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Abstract

The effects of heat stress and symbiosis on pea aphid (Acyrthosiphon pisum) reproduction and survival across life stages

By Emily D. Tran

Acyrthosiphon pisum, the pea aphid, is a tractable insect model system to study cost trade-offs between alternative forms of defense, particularly with the establishment and maintenance of a beneficial, symbiotic relationship with protective microbes. Although there has been research on behavioral and physiological defenses, more understanding is needed in how these defenses trade off with each other as well as how the maintenance of microbial symbionts impact biologically important traits like lifespan and fecundity. These costs can be exacerbated under stress, and heat shock is a common and relevant experimental stressor as climate change is expected to impact many ecological systems.

A previous study observed this defense trade-off phenomenon between the winged polyphenism and symbiotic relationships in adult pea aphid populations when adults were exposed to a heat shock. Here, the aim of our research is to expand on that work and determine whether the age at which aphids are exposed to stress impacts this trade-off.

Two experiments were conducted. Experiment 1 focused on the effects of heat shock in aphids possessing primary symbiont *Buchnera aphidicola* with an alternative strain (CO21) of the symbiont *Regiella insecticola* across 2nd instar, 4th instar, and adult life stages. Experiment 2 expanded on this and utilized genetically identical aphid lines (clone LSR1) with Ui and CO21 strains of *Regiella*. Our results suggest that heat stress at younger life stages in a pea aphid may neutralize negative fitness consequences and possibly improve fitness by adulthood. In addition, there may be an interaction between heat stress and certain symbionts, but it is unclear how exactly they impact host fitness.

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Introduction

In response to environmental stressors, organisms utilize numerous types of defense in order to survive. One defense strategy involves the establishment and maintenance of a beneficial, symbiotic relationship with protective microbes (Parker et al. 2011). Studies have shown that the energy investment in one form of defense often trades off with other types of defense mechanisms, as well as other life history traits that influence an organism's fitness (Rolff et al. 2003), suggesting that investment in symbiosis may lead to trade-offs as well.

Acyrthosiphon pisum, the pea aphid, is a tractable insect model system to study trade-offs between alternative forms of defense, particularly in relation to association with protective symbionts. Most aphid species possess *Buchnera aphidicola*, an obligate bacterial endosymbiont that provides their hosts with essential amino acids missing from the aphids' phloem sap diet (Shigenobu et al. 2000). Aphids can also possess secondary, 'facultative' symbionts that are not necessary for their survival or reproduction. Common facultative bacterial symbionts include S*erratia symbiotica, Hamiltonella defensa,* and *Regiella insecticola*. These influence aphid ecology and evolution by impacting traits such as host-plant utilization, fungal resistance, parasitoid resistance, heat tolerance, reproduction, and development (Oliver et al. 2010). This simple microbiome makes it easier for us to measure the costs of symbiosis and other forms of defense.

In addition to having symbionts that can provide defensive benefits, aphids can also utilize behavioral and morphological defense mechanisms. When threatened by the presence of predators, aphids will drop from their plants while releasing a particular alarm pheromone that can alert other nearby aphids, leading to mass dropping behavior (Roitberg et al., 1978). Other environmental stressors, such as overcrowding, fungal pathogens, and a lack of resources, can trigger the production of winged aphid offspring, an alternative morphological trait useful in allowing the aphids to migrate away from the threat and search for safer plants (Brisson and Stern, 2006). As a result of asexual reproduction, winged and wingless sisters are genetically identical. However, winged aphid offspring require different energy requirements since they must also invest resources into the development and maintenance of their wings and the respective musculature, and this cost may in turn influence other types of defenses (Parker et al., 2017). Studies have demonstrated that winged aphids have weaker immune resistances and are more susceptible to fungal infections compared to their wingless sisters, which suggests that investment in polyphenism-based defenses like wings leads to a trade-off with immune system defenses (Parker et al. 2017).

Although there has been research on behavioral and physiological defenses, more understanding is needed in how these defenses trade off with each other as well as how the maintenance of microbial symbionts impact biologically important traits like lifespan and fecundity. These costs may be exacerbated under stress, and heat shock is a commonly used experimental stressor since it is an easy factor to manipulate and control. Studying the effects of heat stress is extremely relevant as climate change is expected to impact many ecological systems, influencing physiological and behavioral processes in plants, animals and other organisms (Zhao et al., 2017). Increasing intensity and frequency of extreme temperature events may alter individual survival rates, development rates, and fecundity, as well as population growth rates (Meisner et al., 2014).

