Supporting Online Material for

Species Response to Environmental Change: Impacts of Food Web Interactions and Evolution

Jason P. Harmon,* Nancy A. Moran, Anthony R. Ives

*To whom correspondence should be addressed. E-mail: jharmon@wisc.edu

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Species response to environmental change: Impacts of food-web interactions and evolution

Jason P. Harmon¹, Nancy A. Moran², and Anthony R. Ives¹

¹Department of Zoology, UW-Madison, USA and ²Department of Ecology & Evolutionary Biology, University of Arizona, USA

Correspondence: Dr. Jason P. Harmon, Department of Zoology, UW-Madison, Madison, WI 53706, USA. Email: jharmon@wisc.edu

In the SOM, we present more details about (i) our study system, (ii) field surveys used to compare naturally occurring densities of the two predators Coccinella septempunctata and Harmonia axyridis, (iii) the methods and results of field experiments on the interaction effects of predation and heat shocks, (iv) the methods and results of field experiments on aphid evolution (population growth rates of different clones under shocked and non-shocked conditions), and (v) the model that we use to compare ecological and evolutionary processes affecting the anticipated response of pea aphid densities to increasing frequency of heat shocks. These topics are also supported by additional experiments that are not described in the main text to address specific aspects of the experimental designs and conclusions.

1. Aphids, endosymbionts and predators

Aphids – Pea aphids were likely introduced into the New World sometime in the late 1800s from Europe (S1-3). Since introduction they have spread throughout North America where they feed on peas, beans, alfalfa, and other legumes. In Wisconsin, USA, they are parthenogenic over the summer and undergo a sexual generation in the late fall, after which females lay eggs that overwinter (S4). In the asexual generations, females produce live offspring that develop into adults in 8-16 days depending on temperature (S5). Adults produce up to an average of about 5 offspring per day over their lifespan (S6, 7).

Aphids and heat shock – Pea aphids are differentially susceptible to short periods of high temperatures, referred to as heat shocks. Susceptible aphid populations exposed to heat shocks experience substantially lower growth rates that are generated primarily by a reduction in fecundity. Exposure to sufficiently high temperatures (> 40°C) for several hours can sterilize aphids permanently (S8, 9), although our experiments did not approach these high temperatures. Susceptibility to heat shocks can vary depending upon the individual aphids’ genotype and endosymbionts (see below Population growth rates under field heat shocks: Heat shock effects on individual aphids).

Endosymbionts – Pea aphids lack the ability to produce essential amino acids and therefore rely upon primary (obligatory) endosymbionts, specifically Buchnera aphidicola, which has a long, shared evolutionary history with aphids (S10). Pea aphids also contain any of a variety of heritable secondary (facultative) endosymbionts that have been shown to confer resistance to parasitism (Hamiltonella defensa and Serratia symbiotica), expand the range of plant species used by aphids (Regiella insecticola), and provide protection from heat shocks (S. symbiotica) (S11-14). These secondary symbionts are found in many aphid species and show variable frequencies within and among pea aphid populations, based on surveys from Japan, Europe, and North America (S15-19). In the field predation experiments, we used a single green clone, “7-2-1,” that is sensitive to heat due to the presence of Buchnera with the heat sensitive ibpA promoter allele and to the lack of any secondary symbiont. In the field experiments investigating the consequences of heat shocks for evolution, we used two pairs of clones in which pairs differed in heat tolerance due primarily to symbiont effects, as documented previously in laboratory experiments (S20). These clones also differed in color, which is a genetically based polymorphism in pea aphids, not influenced by symbionts, and stable within clones. In Pair A, red aphids were heat-sensitive, green aphids heat-tolerant; in Pair B, green aphids were the heat-tolerant clone. The color polymorphism allowed us to quickly assign clone identity for each individual. In Pair A, the green tolerant clone was “Tucson” which is naturally infected with a strain of S. symbiotica (S6); the red susceptible clone was S5A10, lacking a symbiont and bearing Buchnera with a mutation reducing heat shock response (S21). In Pair B the red tolerant clone was “5A11R” which was experimentally infected during 2001 with S6© that was subsequently vertically transmitted in the lab colony. In pair B the green susceptible clone was S2-1, which was uninfected with secondary endosymbionts and contained Buchnera with the same mutation reducing heat shock response (S21). Thus, for each pair the heat-tolerant clone contained S6© plus Buchnera with the ibpA allele assisting in heat shock protection. Assays to confirm these clone attributes and the endosymbiont complement present in aphids from the field are described in Dunbar et al. (S21) and Sandström et al. (S17). These assays were also used to verify the integrity of laboratory cultures of each aphid clone. To determine whether the Buchnera ibpA allele varied within naturally occurring populations, we compared samples from 1999 (38 aphids) and 2008 (50 aphids) that were taken in alfalfa fields at our study site.

Predators – Both ladybird beetle predators were introduced intentionally into North America as biological control agents for aphids and are now the most abundant aphidophagous predators in southern Wisconsin agroecosystems. These two generalist predators are similar morphologically and are primarily found in the same habitats consuming the same prey species (S22). Despite their broad similarities, they demonstrate specific differences in behavior (S23), physiology (S24), and ecology (S25) that may ultimately produce large differences in these species’ dynamics (S26) and their ability to control specific prey species.

One of the most important of these differences relates to how each species responds to the density of aphid prey; H. axyridis seems better suited to exploit high density aphid colonies, whereas C. septempunctata appears less sensitive to aphid densities. This pattern has been observed within a habitat (Field surveys of aphid and predator populations) and between habitats that differ in the density of focal aphid species (S27). Rather than a single trait or behavior that establishes this connection between predator species and aphid density, there is a suite of correlated traits that makes each predator well suited for a particular aphid density. To summarize relevant results from (S27) and (S28): i. In the lab, C. septempunctata will oviposit even in the absence of aphids provided females are previously fed. In contrast, H. axyridis females only oviposit when stimulated by the presence of large numbers of aphids. ii. In field experiments with cages containing both alfalfa (with pea aphids at lower density) and soybeans (with soybean aphids at higher density), C. septempunctata foraged in both crops whereas
H. axyridis foraged exclusively in soybeans where the aphids were at a higher density and more aggregated distribution.

iii. In the field over normally occurring aphid densities, reproduction of C. septempunctata relative to H. axyridis is much higher in alfalfa on pea aphids than in soybeans with soybean aphids.

iv. In both the field and the laboratory, C. septempunctata is more willing to move among plants, regardless of the presence of aphids. In contrast, H. axyridis is much better at exploiting aphid colonies once it has found them.

v. In field surveys including soybeans in addition to alfalfa, adult H. axyridis showed patterns consistent with selection of fields with high aphid densities, whereas adult C. septempunctata densities were not as strongly associated with densities of either pea aphid or soybean aphids.

