, 17). Aphids with this haplotype lose all or most *Buchnera* following heat exposure and suffer drastic reductions in fecundity; experimental replacement of this haplotype improves heat tolerance (18). Fourth, the facultative symbiont, *Serratia symbiotica*, which ameliorates the negative effects of heat on *Ac.*

Significance

The ability to withstand heat is fundamental to an organism's ecology, and variation in heat tolerance affects the geographic range of species. Many insects have obligate relationships with heat-sensitive bacterial symbionts, raising the question of whether variation in heat sensitivity among symbionts underlies variation in heat sensitivity among their host species. This study compared aphid species with different abilities to withstand heat and showed that titers of the bacterial symbiont *Buchnera* drop rapidly following heat exposure of heat-sensitive aphid species, whereas *Buchnera* titers in heat-tolerant aphid species are unaffected or show delayed responses. While heat-induced responses of aphid hosts themselves also may contribute, symbiont ability to withstand heat appears to be a central determinant of host thermal range.

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[1]To whom correspondence may be addressed. Email: nancy.moran@austin.utexas.edu.

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pisum (19, 20) appears to shield hosts by buffering *Buchnera* populations, possibly by producing protective metabolites (21). Finally, at least, in some cases, levels of *Buchnera* within aphids appear to decline at higher temperatures (22).

Our central questions in this study was whether *Buchnera* limits the heat tolerance of aphid hosts and whether variation among aphid species in heat tolerance is linked to variation in *Buchnera*. Aphid species vary widely in heat sensitivity (23, 24). We documented aphid survivorship and reproduction following heat challenge in 5 widely distributed aphid species chosen on the basis of apparent differences in thermal tolerance, partly reflected in geographic ranges (25) (*SI Appendix*, Table S1). *Aphis craccivora* and *Ap. gossypii* are generalist crop pests distributed worldwide in warm temperate and tropical regions, whereas *Ap. fabae* is a generalist that is most common in cool regions, including northern Europe and Canada. *Ac. kondoi* and *Ac. pisum* specialize on alfalfa and favor cool environments. We included a second *Ac. pisum* line in which *Buchnera* bears a single nucleotide deletion in the *ibpA* heat shock promoter, increasing heat sensitivity (16, 18). We quantified symbiont titers following heat exposure, visualized bacteriocytes with and without heat exposure, and explored the genomic and transcriptional underpinnings of heat responses in both aphid host and *Buchnera*.

Results

Effects of Heat Exposure on Aphid Survival, Developmental Time, and Fecundity. We first measured effects of second-day sublethal exposure to 38 °C on later survival. As expected, we found significant differences among the 6 aphid lines (Fig. 1*A*, $\chi^2 = 42.811$, df = 5, $P < 0.001$). *Ap. craccivora* and *Ap. gossypii* had zero mortality following heat exposure, while *Ap. fabae* survival dropped to 82.4%. *Ac. pisum* AusC in which *Buchnera* carries the *ibpA* promoter mutation had the lowest survival rate at 58.9% at the end of the trial, significantly lower than that of *Ac. pisum* 5AYC at 76.1% ($P = 0.048$). Controls showed no mortality except for 1 *Ap. craccivora* individual, resulting in its heat treatment survival score over 100% after control calibration.

The aphid lines also differed in how heat exposure affected time to maturity and fecundity (Fig. 1 *B* and *C*). Strikingly, *Ap. gossypii* performed better under heat exposure; it did not delay first reproduction (developmental time: W = 105.5, $P = 0.604$; fecundity: W = 51.5, $P = 0.012$). *Ap. craccivora* was also tolerant to heat exposure, which had no significant effects on developmental time or fecundity. In contrast, all *Acyrthosiphon* lines were negatively affected. *Ac. kondoi* suffered both delayed maturity and reduced fecundity (developmental time: W = 0, $P < 0.001$; fecundity: W = 212.5, $P < 0.001$). Both lines of *Ac. pisum* showed delayed maturity after heat shock (developmental time: 5AYC: W = 10, $P < 0.001$; AusC: W = 0, $P < 0.001$). However, the *Ac. pisum* lines differed sharply in the effect of heat on fecundity. *Ac. pisum* AusC with the heat-sensitive *Buchnera ibpA* genotype showed a steep drop in fecundity (W = 225, $P < 0.001$); whereas *Ac. pisum* 5AYC with the relatively heat-tolerant *Buchnera ibpA* genotype showed no change in fecundity (W = 110, $P = 0.933$).