A previous study observed the defense trade-off phenomenon between the winged polyphenism and symbiotic relationships in adult pea aphid populations when adults were exposed to a heat shock (Reyes et al. in prep). Reyes *et al.* specifically demonstrated that winged adult aphids had lower lifespans and reproductive rates than their wingless sisters. In addition, aphid lines with certain symbionts performed worse than others. *Here, the aim of our research is to determine whether the age at which aphids are exposed to stress impacts this trade-off.* We use genetically identical aphid lines (clone LSR1) with alternative strains of the symbiont *Regiella insecticola* (Ui and CO21). The Ui strain protects their hosts against *Pandora neoaphidis,* a fungal pathogen. On the other hand, the CO21 strain is non-protective. We also include line LSR1-01 who possess *Buchnera aphidicola*, their primary symbiont, but not *R. insecticola.*

Developmental life stages in aphids include 1st, 2nd, 3rd, and 4th instars before adulthood. *Since 2nd instar aphids are not fully developed, we hypothesize that the effects of heat stress will be compounded when they are exposed at the 2nd instar stage, resulting in decreased lifespan and reproduction rates in those individuals exposed to heat stress at the 2nd instar as compared to those exposed to heat stress at the 4th instar.* Taking symbiosis as a defense trade-off into account, we further hypothesize that heat stress will have the greatest impact on 2nd instars who harbor the Ui *Regiella*, which has negative fitness consequences for aphids in the absence of heat shock (Reyes et al., in prep), resulting in the shortest lifespans and poorest fecundity.

Methods

Aphid Rearing

All experimental pea aphids were reared on fava bean plants at 20 degrees Celsius in summer light conditions. In order to maximize plant health and reduce variation from other environmental factors, only 10-day old plants were used in aphid rearing. A maximum of 15 aphids were kept on each plant.

Experiment 1 Protocols

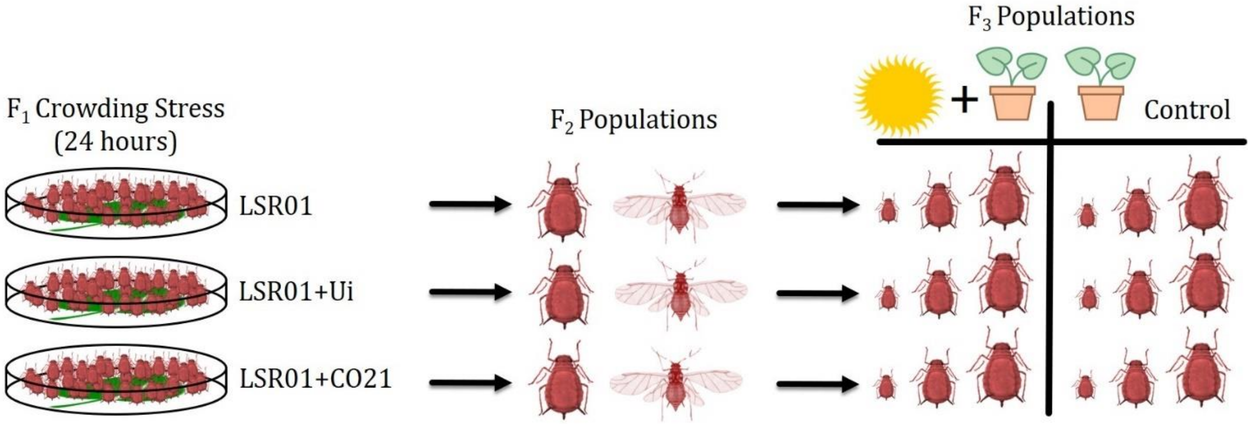

Fig. 1. Reyes et al. workflow. Experiments 1 and 2 closely followed this workflow established by Reyes et al., with the exception of crowding of F_1 wingless mothers and separating winged and wingless F_2 progeny across the three symbiont lines. Instead, only wingless F_2 individuals were considered and their F_3 progeny were separated by life stages (2nd instar, 4th instar, and adult) per line and placed into control and heat shock treatment groups. After the heat shock treatment, the aphids were split onto individual plants to monitor offspring production and survival.