2. Field surveys of aphid and predator populations

Field surveys were conducted to determine the relative abundances of the two ladybeetle species as a function of pea aphid densities within alfalfa fields over the course of five summers, 2003-2007. The specific question we addressed is whether C. septempunctata maintains high density at low aphid density in comparison to H. axyridis.

Methods – Alfalfa fields were sampled roughly weekly in the summers of 2003-2007. Five fields were sampled in each year except 2006 when 9 fields were sampled, to give 431 sample points from 29 field-years. Established alfalfa fields are cut three times in the summer. In the text we present data for the second harvest cycle, which is between the first and second harvests, and corresponds approximately to the month of June, and for the third harvest cycle which corresponds approximately to the month of July. These two harvest cycles encompass the primary period of aphid-predator interactions. Moreover, we chose these periods because we only have data from before the first harvest in years 2005 and 2007, and after the third harvest the number of H. axyridis is episodically dominated by strong migration from soybean fields as soybeans senesce. Here, we analyze both the entire data set and the data limited to the second and third harvest cycles.

Each sample consisted of sweep netting for pea aphids and adult ladybeetles, and 10-minute visual scan samples for ladybeetles. The intensity of sweep netting ranged from 9 to 900 sweeps per sample, depending on aphid density. For analysis, the counts of ladybeetle densities from netting and scans were added, because these were of similar magnitude within a field.

Analyses – The data were analyzed using a Generalized Linear Mixed Model (GLMM) whose fixed effects are given by

$$X = S + \log_{10} A + C + S \times \log_{10} A + S \times C + C \times \log_{10} A$$

where X is the number of ladybird beetles, S is a categorical variable corresponding to C. septempunctata vs. H. axyridis, $\log_{10}$ A is the log-transformed pea aphid density, and C is a categorical variable for alfalfa harvest cycle. The model also included two random effects. To account for correlations in the number of beetles captured in the same field during the same harvest cycle, we included a random effect grouping data by field-cycle. To account for correlations between the numbers of C. septempunctata and H. axyridis caught in the same sample, we included a random effect grouping data by samples. Finally, we assumed that the number of ladybeetles is Poisson distributed and used the “family = quasipoisson” option in the R function lmer() (529). In the quasipoisson option, the analyses use the estimated scaling factor from the GLMM rather than forcing the scaling factor to equal one; this allows for greater-than-Poisson variance in the dependent variable.

Results – There were strong S * $\log_{10}$ A interaction effects indicating that C. septempunctata and H. axyridis differed in their responses to pea aphid density both for the full data set and the data limited to the second and third cycles (Table S1).

For figure 1, we used data from only the second and third harvest cycles. To account for differences among years and harvest cycles in the total number of ladybeetles, the densities of each species of beetle in each cycle-year were standardized to have mean 1, and then the square-root was taken to homogenize variances. Aphid densities were transformed using $\log_{10}(1+x)$ where x equals the number of aphids per 10 sweeps.

Predation pressure on pea aphids, to a first approximation, is proportional to the density of each ladybeetle species. Ladybeetles that are sampled in field surveys using sweep nets and visual scans are foraging actively, rather than sitting inactively. Furthermore, the densities of aphids (< 1 per alfalfa stem) and ladybeetles (< 0.1 per sweep, < 2 per minute scan) diminishes the chances of both predator satiation and direct interactions among predators.

3. Field predation experiments

Exposing pea aphids to heat shocks can have detrimental effects to their growth rate in the laboratory (S8, 20, 21) and in the field (see Population growth rates under field heat shocks below). Similarly, ladybeetles can reduce aphid numbers by direct predation. What is not known is how these two forces may interact. We performed field experiments to determine how the combination of these two negative effects – heat shocks and predation – interact to influence pea aphid dynamics. We used large field cages that were either kept at ambient field conditions or were subjected to periodic experimental heat shocks, and crossed this treatment with the presence or absence of predators. We then compared the number of aphids in a cage across all four treatments, specifically looking for an interactive effect of heat shock and predation. We repeated the experiment using two different predators, C. septempunctata and H. axyridis, each of which we expect to respond differently to the number of aphids available.

The effects of heat shock often take 2-3 weeks to manifest (see section below: Potential for evolution: The phenology of heat shock effects on laboratory populations). This is primarily because heat shocks have the greatest effect when aphids are young, but there are little consequences of the heat shocks until young aphids become adults and have lower fecundity than unshocked aphids (S8, 21). In an attempt to speed up this effect and maximize the amount of time the forces of shocking and predation could interact, we preliminarily shocked aphids in the laboratory before the start of the experiment and used these aphids to stock field cages in treatments including heat shocks. The efficacy of this pre-shock treatment was tested in an ancillary experiment (see Confirmation of the effects of initial shocking in the laboratory below). We also tested to see whether either ladybeetle species had different predation rates on shocked vs. unshocked aphids, as this could affect the interpretation of the field predation experiments (see Predation rates on shocked and control aphids below).

Methods – On June 6, 2007 we set up 20 2x2x2m Lumite-screened field enclosures at the Arlington Agricultural Research Station, Arlington, WI, USA. Enclosures were placed over alfalfa shortly after it was cut and harvested, and we used a vacuum sampler (d-vac) to remove insects from the cages prior to the start of the experiment. Cages were randomly assigned to treatments in a 2x2 design: with heat shocks or at ambient temperature, and with or without predators. On June 9, 2007, we added 150 aphids from a homogenous colony of green susceptible aphids (strain 7-2-1) to each cage and 5 adult H. axyridis to cages assigned to treatments with predators. Aphids for the treatments with heat shocks were...
shocked before the experiment as follows. We split the original aphid line into two groups, each of which was spread over numerous potted plants in a greenhouse. One of these groups was maintained at a constant temperature of approximately 19°C. Aphids from this group were used to stock cages in the ambient temperature treatment. The other group of aphids was also kept at 19°C except during heat shocks, when they were exposed to 35°C for 4h. We shocked these aphids 4 times over 9d to ensure that all life stages of aphids were exposed to shocks and that all of the aphids put into the field cage would have lower fecundity as adults (see Verifying the effect of shocking on aphid growth rate for confirmation).