Effect of Heat Exposure on *Buchnera* Titer. To address whether the effects of heat on hosts reflect impacts on obligate symbionts, we measured effects of heat exposure on the *Buchnera* titer in each of the aphid lines (Fig. 2 and *SI Appendix*, Table S2). *Buchnera* of *Ap. gossypii* was least affected, and *Buchnera* titers were unaffected by heat exposure at any time point. *Buchnera* titers in the 3 *Aphis* lines were not affected at Day 3 (24 h after exposure); but *Buchnera* titers in heat-exposed *Ap. craccivora* and *Ap. fabae* were reduced at Days 6 and 9 (Fig. 2 *A*–*C*). In contrast to all *Aphis* lines, *Buchnera* in all *Acyrthosiphon* lines underwent reductions within 24 h after heat exposure (Day 3) (Fig. 2 *D*–*F*). The effect on *Ac. pisum* AusC was especially persistent as heat exposure reduced titers at all sampling points.

Titers were measured as the ratio of *Buchnera* genome copies to aphid genome copies in the entire aphid and, therefore, reflected changes in bacteriocytes of both mother and embryos during development. As expected, aphid age has a large effect on this ratio (*SI Appendix*, Table S2). The average titer and the shifts in titer with age vary across aphid species, reflecting species-specific differences in developmental patterns. Overall, species in which *Buchnera* declined in response to heat exposure were species in which heat caused subsequent reductions in fitness.

Fluorescent in situ Hybridization Visualization of Effects of Heat Exposure on *Buchnera* in Whole Aphids. The *Buchnera* titer alone may not capture how heat disrupts bacteriocytes and does not allow discrimination between effects on maternal and embryonic

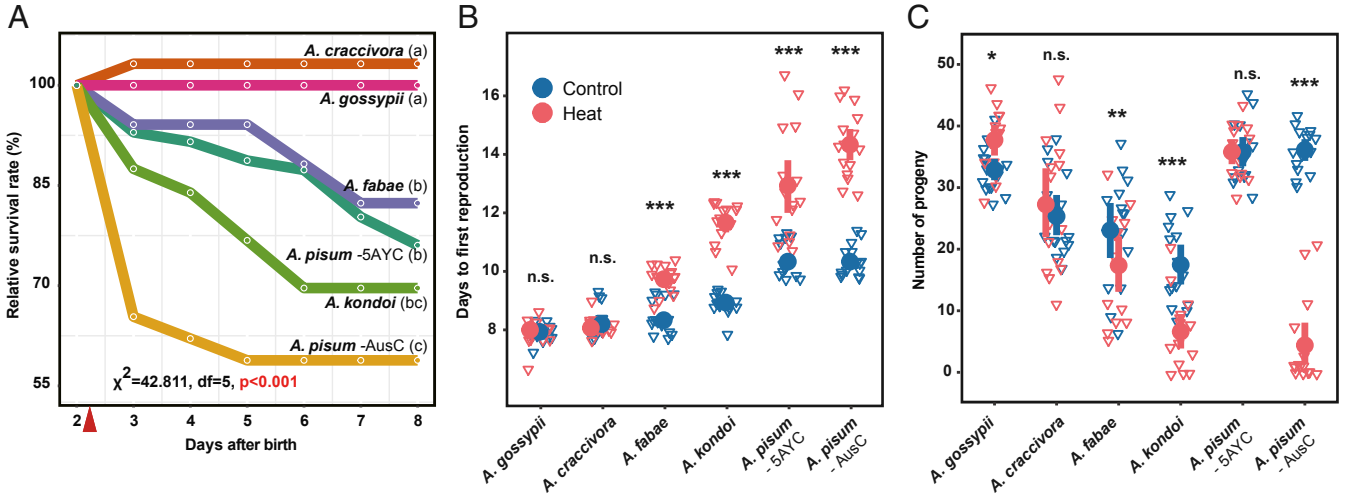

Fig. 1. Effects of heat exposure on aphid fitness. (*A*) Relative daily survival rate of 6 aphid lines after sublethal 38 °C on day 2. Survival calibrated by mortality of controls at constant 20 °C. The red triangle indicates time of heat exposure. The different letters following each aphid line indicate significant differences ($P < 0.05$) among lines with post hoc pairwise χ^2 tests. (*B*) Time from birth to first reproduction and (*C*) number of progeny during first 7 d of reproduction. The bars above and below the dots indicate the SEs. The asterisks indicate significant differences between control and heat treatments (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and n.s. nonsignificant).