We maintained aphids at 20 degree C under long day lengths (16 hours). Following established protocols from Reyes et al. (in prep), we focused on the LSR1-CO21 symbiont and produced a desirable sample size from wingless mothers and separated 28 individuals of the 2nd instars, 4th instars, and adult age groups each: 14 aphids in the control and heat-shock groups respectively. The treatment groups from all ages were subjected to heat shock at 30 degrees Celsius for six hours. Individuals that survived the heat stress developed into adults on separate plants, and at the adult stage, we conducted daily checks for survival and offspring production, measuring total fecundity for 30 days or until they all died.

Experiment 2 Protocols

Experiment 2 followed similar protocols from Reyes et al. with the exception of crowding the F_1 population, wingless F_2 progeny reproduced a desirable F_3 sample size of offspring across three symbiont lines, LSR1-01, LSR1-Ui, and LSR1-CO21. We separated 20 2nd instars and 20 4th instar aphids per line: 10 aphids in the control and heat-shock groups respectively (Fig. 1). The treatment groups were subjected to heat shock at 30 degrees Celsius for six hours. Individuals that survived the heat stress developed into adults on separate plants, and at the adult stage, we conducted daily checks for survival and offspring production, measuring total fecundity for 32 days or until they all died.

Unlike Experiment 1, Experiment 2 did not have adult aphid groups due to its large sample size as well as restrictions in time and resources. Reyes et al. (in prep) produced a heat-shock study previous to this work that focused on adult aphids from the same three symbiont lines produced by wingless mothers. Therefore, we utilized their results to analyze the data across age groups.

Statistical Analysis

Reproduction was analyzed via general linear models (GLM) ANOVAs with heat-shock treatment, aphid line and aphid age (instar phase) as fixed factors. Survival was measured via multiple sample Kaplan & Meier survival distributions. All analyses were conducted using the Statistica Ver. 12.0 software package (StatSoft® 1984-2015).

Results

Experiment 1

Reproduction

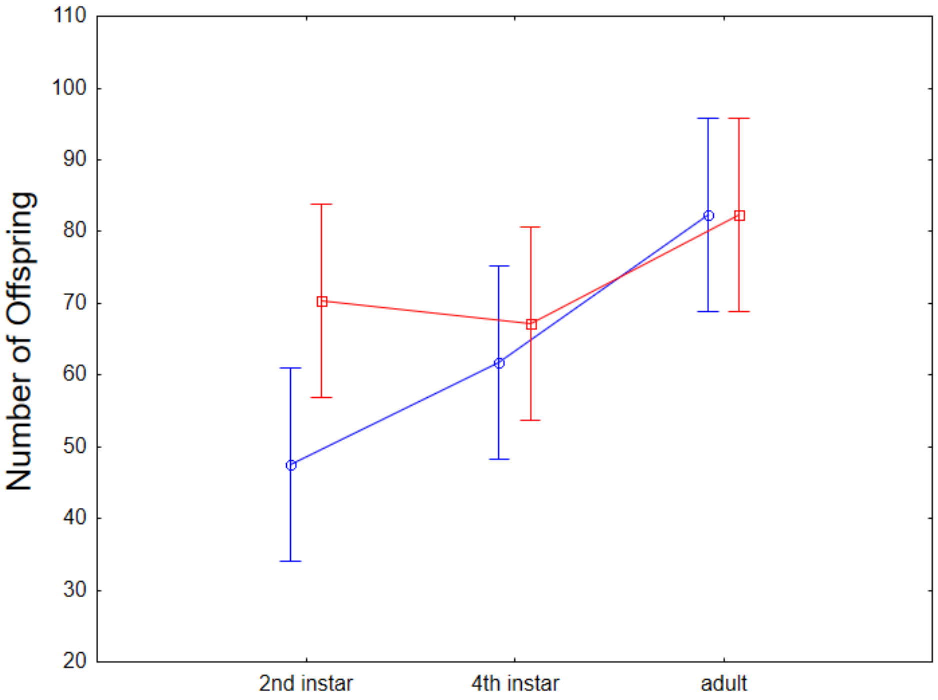

Fig. 2. Total reproduction across age groups of exposure to heat stress for LSR1-CO21. Number of offspring produced by the control (blue) and treatment groups (red) across different ages of heat shock exposure. Analysis using F-test yielded p=.002390.