We continued to manipulate cages during the experiment to help maintain both the shocking and predation treatments. Cages in the shocked treatments were wrapped in clear plastic for 4h at mid-day. We performed the heat-shock treatment starting on day 2 of the experiment and continued 3 times per week for the duration of the experiment. This manipulation increased peak temperatures in the enclosures by an average of 5°C (range: 2.7-7°C) and the average temperature difference during the heat shock period in adjacent shocked and ambient temperature cages was on average 3.7°C (range: 1.2-5.9°C); figure S1 gives an example from one heat shock manipulation. To help maintain the predator treatment, 3 additional H. axyridis adults were added on day 4 of the experiment and 2 non-shocked aphids were added on day 6. Throughout the experiment, we determined aphid densities by sampling each cage every 2-3d by inspecting 50-200 alfalfa stems. The experiment was terminated on day 10 after the alfalfa had reached approximately 70cm and was flattened by a heavy rain storm.

On July 10, 2007, 20 cages were again set up for an experiment of the same design but using the second species of ladybeetle, C. septempunctata. On July 13, 2007, 150 pre-shocked or control aphids were added to shock and ambient cages respectively, and 5 adult C. septempunctata were added to each cage in the predator treatment. The experimental methods were identical to the previous run except we were able to maintain the experiment for 16d due to favorable weather.

Analysis – We analyzed the data using a GLMM for each of the experiments performed with one of the predator species. The fixed effects for each model are given by

\[ Y = \text{Shock} + \text{Pred} + \text{Day} + \text{Shock}^{*}\text{Pred} + \text{Shock}^{*}\text{Day} + \text{Pred}^{*}\text{Day} + \text{Shock}^{*}\text{Pred}^{*}\text{Day} \]

where \( Y \) is the number of pea aphids, Shock is a categorical variable corresponding to the experimental heat-shock treatment, Pred is a categorical variable corresponding to the experimental predation treatment, and Day is a categorical variable corresponding to the day of experiment. The model also included a random effect to account for correlations in the number of pea aphids within the same cage sampled repeatedly; this manner of incorporating correlations due to repeated measurements effectively assumes that the autocorrelation among samples within the same cage is one. We fit the GLMM model using the R function lmer() (S29) with the “family = quasipoisson” option. In the quasipoisson option, the analyses use the estimated scaling factor from the GLMM rather than forcing the scaling factor to equal one; this allows for greater-than-Poisson variance in the dependent variable.

Results – For C. septempunctata, there was a statistically significant Shock*Pred*Day interaction, indicating that there was an interaction between the two treatments that differed among days of the experiment (Table S2A). This three-way interaction occurs due to the negative Shock*Pred interactions in the middle of the experiment (Fig. 2A). Confining the analysis to the final 4 days of the experiment reveals no Shock*Pred*Day interaction (Table S2B). In contrast, H. axyridis showed a strong Shock*Pred*Day interaction (Table S2C) that was positive. This indicates that the impact of the heat-shock treatment was relatively less than expected in the presence of H. axyridis predators (Fig. 2B). If the C. septempunctata data set is confined to the same length as the H. axyridis data (12 days, 5 samples), there is a significant negative interaction (Table S2C), further supporting our argument for species-specific effects between these predators.

For presentation of the results in Table S2, we have only included the test statistic for the highest-order interaction; if the 3-way interaction Shock*Pred*Day is statistically significant, then all subordinate interactions and main effects must be considered in the model. Because Day is categorical, the models contain large number of coefficients; for example, the model for C. septempunctata contains 32 coefficients for fixed effects. Rather than include these in Table S2, instead we present them graphically in Fig. 2; the plotted values are the expectations computed directly from the fixed effects coefficients. The line depicting the expected aphid abundance in the absence of the interaction effect (labeled “both\text{no}”) is computed by removing the coefficients for Shock*Pred*Day and Shock*Pred interactions.

Confirmation of the effects of initial shocking in the laboratory – We performed an additional laboratory experiment to verify the effect of preliminary laboratory shocking on the aphids used in the field predation experiments (above). This involved taking a subsample of aphids from the stocks used to initiate the field predation experiments and measuring their population growth rate in the laboratory in the absence of further heat shocks. A lower population growth rate of the aphids used to stock the field cages in treatments with heat shocks would confirm the desired effect of the treatment before the field component of the experiment was initiated.

Methods – A random subsample of juvenile and adult aphids that had been prepared for the field predation experiment were selected from both treatment groups, those that had undergone 4h heat shocks 4 times over 9d, and those that were kept at the ambient 19°C. On the same day that the field predation experiment was set up, this laboratory experiment was also initiated. We placed 5 aphids (2 adults and 3 juveniles) onto individual fava bean plants and covered each plant with a cage made from a Mylar tube and mesh screening. Sixteen plants were given shocked aphids and 16 plants were given ambient temperature control aphids. All plants were kept in the constant environment they had been raised in previously (19°C). We counted the number of aphids on all plants after 7d.

Analysis – We used ANOVA to analyze the data with the response variable being the total number of aphids on a plant and the independent variable being whether the aphids were in the preshock or ambient temperature treatment.

Results – There were fewer aphids on plants that had shocked aphids compared to plants with aphids kept in the constant, control environment (average ± 1 SEM: 40.8 ± 3.5 vs. 63.9 ± 5.9; \( F_{1,30}=11.5 \ p=0.002 \)). These results indicate that shocking aphids before the experiment reduced aphid growth rates, thereby confirming the effectiveness of the shocking treatment used on aphids before the field component of the experiment was initiated.