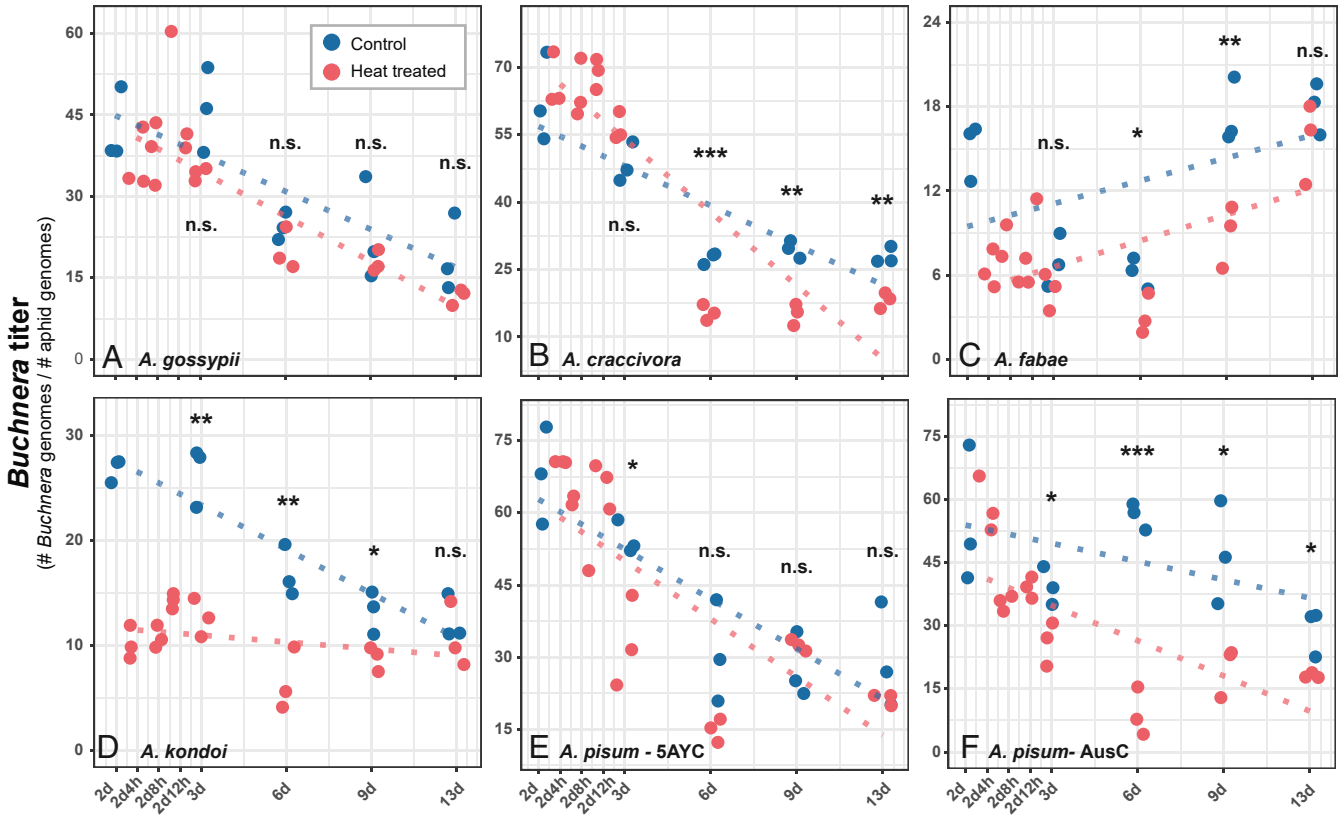

Fig. 2. Effect of heat exposure on the *Buchnera* titer for 6 aphid lines: (*A*) *Ap. gossypii*, (*B*) *A.p craccivora*, (*C*) *Ap. fabae*, (*D*) *Ac. kondoi*, (*E*) *Ac. pisum*-5AYC, and (*F*) *Ac. pisum*-AusC. Heat exposure occurred on Day 2. Each dot represents a biological replicate, at least, 10 aphids for each sampling timing. The asterisks indicate significant differences between control and heat treatments (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and n.s. nonsignificant).

bacteriocytes. Heat exposure had no visible effect on the bacteriocytes of heat-tolerant aphid species *Ap. craccivora* and *Ap. gossypii* (Fig. 3 *A* and *B*). The embryonic bacteriomes of heat-sensitive species *Ap. fabae* and *Ac. kondoi*, however, were consistently smaller and irregularly shaped after heat treatment, and adult aphid body size was also reduced after heat exposure (Fig. 3 *C* and *D*). In *Ac. pisum* 5AYC, heat treatment had no visible effect on bacteriocytes (Fig. 3*E*). In contrast, heat caused dramatic disruption in both maternal and embryonic bacteriocytes in *Ac. pisum* AusC in which heat reduced aphid body size and lowered *Buchnera* titers (Fig. 3*F*). Surprisingly, *Ac. pisum* AusC and *Ap. fabae* both gave a signal indicating *Buchnera* rRNA in or on their midguts after heat exposure; the basis for this signal is not clear (Fig. 3, C2, F2). Generally, the FISH results correlate well with qPCR-based measures of the titer, and they reveal that effects on embryonic bacteriocytes are particularly severe.