Predation rates on shocked and control aphids – We designed an experiment to rule out the possibility that predators (either C. septempunctata or H. axyridis) have different predation rates on shocked vs. unshocked aphids. Our interpretation of the results of the field predation experiment is that differences in predation rates between treatments with shocked and unshocked aphids were caused by differences in aphid densities. An alternative explanation is that the two predators had different predation rates on shocked vs. unshocked aphids. For example, if H. axyridis preferred unshocked aphids over shocked aphids whereas C.
Methods – The experiment was set up as a paired design using adult predators and aphids in 9cm diameter Petri dishes. All predators were from recently established colonies that originated from alfalfa and soybean fields in southern Wisconsin. Before the experiment, all predators were satiated and then starved for 24h to standardize each predator’s hunger level and enhance the likelihood of predation. Half of the predators of each species were given 20 shocked aphids for 2h, then satiated and starved for another 24h, and finally given 20 control aphids for another 2h. We switched the order for the other half of the predators so that they were given control aphids first and then shocked aphids. Performing the experiment as a paired design allowed us to account for individual differences in predation rates. Both aphid types were from the same heat-shock susceptible aphid line (strain 7-2-1). Control aphids were kept at a constant temperature (19°C), and shocked aphids had been kept at the constant temperature except for repeated, regular exposures to high temperature shocks (35°C). Predation was quantified after 2h, at which point most predators were beginning to satiate. We performed the paired experiment with 40 H. axyridis and 40 C. septempunctata.

Analysis – To determine whether shocking affected predation, we calculated the difference in how many aphids of each type were consumed by each predator and used t-NOVA to determine if predator species or sex influenced the difference in predation between aphid treatments. We then compared the distribution of differential predation to a hypothesized mean of zero using a t-test.

Results – The difference in the number of control aphids vs. shocked aphids did not vary between the species of predator used (F1,39=0.71 p=0.40) or the sex of the predator (F1,39=1.07 p=0.30). Since there were no statistical differences in predator species or sex, we combined all the data and looked to see whether the difference in predation between the two types of aphids differed from zero. We found that our predator species tended to consume more control aphids than shocked aphids (average difference in aphids consumed ± SEM: 2.24 ± 0.50; aphids consumed by C. septempunctata: 13.75 ± 0.57 control and 13.70 ± 0.76 shocked; aphids consumed by H. axyridis: 9.55 ± 0.90 control and 9.35 ± 0.75 shocked) and that this difference in consumption was significantly higher than a hypothesized mean value of 0 (no difference in predation) (t22=4.49, p<0.0001).

These results suggest that something about the shocking process reduces the aphids’ exposure or attractiveness to our predators. This small difference may help ameliorate some of the negative effects of aphid growth rate that occur when aphids are shocked. However, this difference was the same for the two predator species; therefore, it cannot explain why the results for C. septempunctata and H. axyridis differed when they were the focal predators in the field experiments.

4. Potential for evolution
In laboratory experiments using individual pea aphids, heat shocks reduce fecundity, increase mortality, and slow development time, and the extent of these effects depends on the aphids’ primary symbionts and predators above. Placing both sensitive and tolerant clones within single cages allowed us to control for the cage-to-cage variation that can occur in field experiments. Pea aphids have two color morphs, green and red, that are genetically determined and not influenced by symbionts. Within each of our experimental pairs, one clone was green, and the other was red; in pair A the green clone was tolerant and in pair B the red clone was tolerant. Pairing clones of different colors allowed us to easily distinguish the two clones within the same cage for sampling.

Below we present the details of the experiment and analyses. We then perform an analysis of laboratory data on the response of individual pea aphids to heat shocks to compare with the estimates we obtained from field cages (see Predictation rates on individual aphids below). Next, we present a laboratory experiment in which we estimate the delay between the initiation of shocking and the effect on pea aphid population growth rates (see The phenology of heat shock effects on laboratory populations below). We expect a delay, because the main effect of heat shocks is on fecundity, and aphids are most susceptible to heat shocks when they are in early instars. Therefore, the effect of heat shocks on aphid population growth will be delayed for the time it takes for aphids shocked in the early instar to become the majority of reproductive females. Finally, we tested to see whether either lady beetle species had different predation rates on susceptible vs. tolerant aphids as this could affect the evolution of tolerance to heat shocks (see Predation rates on susceptible and tolerant aphids below).

Methods – On June 23, 2006 we set up 24 2x2x2m Lumite-screened field cages at the Arlington Agricultural Research Station, Arlington, WI, USA. Cages were placed over alfalfa shortly after it was cut and harvested, and we used a vacuum sampler (d-vac) to remove insects from the cages prior to the start of the experiment. On June 27, 2006, when alfalfa was approximately 20cm high, we added 40 aphids from each of two clones to each cage. Pair A consisted of a green tolerant clone (Tucson) and a red sensitive clone (5A10), and pair B consisted of a red tolerant clone (5A11R) and a green sensitive clone (7-2-1). To determine population growth rates, aphid densities were sampled every 2-3d for 29d by inspecting 50-100 alfalfa stems. The experiment was terminated when the alfalfa reached approximately 70cm.

Six randomly selected cages for each clone pair were given a heat-shock treatment by wrapping the cages in clear plastic for 4h at mid-day. We performed the heat-shock treatment on day 2 of the experiment and continued twice per week on sunny days to ensure the effectiveness of the treatment. The treatment increased peak temperatures in the cages by about 5°C (2-7°C), and the average temperature differential during the heat shock period in adjacent shocked and unshocked cages was 3.7°C (1.2-5.9°C) (Fig. S1).

Analysis – We analyzed the data to estimate the population growth rates (r) of each clone in the experiment and the effect of the heat-shock treatment on the growth rates. The analysis presented three challenges. (i) The data represent separate time series for each clone in each cage, and this necessitated statistically modeling the population growth of each clone in each cage separately. Nonetheless, we anticipated that the population growth of clones in the same cages would be statistically correlated. (ii) We anticipated a delayed effect of the heat-shock treatment on population growth rates, because prior laboratory experiments demonstrated that the main effect of heat shock was to reduce the fecundity of aphids that experienced heat shock during their larval development, in the second and third instars. We anticipated a delay in detectable effects of the heat shock treatment of roughly 2-3wk. Before this delayed effect, we anticipated that the population growth rates of clones in the heat-shock treatment would be the same as in the no-heat shock treatment (see The

Aphids, endosymbionts and predators above).
phenology of heat shock effects on laboratory populations below).

(iii) Aphid populations at the beginning of the experiment were small. This uncertainty is compounded by sampling error.