Gene Expression Differentiation and Ontology Enrichment. Comparison of transcriptional responses to heat exposure was based on a total of 379,988,517 clean reads from 24 libraries derived from 4 aphid lines, *Ap. gossypii*, *Ap. fabae*, *Ac. pisum* AusC, and *Ac. pisum* 5AYC (*SI Appendix*, Table S3). Of these, 10–25% of reads mapped to aphid or *Buchnera* protein-coding genes; most other reads came from aphid rRNA that was not effectively removed by the animal rRNA depletion kit. Among all significantly up-regulated genes, the heat shock proteins of both aphids and *Buchnera* showed the most prominent responses in all aphid lines (Fig. 4 and *SI Appendix*, Table S4). Within the Hsp70 superfamily of the aphids, 1 orthologous gene Hsp68-like cluster and 1 Hsp70 A1-like cluster of genes were strikingly up-regulated in all lines. However, lines differed in the numbers of paralogous copies present with 6 paralogs of these heat-responsive genes in both *Ap. gossypii* and *Ap. fabae* and only 3 paralogs in the *Ac. pisum* lines. The other 2 orthologous genes (Hsp70 cognates and Hsp70 protein 4) in the Hsp70 superfamily showed little or no response to heat exposure with a maximum fold change of 2 in the *Ac. pisum* 5AYC line.

In *Buchnera*, genes encoding heat shock proteins, including *groEL*, *groES*, *dnaK*, and *dnaJ*, were the top up-regulated genes in all lines. The small heat shock protein encoded by *ibpA* (Hsp20) showed strong responses in *Buchnera* of both *Ap. gossypii* and *Ac. pisum* 5AY. Unexpectedly, this locus was absent from the genome of *Buchnera* of *Ap. fabae*. PCR and Sanger sequencing spanning the flanking genes (*hslU-fpr*) were used to verify the absence of *ibpA* in further samples from our experimental laboratory line (originating from Belgium) and from additional *Ap. fabae* collections from Germany (*SI Appendix*, Table S1), indicating that this gene loss is widespread in *Buchnera* of *Ap. fabae*. *Buchnera* of the *Ac. pisum* AusC line had an intact *ibpA* but showed little *ibpA* transcriptional response. In *Ac. pisum* AusC, *ibpA* transcripts increased only 1.9 fold after heat exposure in contrast to a 27.3 fold increase in *Ac. pisum* 5AYC. This lack of response in *Ac. pisum* AusC corresponds to the presence of a single base deletion in the *ibpA* promoter, resulting in a reduced spacer length between the −10 and the −35 binding sites for the heat shock promoter and, consequently, a reduced responsiveness to heat (16). We verified the difference in an *ibpA* promoter between *Buchnera* of *Ac. pisum* AusC and *Ac. pisum* 5AYC using PCR and Sanger sequencing with DNA from our experimental aphid lines.

By clustering the profiles of up-regulated aphid genes to ontology enrichment terms, the 2 *Aphis* species were found to have many up-regulated genes with terms related to chaperone binding (e.g., GO:0031072) and chaperone-mediated protein