To address these challenges, we analyzed the data using a state-space time-series model that has two components: a process equation that describes the underlying changes in the population density and a measurement equation that describes the sampling of populations and the associated uncertainty in sampling. For each clone pair, we fit the model

\[ y_t = \mathcal{g}(\text{treat}) y_{t-1} + \mathbf{a}, \quad \mathbf{Y} = h(y_t) + \alpha + \epsilon \]  

Here, \( y_t \) is the 48x1 vector of log densities of each clone in each cage, and \( \mathcal{g} \) is the 48x1 vector of population growth rates. Each clone is given a separate population growth rate, \( r_t \) and \( r_s \), for the heat-sensitive and heat-tolerant clones, respectively. In the heat-shock treatment enclosures, after \( T \) days the population growth rates change from \( r_t \) and \( r_s \) to \( r_t(\text{shock}) \) and \( r_s(\text{shock}) \). We statistically explored different values of \( T \) for pair A the conclusions were the same for \( T = 14, 16, \) and 18 days, although for pair B the effect of heat-shock treatment on the sensitive clone only became statistically significant at \( T = 18 \) days. For the process error \( \mathbf{a} \), we assumed that the variance had two sources, a within-cage variance of \( \sigma_{\text{cap}}^2 \), that affected each clone in the same cage and a separate population variance \( \sigma_p^2 \) that allowed each population to differ; thus, the covariance matrix of \( \mathbf{a} \) contained diagonal elements \( \sigma_{\text{cap}}^2 + \sigma_p^2 \) and off-diagonal elements corresponding to different clones within the same cages \( \sigma_{\text{cap}}^2 \).

In the measurement equation (Eq. S2), \( Y_t \) is the 48x1 vector giving the observed number of aphids of each population in the sample of day \( \tau \). Note that while the process equation (Eq. S1) is iterated on a daily time scale, the measurement equation is only implemented on days on which samples were taken. We assumed that the distribution of sampled aphids follows a Poisson distribution with mean \( h(y_t) = \exp(\gamma_t) \). Hence, the variance of the measurement error \( \epsilon_t \) also equals \( \exp(\gamma_t) \). We assumed there is no covariance in the measurements for different clones.

We fit the state-space model given by equations (Eq. S1) and (Eq. S2) using an extended Kalman filter that can accommodate the nonlinear measurement equation. For initial values of \( y_0 \), we assumed values are normally distributed with mean \( y_{\text{init}} \) and variance \( \sigma_{\text{init}}^2 \). The extended Kalman filter gives the approximate log-likelihood function for the state and model, and we maximized this to obtain maximum likelihood parameter estimates. We tested the hypothesis that clones within the same pair responded differently to the heat-shock treatment using a likelihood ratio test which compares the maximum likelihoods under the assumptions that both species either have the same or different population growth rates after time \( T \) in the heat-shock treatment enclosures. We also computed approximate standard errors of the estimates by inverting the information matrix obtained from the likelihood functions.

**Results** – The results for pairs A and B were very similar (Table S3). In the absence of heat shocks (no-shock treatment) or before the shocks have an effect (shock treatments), the population growth rates of the sensitive clones were slightly higher than the tolerant clones. However, starting at 18 days in the shock treatment, the population growth rates of the sensitive clones decreased substantially while those for the tolerant clones did not. The difference in responses to the heat shock between sensitive and tolerant clones was statistically significant (pair A: \( \chi^2 = 12.1, P < 0.001 \); pair B: \( \chi^2 = 6.96, P < 0.01 \)).

The process error (variance of \( \mathbf{a} \)) was divided into two roughly equal components, one corresponding to differences among cages \( \sigma_{\text{cap}}^2 \) and one corresponding to differences among clones \( \sigma_p^2 \). The estimate for the initial number of aphids of each clone was slightly different between pairs A and B, although this difference was not great, translating to mean numbers of individuals per stem of equal to 4.12 = 0.016 and 0.014 for pairs A and B, respectively. The range of initial starting values for pair A was wide, with the 66% inclusion interval for the initial starting densities given by \( \exp(-4.12 \pm 0.516) \approx [0.0097, 0.0284] \).

**Heat shock effects on individual aphids** – We compared the results of our field experiments with prior laboratory experiments to determine whether our results are consistent with detailed studies on the effects of heat shock on individual pea aphids. Russell and Moran (2020) studied the effects of three lines of \( S. symbiotica \) within the aphid clone ‘5A’ that was isolated in Madison, WI, and naturally had no secondary endosymbionts. Here we focus on two aphid lines. The first is the parent ‘5A’ line that is sensitive to heat shocks because it contains \( B. aphid promoter allele and does not contain secondary endosymbionts. The second is resistant to the effects of heat shocks because it does not contain \( B. aphid promoter allele, and Russell and Moran inoculated the aphid line with the \( S. symbiotica \) line isolated from Arizona (\( S^a \)). These two aphid lines correspond to the red sensitive clone in pair A and the red tolerant clone in pair B. Aphids from the two clones were used in three treatments: no heat shock, heat shock at day 2, and heat shock at day 38.2°C in an ambient temperature of 18°C to 37.5°C over 2h, maintaining the high temperature for 4h, and reducing the temperature back to 18°C over 2h. This treatment was more severe than that achieved in the field experiments, where we raised temperatures by about 5°C and on average reached a maximum temperature of 33.7°C (range 25.6 – 38.2°C). After the treatments, Russell and Moran (2020) determined the development time (time from birth to adulthood), survival to adulthood, and fecundity of surviving individuals measured at 5 and 9d after they reached adulthood.

Using laboratory data on development time, survival, and fecundity measured on individual aphids, we constructed Leslie matrix models to predict the population growth rates of the two aphid lines used in our field experiments. To tease apart the effects of shocks through development, survival, and fecundity, we computed the population growth rates including the separate effects of shocks on development, survival and fecundity while keeping the other life-history characteristics the same; thus, the effect of shocks through development was calculated using the development schedules of the heat shocked individuals but the survival and fecundity schedules of the non-shocked individuals (Table S4). The effects of heat shocks on the sensitive ‘5A’ clone were severe, reducing the population growth rate from 0.250 to 0.053 and 0.061 in the day 2 and day 6 shock treatments. The main effects of the heat shocks were through fecundity, which alone would have decreased population growth rates to 0.136 and 0.097. The tolerant aphid line, ‘5A’ with the \( S^a \) endosymbiont was also affected by both shock treatments, although to a lesser extent than the sensitive ‘5A’ line (Table S4).