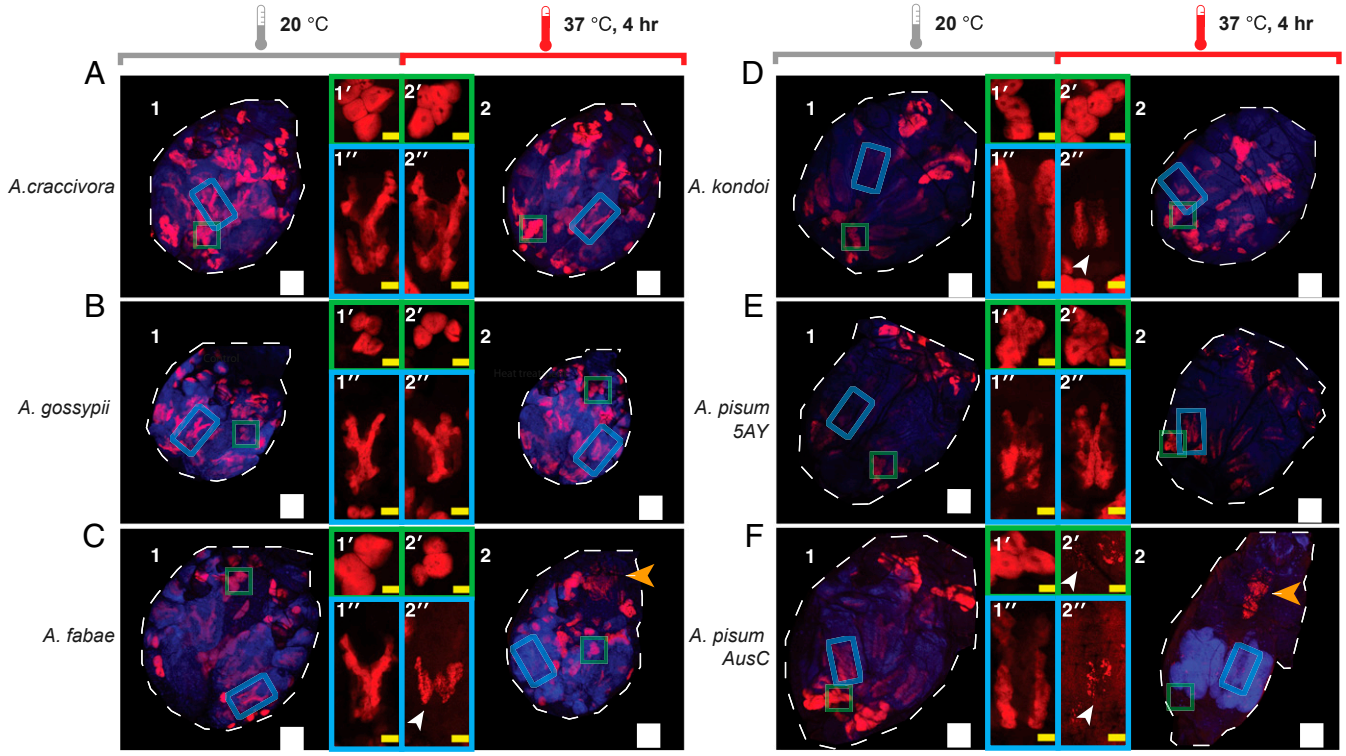

Fig. 3. Laser confocal images of whole-mount fluorescent in situ hybridization (FISH) of aphid lines in control (1) and after treatment of 4 h at 37 °C as juveniles (2). Whole aphid images are maximum intensity projects of merged DAPI (blue) and *Buchnera* (red) channels (see *Materials and Methods* for full display information). *Insets* 1′ and 2′ are enlarged maternal bacteriocytes (green squares), and 1″ and 2″ are enlarged embryonic bacteriocytes (blue rectangles). Species shown include *Ap. craccivora* (*A*), *Ap. gossypii* (*B*), *Ap. fabae* (*C*), *Ac. kondoi* (*D*), *Ac. pisum*-5AYC (*E*), and *Ac. pisum*-AusC (*F*). Images are representative of 4 to 5 aphids inspected for each condition. The white arrowheads indicate maternal or embryonic bacteriocytes showing gross deformity after heat treatment (*C*, *D*, and *F*). The orange arrowheads indicate observed *Buchnera* DNA in the midgut of *Ap. fabae* and *Ac. pisum*-AusC after heat treatment. The white Scale bars are 150 microns, and the yellow Scale bars are 50 microns.

folding (e.g., GO: 0061077); these terms were absent from genes up-regulated in *Ac. pisum* (*SI Appendix*, Fig. S1). The *Ac. pisum* 5AYC line was enriched in neurotransmitter and signal release ontology terms, suggesting differences in responses to heat stimuli among aphid species.

Discussion

Aphid species differ dramatically in the effects of brief heat exposure on later survivorship, time to maturity, and fecundity (Fig. 1). Our results reveal that heat sensitivity of their obligate symbiont *Buchnera* is key in determining the impact of heat on

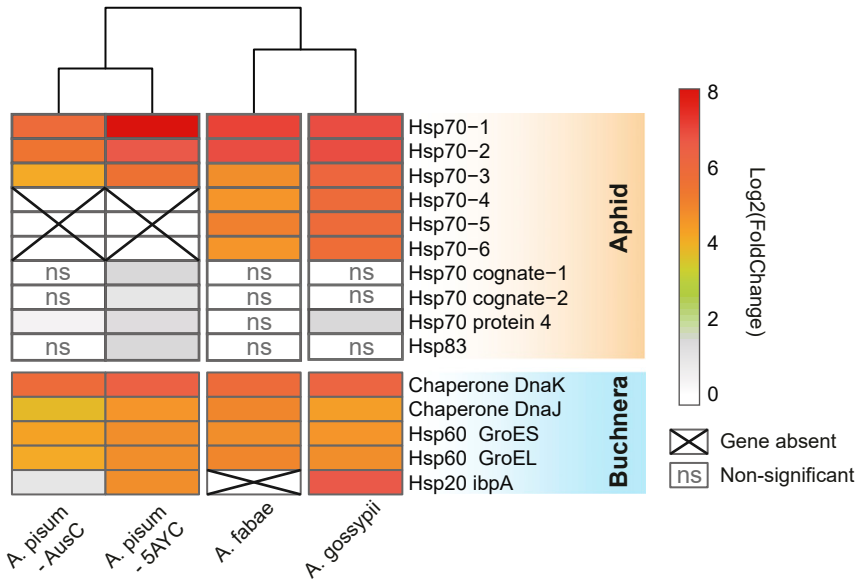

Fig. 4. Transcriptional responses following heat exposure of aphid and *Buchnera* genes for heat shock proteins in 4 aphid lines.

aphid fitness. At 1 extreme, *Buchnera* populations in *Ap. gossypii* were unaffected by heat exposure, and hosts also suffered no negative effects: Survivorship or time to maturity were unaffected, and fecundity even increased. At the other extreme, the *Buchnera* of *Ac. kondoi* and of *Ac. pisum* AusC dropped within a day of exposure and remained significantly depressed a week after exposure (Fig. 2), and aphids in both of these lines suffered delayed maturity and sharply reduced fecundity (Fig. 2).

Ac. pisum AusC provides a striking contrast to *Ac. pisum* 5AYC in heat tolerance of both *Buchnera* and host. The *Buchnera* of these lines differ in the presence of a single base deletion that reduces the transcriptional response of *ibpA* to heat exposure, and that increases the sensitivity of *Buchnera* to heat with populations in AusC but not 5AYC being dramatically depressed. A similar negative effect of this mutation on *Buchnera* titers following heat exposure was reported previously for different combinations of *Ac. pisum* and *Buchnera* (16, 18). This suggests that this mutation, rather than other genotypic differences of symbiont or host, underlies the sensitivity of AusC to heat, but some effect of other genotypic differences cannot be entirely ruled out. While both lines have reduced survivorship and delayed maturity following heat, 5AYC fecundity is unaffected, whereas AusC fecundity is drastically reduced, and many AusC individuals failed to reproduce. IbpA is a small heat shock protein found in almost all organisms, including prokaryotes and eukaryotes; it acts within the cytoplasm to bind misfolded proteins and prevent them from forming irreversible aggregates (26). Thus, the heat-induced drop in *Buchnera* numbers in the AusC line represents an impact first on *Buchnera* with a consequent impact on aphid fecundity.

Previous studies have documented distinct responses to heat in different clones of *Ac. pisum* from field populations (27). Potentially this variation reflects the ability of *Buchnera* populations to withstand heat due to genotypic differences in either *Buchnera* (exemplified by the *ibpA* promoter mutation) or aphid.

Whereas heat has no negative effect on any component of fitness in 2 *Aphis* species, it has negative effects for survivorship, developmental time, and fecundity in *Ap. fabae* (Fig. 1). *Ap. fabae* also undergoes a drop in *Buchnera* numbers following heat (Fig. 2), unlike the other 2 *Aphis* species. A potential factor contributing to heat sensitivity of the *Ap. fabae Buchnera* is the loss of *ibpA*, which has otherwise been retained in sequenced *Buchnera* within the Aphidinae (the largest aphid subfamily, containing most aphid species) (10). This loss likely contributes to the heat sensitivity of this *Buchnera* and, in turn, of *Ap. fabae*. *Ap. fabae* also displays an unusual *Buchnera* titer, measured as the ratio of *Buchnera* to aphid genome copies in the whole aphid. This value decreased during development to adulthood in all species except *Ap. fabae* in which it increased possibly reflecting interspecies developmental differences.