These laboratory results cannot be compared directly to our field results, although they are instructive. The treatments in the laboratory were both more severe, changing temperature by almost 20°C, and more extreme, increasing temperatures to 37.5°C. Furthermore, the shocks were only applied once to individual aphids of a given age, rather than the twice-weekly shocks we administered to entire, mixed-age aphid populations in the field. Nonetheless, the population growth rates estimated for non-shocked lab individuals are very similar to those for non-shocked conditions in the field, which ranged from 0.214 to 0.269. In contrast to the laboratory experiments, however, in the field there was the suggestion of a cost to exhibiting heat tolerance, with the population growth rates slightly lower for the tolerant clones than sensitive clones in each of pair A and B. This difference parallels experimental results for pea aphid symbioses conferring parasite resistance; costs of symbiont presence in the absence of parasites were observed in experiments using mixed population cages but not in experiments based on individual aphids (S31).
The population cages are more likely to represent conditions in field populations. A further difference is that the more severe heat shocks administered in the laboratory caused greater declines in population growth rates of the sensitive aphid clone than observed in the field. Furthermore, even the tolerant clone in the laboratory experienced a decline in population growth rate with heat shock, particularly when the shock was delivered on day 6. In contrast, there was no discernable effect of heat shock on the tolerant aphid clones in the field, possibly due to the less extreme heat treatment in the field experiments. Finally, in the field experiment there was a distinct delay between the initiation of shocking and the detection of a decline in population growth rates. This is what we anticipated from the laboratory results, which show that the greatest effects of heat shocks on population growth rates occur through reduced fecundity once exposed juveniles become adults (The phenology of heat shock effects on laboratory populations below).

The phenology of heat shock effects on laboratory populations – As summarized above, aphid life stages are differentially susceptible to the effects of heat shocks; shocking young aphids has the greatest detrimental effect whereas shocking adults has almost no effect. Moreover, because the strongest effects of heat shocks occur on young aphids and appear via changes in aphid fecundity, differences do not appear until young aphids become adults and display diminished fecundity. Both of these factors strongly influence the phenology of heat shock effects on aphid dynamics. To characterize the timing of the heat shock response, we performed a laboratory experiment where a small number of adult aphids was allowed to initiate a small population of aphids on a plant. These small aphid groups were shocked regularly, and their growth and densities were monitored through time in the laboratory.

Methods – We initiated small colonies of aphids on individual plants and determined their growth through time. We used aphids that corresponded to pair B in the field experiment on the potential evolution of heat-shock tolerance: a red tolerant clone (5A11R) and a green sensitive clone (7-2-1). We randomly assigned individual plants to one of the two clones. To initiate the experiment, we placed 3 adult aphids from either strain onto an individual fava bean plant and covered each plant with a cage made of a Mylar tube with mesh screening. These adult aphids were from clones that had been kept in a constant 18˚C environment. There were 16 plants for each of the two clones. All plants were kept at 18˚C except for regular exposures to heat shocks. Heat shocks were applied by gradually increasing the temperature to 37.5˚C over 2h, maintaining the high temperature for 4h, and then gradually decreasing the temperature back to 18˚C. Aphids were shocked on day 2 of the experiment and then every third day until the end of the experiment. Counts of all aphids on a plant were performed every other day throughout the experiment. The experiment was terminated after 16d when the plants begin to deteriorate, at which time aphid population growth declines.

Results – After repeated, regular exposure to heat shocks in the laboratory, heat-shock tolerant aphid populations were larger than heat-shock susceptible aphid populations (Average ± 1 SEM 221.8 ± 26.9 heat-shock tolerant aphids vs. 142.8 ± 23.8 heat-shock susceptible aphids; t\_\_2=2.21 p=0.036). This difference, however, was not detectable until day 12 (heat tolerant line: 64.8 ± 5.1 vs. heat susceptible line: 53.6 ± 4.8; t\_\_2=1.60, p=0.12) and not statistically distinguishable until day 14 (heat tolerant line: 143.6 ± 14.2 vs. heat susceptible line: 85.4 ± 13.2; t\_\_2=3.01, p=0.006). These results are consistent with the results of the field cage experiment in which the effects of experimentally augmented heat shocks were not observed for 14-16d following the initiation of the experiment.

Predation rates on susceptible and tolerant aphids – If either C. septempunctata or H. axyridis preys upon susceptible aphids at a different rate than they prey upon tolerant aphids, predators could affect the evolution of tolerance to heat shocks. Specifically, a higher predation rate on heat-shock tolerant aphids would decrease the potential for evolution of tolerance. To address this possibility, we performed an experiment on the predation rates of both ladybeetle species on heat-tolerant vs. sensitive aphids.

Methods – The experiment used a paired design with adult predators and aphids in 9cm diameter Petri dishes. All predators were from recently established colonies that originated from alfalfa and soybean fields in southern Wisconsin. Before the experiment, all predators were satiated and then starved for 24h to standardize each predator’s hunger level and enhance the likelihood of predation. Half of the predators of each species were given 20 susceptible aphids for 2h, again satiated and starved for 24h, and then given 20 tolerant aphids for another 2h. Predation was quantified after 2h, at which point most predators were beginning to satiate. We switched the order for the other half of the predators so that they were given tolerant aphids first and then susceptible aphids. Performing the experiment as a paired design allowed us to account for individual differences in predation rates. Aphids used in this experiment were the two red morphs used in the Potential for Evolution field experiments. Susceptible aphids were from the ‘5A10’ line and tolerant aphids were from the ‘5A11R’ line. Both aphid lines were kept at a constant temperature (19˚C) prior to the experiment. We performed the paired experiment with 20 H. axyridis and 20 C. septempunctata.

Analysis – To determine whether predation rates varied between the tolerant and sensitive clones, we calculated the difference in how many aphids of each type were consumed by each predator and used ANOVA to determine if predator species or sex influenced the difference in aphid predation. We then compared the distribution of predation differences to a hypothesized mean of zero using a t-test.

Results – The difference in predation on tolerant and sensitive aphids did not vary between predator species (F\_\_2=0.01, p=0.89) or with the sex of the predator (F\_3\_6=1.08, p=0.30). Since there were no differences in predator species or sex, we maximized statistical power by combining all the data and determined whether the difference in predation between the two aphid clones differed from zero. We found no difference in predation rates on tolerant vs. sensitive clones (average difference in the number of heat shock tolerant aphids consumed – heat-shock sensitive aphids consumed ± 1 SEM: C. septempunctata 0.05 ± 0.77; H. axyridis 0.2 ± 0.77; overall 0.13 ± 0.54) (Fig S2). The mean value of the combined distribution was not different from 0 (t\_\_2=0.23, p=0.82).