In general, the heat sensitivities of aphids (Fig. 1) and their *Buchnera* (Fig. 2) correspond to the seasonal and geographic distributions of these aphid species. We note that geographic distribution as indicated by collection records is not a simple indicator of thermal range in the field since aphids are highly mobile and can colonize geographic regions for limited periods during favorable conditions, migrating long distances among regions. For example, *Ac. pisum* and *Ac. kondoi* can be found in warm locations, such as Texas and southern Arizona (USA) but only during the winter months, whereas *Ap. craccivora* and *Ap. gossypii* are abundant in the same locations during hot summer months. Our results would suggest that *Ap. fabae* cannot live in hot places; however, this might only apply to particular subspecies of *Ap. fabae*, which is widespread, may include subspecies or sibling species, and is often misidentified due to its resemblance to the related black species of *Aphis*, such as *Ap. craccivora* and *Aphis rumicis* (25). Our *Ap. fabae* line originated in northern Europe, and the other *Ap. fabae* samples confirmed as lacking *ibpA* were from northern Germany (*SI Appendix*, Table S1). Thus, at least, some widespread matrilines of *Ap. fabae* are likely confined to relatively cool geographic regions due, at least, in part, to their *Buchnera* undergoing an irreversible loss of the gene encoding this small heat shock protein. While its geographic range is difficult to ascertain from published records due to misidentification or possible sibling species, *Ap. fabae* appears to be largely absent from warm tropical or subtropical regions, whereas *Ap. gossypii* and *Ap. craccivora* are common in warm regions worldwide (25).

The 2 lines that had little or no *ibpA* response, *Ac. pisum* AusC and *Ap. fabae*, showed an unexpected similarity in *Buchnera* localization following heat shock. In both, heat caused *Buchnera* titers to decline in both maternal and embryonic bacteriocytes, coinciding with the qPCR results showing a drop in the overall titer. And in both, the midgut region acquired a *Buchnera* signal following heat shock (Fig. 3). Because the FISH probe hybridizes with *Buchnera* rRNA and because RNA molecules are not expected to be intact once cells are disrupted, the signal should indicate the presence of intact *Buchnera* cells. However, the basis for this midgut signal is not clear.

Our results indicate that obligate endosymbionts are sometimes more sensitive to heat than their hosts and that they can represent an "Achilles heel" limiting host ability to live under hot conditions as observed for gut symbionts of stinkbugs (3). However, obviously, many insect species with obligate endosymbionts do live in hot places as exemplified by 2 aphid species in our study: *Ap. gossypii* and *Ap. craccivora*. In *Ap. gossypii, Buchnera* experienced no decline following heat exposure, which boosted rather than suppressed host fecundity. One interesting and unresolved question is whether this resilience to heat exposure represents adaptation by *Buchnera* itself or adaptation by the host to protect and support its *Buchnera* population. In *Ap. craccivora*, the *Buchnera* titer did drop several days after heat exposure, but this reduction in symbiont numbers did not affect host fitness, suggesting that this host is adapted to be resilient in the face of temperature-induced fluctuations in *Buchnera* populations.

Continuous selection to maintain fitness under hot environments will select for ability to cope with heat on the part of both symbiont and host; failure of either to adapt will result in extinction or range contraction. However, *Buchnera* appears to often be more heat sensitive than hosts, suggesting that its small asexual genome is less able to respond to selection for maintaining thermal tolerance. When *Buchnera* heat sensitivity reflects a gene loss as appears to be the case with *ibpA* in *Ap. fabae*, then the loss of heat tolerance may be irreversible as *Buchnera* genomes do not regain genes (10). Thus, dependence on obligate endosymbionts could impose inflexibility on thermal adaptation such that temporary relaxation of selection for heat tolerance, for example, during a cool period or during movement to a cooler location, might result in permanent shifts. Most aphid species are confined to north temperate regions, and the lack of aphid species in the tropics and even in temperate regions in the Southern Hemisphere has been hypothesized to reflect an inability of aphid lineages in north temperate regions to colonize in tropical latitudes (28). Potentially, *Buchnera* sensitivity to heat contributes to this constraint (29). Interestingly, the few cases of permanent loss of *Buchnera* are in aphid species living in unusually warm climates, including several losses within Cerataphidini species in subtropical and tropical Asia (30) and 1 within *Geopemphigus* in Mexico (31). In both cases, *Buchnera* is replaced with a novel symbiont type. Replacement of an ancient heritable symbiont, such as *Buchnera*, is a potential evolutionary route to escape the limitations of a poorly functioning partner (e.g., ref. 32). However, such replacements are rare, occurring only a few times in the >200 million-year history of the sap-feeding homopteran insects (33).

Materials and Methods

Additional details of Methods are included in the *SI Appendix*.

Aphid Clonal Lines and Rearing. We used 6 clonal lines representing 5 aphid species, including *Ac. pisum*, *Ac. kondoi*, *Ap. fabae*, *Ap. craccivora*, and *Ap. gossypii* (*SI Appendix*, Table S1). All lines were initiated with 1 field-collected female and maintained in the laboratory at constant 20 °C with a 16L/8D daily light cycle. Aphid lines were maintained on plants in 4-in pots and enclosed by an inverted plastic cup modified with a mesh top.