5. Model
To investigate the combination of ecological and evolutionary processes affecting the response of pea aphids to increased frequency of heat shocks, we used a population dynamics model that we parameterized from our field data. In the model pea aphid dynamics are given by

\[ x_{t+1} = x_t \exp(r \cdot y(t)) \]

where \( x_t \) is the density of pea aphids, \( r \) is the population growth rate that differs between heat-tolerant and heat-sensitive clones and between environmental regimes with and without heat shocks, and \( y(t) \) is the abundance of predators as a function of pea aphid density. Values of \( r \) were obtained by averaging values for the two heat-sensitive and two heat-tolerant strains used in the field experiments (Table 1), specifically, in the regime without augmented heat shocks, the values of \( r \) were 0.2560 and 0.2305 for heat-sensitive and heat tolerant strains, and in the regime with
augmented heat shocks they were 0.1420 and 0.2210, respectively.

Predator abundances $y(x_t)$ were assumed to be proportional to the
densities of either predator from the field survey (Fig. 1). We used
the field survey data, rather than the field experiments, because
these give the larger-scale pattern of predation that incorporate the
suite of traits that determine the foraging of ladybeetles on aphids
(see 1. Aphids, endosymbionts and predators, Predators above).
The equation for predator abundance is

$$y(x_t) = c \exp[b_0 + b_1 \log_{10}(x_t)].$$

The values of $b_0$ and $b_1$ were estimated from the field survey data,
giving values of $-0.6147$ and $0.1246$ for $C. \text{septempunctata}$ and $-1.6181$ and $0.4374$ for $H. \text{axyridis}$. The proportionality constant $c$
was selected to give a pea aphid population density of 500 in the
non-heat-shock environmental regime to correspond to the average
pea aphid density observed in the field survey data (Fig. 1).

Although we assume that the predation rate by the two
ladybeetle species is proportional to abundance in the field survey,
the qualitative results of the model do not strongly depend on this
assumption. For example, assuming the predation pressure scales
with density to the power $\frac{1}{2}$, as would be the case of a strong type
2 functional response, does not change the qualitative contrast
between the effects of ladybeetle species on pea aphid dynamics.

Finally, the model assumes that the predation rates on
sensitive and tolerant aphids do not differ. While this is consistent
with small-scale laboratory experiments (see 4. Potential for
Evolution, Predation rates on susceptible and tolerant aphids), this
assumption is difficult to test in the field under natural conditions.
If predators do select sensitive or tolerant aphid clones, this will
affect the evolution of tolerance to the degree of the differential
selection. Nonetheless, predator selection would have to be very
pronounced for it to be measurable against the large direct effect of
heat shocks on the selection differential between sensitive and
tolerant clones.

![Fig. S1: A 24h sample of temperatures for field cages manipulated
to produce a heat shock by wrapping the cage in plastic (red lines)
and ambient control cages (black lines). Temperature was
recorded every 5 minutes by data loggers placed at the height of
the alfalfa canopy. The heat-shock manipulation was performed
from 1000 – 1330 hours.](image)

![Fig S2: The difference in predation on heat-shock tolerant aphids
minus predation on sensitive aphids for individual $C. \text{septempunctata}$ and $H. \text{axyridis}$. Circles are points for individuals
and bars are means (+/- SEM) for each predator. Points have been
spread horizontally to show all of the data.](image)
and the last 4 sample days, (B) Since these interactions were significant, limited to the first 5 sample days, statistical model is a GLMM performed by the lmer() function in R assuming that the response variable (density of ladybeetles) is Poisson distributed. The “family=quasipoisson” option was used that can incorporate greater-than-Poisson variability in the samples. The term S is a binary factor (C. septempunctata = 0, H. axyridis = 1); C is a factor denoting the harvest cycle of alfalfa (first through fourth), with different levels given for cycles in different years; Sample is the day of sample; and Field-year is a factor that distinguishes samples taken in the same field during the same year. Sample and Field-year are used as grouping variables to structure the random effects in the model; these variabilities are accounted for non-independence of C. septempunctata and H. axyridis counts within the same sample and within the same field-cycle. The differences in ladybeetle species responses to aphid density is captured in the S * log₁₀ A interaction term. For the analysis of the interaction effects S * C and C * log₁₀ A, statistical significance was determined using a likelihood ratio test for the models with and without the interactions. Since these interactions were significant, we did not test the significance of the main effect of C.

Table S1.

A. All harvest cycles

<table>
<thead>
<tr>
<th>Random effects</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample (N=431)</td>
<td>0.811</td>
</tr>
<tr>
<td>Field-cycle (N=84)</td>
<td>0.53</td>
</tr>
<tr>
<td>Residual</td>
<td>1.08</td>
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</tbody>
</table>

Random effects

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Coefficient</th>
<th>Standard Error</th>
<th>t-value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>-3.35</td>
<td>0.46</td>
<td>-7.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>log₁₀ A</td>
<td>2.85</td>
<td>0.79</td>
<td>3.60</td>
<td>0.005</td>
</tr>
<tr>
<td>S * log₁₀ A</td>
<td>0.752</td>
<td>0.10</td>
<td>7.39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>S * C</td>
<td>-2.54</td>
<td>0.42</td>
<td>5.90</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C * log₁₀ A</td>
<td>1.52</td>
<td>0.30</td>
<td>5.03</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

B. The same analysis as in (A), but for only second and third harvest cycles

<table>
<thead>
<tr>
<th>Random effects</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample (N=310)</td>
<td>1.00</td>
</tr>
<tr>
<td>Field-year (N=54)</td>
<td>0.78</td>
</tr>
<tr>
<td>Residual</td>
<td>1.34</td>
</tr>
</tbody>
</table>

Random effects

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Coefficient</th>
<th>Standard Error</th>
<th>t-value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>-4.85</td>
<td>0.50</td>
<td>-9.71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>log₁₀ A</td>
<td>0.398</td>
<td>0.11</td>
<td>3.47</td>
<td>0.005</td>
</tr>
<tr>
<td>C</td>
<td>-1.11</td>
<td>0.33</td>
<td>-3.35</td>
<td>0.005</td>
</tr>
<tr>
<td>S * C</td>
<td>1.52</td>
<td>0.30</td>
<td>5.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>S * log₁₀ A</td>
<td>0.940</td>
<td>0.13</td>
<td>7.25</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table S2: Results of two experiments on the interaction between heat shocks and predation using (A) C. septempunctata, (B) C. septempunctata but confining the analysis to the last 4 sample days, (C) C. septempunctata but confining the analysis to the first 5 sample days, the same length as the H. axyridis data set, and (D) H. axyridis. The statistical model is a GLMM performed by the lmer() function in R assuming that the response variable (density of pea aphids) is Poisson distributed. The “family=quasipoisson” option was used that can incorporate greater-than-Poisson variability in the samples. The treatments are given by the categorical variables “Shock” and “Pred,” and day-of-experiment was treated as a categorical variable “Day.” The model included a random effect “Cage” to account for repeated measurements of the same cages. For the analysis of the interaction effect Shock*Pred including Day (= day of experiment), statistical significance was determined using a likelihood ratio test for the models with and without the interactions Shock*Pred and Shock*Pred*Day.

Table S2.

A. C. septempunctata

<table>
<thead>
<tr>
<th>Random effects</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cage (N=20)</td>
<td>5.80</td>
</tr>
<tr>
<td>Residual</td>
<td>7.04</td>
</tr>
</tbody>
</table>

Random effects

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Test score</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shock<em>Pred</em>Day</td>
<td>χ² = 76.8</td>
<td>&lt;10⁻⁸</td>
</tr>
</tbody>
</table>

B. C. septempunctata limited to the last 4 sample days

<table>
<thead>
<tr>
<th>Random effects</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cage (N=20)</td>
<td>4.34</td>
</tr>
<tr>
<td>Residual</td>
<td>3.99</td>
</tr>
</tbody>
</table>

Random effects

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Test score</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shock<em>Pred</em>Day</td>
<td>χ² = 2.4</td>
<td>&gt;0.1</td>
</tr>
</tbody>
</table>

C. C. septempunctata limited to the first 5 sample days

<table>
<thead>
<tr>
<th>Random effects</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cage (N=20)</td>
<td>3.33</td>
</tr>
<tr>
<td>Residual</td>
<td>3.64</td>
</tr>
</tbody>
</table>

Random effects

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Test score</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shock<em>Pred</em>Day</td>
<td>χ² = 54.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

D. H. axyridis

<table>
<thead>
<tr>
<th>Random effects</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cage (N=20)</td>
<td>1.66</td>
</tr>
<tr>
<td>Residual</td>
<td>5.90</td>
</tr>
</tbody>
</table>

Random effects

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Test score</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shock<em>Pred</em>Day</td>
<td>χ² = 41.8</td>
<td>&lt;10⁻⁸</td>
</tr>
</tbody>
</table>

¹The best-fitting model did not include a C * log₁₀ A interaction.
Table S3: Population growth rate $r$ calculated from laboratory measurements on the development time, survival, and fecundity of two aphid lines differing in sensitivity to heat shocks (S20). The sensitive aphid line is ‘5A’ and the tolerant line is ‘5A’ that had been inoculated with the secondary endosymbiont S. symbiotica (strain S6d2). The population growth rates $r$ were calculated to include the effect on development only, survival only, fecundity only, and all three life history characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pair A</th>
<th>Pair B</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_s$</td>
<td>0.243 ± 0.009</td>
<td>0.269 ± 0.008</td>
</tr>
<tr>
<td>$r_t$</td>
<td>0.214 ± 0.007</td>
<td>0.247 ± 0.010</td>
</tr>
<tr>
<td>$r_s$(shock)</td>
<td>0.155 ± 0.027</td>
<td>0.129 ± 0.033</td>
</tr>
<tr>
<td>$r_t$(shock)</td>
<td>0.234 ± 0.023</td>
<td>0.208 ± 0.031</td>
</tr>
<tr>
<td>$\sigma^2_{\text{cage}}$</td>
<td>0.187 ± 0.036</td>
<td>0.218 ± 0.043</td>
</tr>
<tr>
<td>$\sigma^2_{\text{shock}}$</td>
<td>0.180 ± 0.033</td>
<td>0.195 ± 0.045</td>
</tr>
<tr>
<td>$\gamma_{\text{init}}$</td>
<td>-4.12 ± 0.085</td>
<td>-3.56 ± 0.075</td>
</tr>
<tr>
<td>$\sigma^2_{\text{init}}$</td>
<td>0.516 ± 0.143</td>
<td>0.073 ± 0.060</td>
</tr>
</tbody>
</table>

For both pair A and pair B, the decrease in $r_s$ due to heat shocking was greater than the decrease in $r_t$; likelihood ratio test, pair A: $\chi^2 = 12.1, P < 0.001$; pair B, $\chi^2 = 6.96, P < 0.01$.

Table S4: Population growth rate $r$ calculated from laboratory measurements on the development time, survival, and fecundity of two aphid lines differing in sensitivity to heat shocks (S20). The sensitive aphid line is ‘5A’ and the tolerant line is ‘5A’ that had been inoculated with the secondary endosymbiont S. symbiotica (strain S6d2). The population growth rates $r$ were calculated to include the effect on development only, survival only, fecundity only, and all three life history characteristics.

<table>
<thead>
<tr>
<th>Aphid line</th>
<th>Treatment</th>
<th>Development</th>
<th>Survival</th>
<th>Fecundity</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘5A’</td>
<td>No shock</td>
<td>0.250</td>
<td>0.250</td>
<td>0.250</td>
<td>0.250</td>
</tr>
<tr>
<td></td>
<td>Shock at day 2</td>
<td>0.208</td>
<td>0.173</td>
<td>0.136</td>
<td>0.053</td>
</tr>
<tr>
<td></td>
<td>Shock at day 6</td>
<td>0.240</td>
<td>0.214</td>
<td>0.097</td>
<td>0.061</td>
</tr>
<tr>
<td>‘5A’ with S6d2</td>
<td>No shock</td>
<td>0.250</td>
<td>0.250</td>
<td>0.250</td>
<td>0.250</td>
</tr>
<tr>
<td></td>
<td>Shock at day 2</td>
<td>0.230</td>
<td>0.186</td>
<td>0.230</td>
<td>0.153</td>
</tr>
<tr>
<td></td>
<td>Shock at day 6</td>
<td>0.241</td>
<td>0.186</td>
<td>0.157</td>
<td>0.093</td>
</tr>
</tbody>
</table>

References