We included 2 *Ac. pisum* strains: 5AYC (heat resistant *Buchnera* genotype) and AusC (heat-sensitive *Buchnera* genotype). *Ap. fabae*, *Ap. craccivora*, and *Ac. pisum* were maintained on *Vicia faba*, *Ac. kondoi* were maintained on *Medicago sativa* (alfalfa), and *Ap. gossypii* were maintained on *Cucumis melo* (melon). We verified that all these aphid clones harbor *Buchnera* but lack secondary symbionts as confirmed by PCR based on universal primers spanning the 16S-23S ribosomal RNA spacer (34).

Thermal Fitness Measurement. To determine aphid species survivorship following a heat treatment, we exposed second-day nymphs to 38 °C for 3 h in a thermal incubator, then monitored aphid survival daily for a week. To reduce impacts of plants and soil on the microclimate within cup cages, 2 to 3 aphid adults were transferred to seedlings in plastic screen cages ($10 \times 4 \times 25$ cm) to reproduce for 1 day at 20 °C. After heat treatment on the second day, the nymphs were moved to intact host plants in cup cages for further development. For each line, about 30 nymphs were treated, and about 30 nymphs were kept at constant 20 °C as controls.

To determine developmental time and fecundity following heat exposure, we used a similar setup and procedure for a second set of aphids, exposing them to 37 °C for 4 h, a treatment that stresses but does not kill the aphids. We then recorded survivorship, developmental time (days from birth to first reproduction), and fecundity (total offspring during the first 7 d of reproduction). Twelve to 15 adults were tested for treatment and for control in each line, respectively.

Real-Time qPCR of the *Buchnera* Titer. To estimate dynamics of *Buchnera* populations following heat exposure, the ratio of *Buchnera* genome copies to aphid genome copies was determined by real-time qPCR using single-copy genes from both genomes. Aphids were heat exposed as described above (37 °C for 4 h on the second day) and sampled at 4, 8, 12, and 24 h (equivalent to the third day), sixth day, ninth day, and 13th day. Control aphids were kept at 20 °C for the duration of the experiment. At least, 10 aphids were collected as a replicate in each time point; at least, 30 individuals were sampled in the early stages due to the small size of young nymphs. For each time point, 3 biological replicates were prepared; for each biological replicate, we performed 3 technical replicates. PCR primers, and conditions are in *SI Appendix*, Table S2.

FISH to Determine Effects of Heat on Bacteriocytes. Whole-mount in situ hybridization followed published protocols (35, 36). Control and heat-exposed aphids were treated as described above. Newly matured (Day 1) adults were sampled and fixed.

***Buchnera* Genome Sequencing.** *Buchnera* genomes from *Ap. fabae* and *Ap. gossypii* were sequenced de novo and assembled to be used as reference genomes for this study.

Transcriptional Responses to Heat Exposure. To explore transcriptional responses to heat exposure in both aphids and *Buchnera*, we sampled 2-d-old nymphs with and without exposure to 37 °C for 4 h. The treated aphids were allowed to recover at 20 °C for 2 h before sampling. About 40–50 nymphs were collected for each replicate and then flash frozen in liquid nitrogen before storing at −80 °C prior to RNA extraction. Three biological replicates each for control and heat treatment were prepared for 4 aphid lines: *Ap. gossypii*, *Ap. fabae*, and the 2 lines of *Ac. pisum*.

Statistical Analyses. All statistical analyses were performed using R 3.5.1 (37). We used the Cox proportional hazard model in the "survival" package to compare the survival curves after heat shock among aphid lines. Because none of the lines dropped survival to 50% within 7 d, we also compared the survival values on the last day by χ^2 distribution in the "rcompanion" package. Pearson's χ^2 test and post hoc pairwise χ^2 tests are presented in Fig. 1 with the significance threshold of $P < 0.05$. To test aphid fitness after heat exposure, we used generalized linear models for developmental time and fecundity measurements with aphid lines and treatment as fixed effects. When treatment was significant as a main effect, we followed this test with unpaired 2-sample Wilcoxon rank sum tests. Because data were not normally distributed, we used the continuity correction between control and heat treatment for each aphid line in the "dplyr" package. Additionally, tests for effects of heat exposure on the *Buchnera* titer were conducted using ANOVA under a general linear model, and independent T tests were used to compare control and treatment for each time point.

Data Availability. All sequence data are available at National Center for Biotechnology Information. *Buchnera* genome sequences for *Ap. fabae* and *Ap. gossypii* are deposited under accession numbers CP042426 and CP042427. RNA-Seq data are deposited under BioProject ID PRJNA577642, SRA accession numbers SRR10291791–SRR10291814.

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