There was not a significant interaction between age of exposure and treatment groups. For the adults and 4th instars respectively, the control and heat-shock groups had similar fecundities. On the other hand, there is a large gap between the controls and treatment group exposed to heat shock as 2nd instars in which the treatment group had greater reproduction than the controls (Fig. 2).

Survival

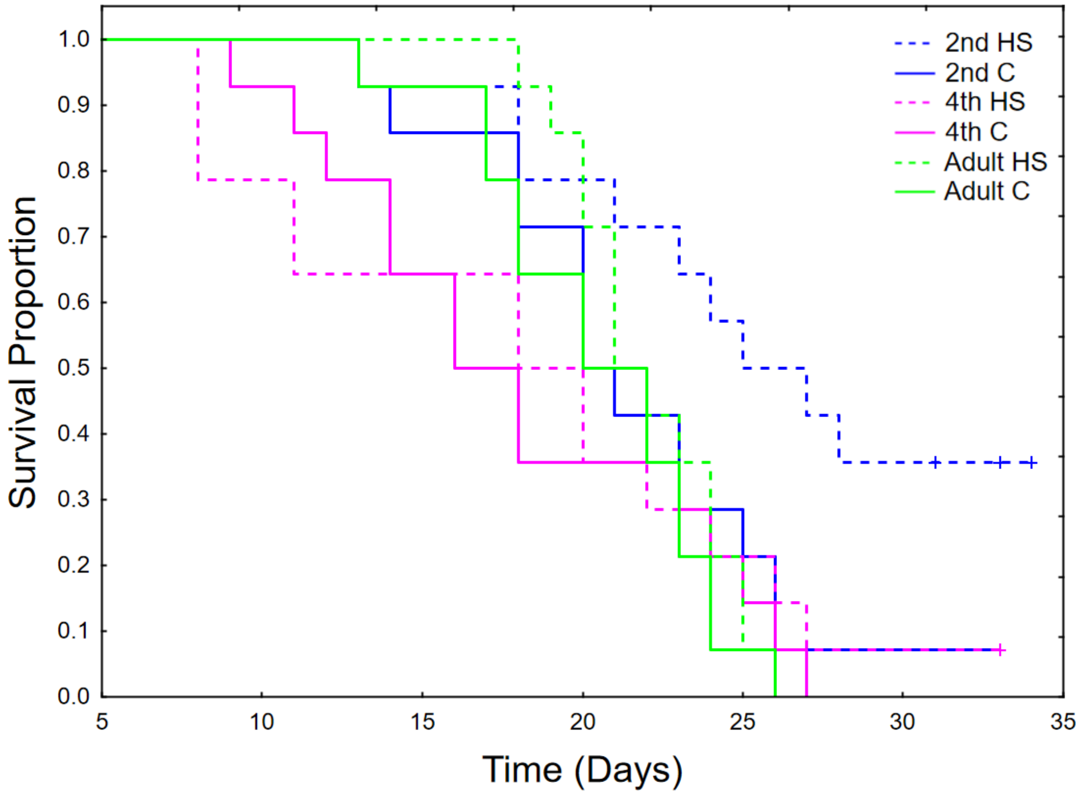

Fig. 3. Survival plot of all age and treatment groups for LSR1-CO21 aphids. Control groups are indicated by solid lines while heat shock treatment groups are in dashed lines. 2nd instars are indicated in blue, 4th instars in magenta, and adults in light green.

In Experiment 1, aphids exposed to heat stress at the 2nd instar stage generally performed better than those at 4th instar and adult stages. The heat-shocked 2nd instars had the highest survival rates with 40% of the population still alive at day 30, while the population of their control counterparts dwindled to 10% of the initial population by the last day. For the 4th instars, the treatment group performed similarly to the 2nd instar controls while the 4th instar controls all perished by day 26. Among the adults, the control and treatment group shared similar trends to the 4th instar control group (Fig. 3).

Experiment 2

Reproduction

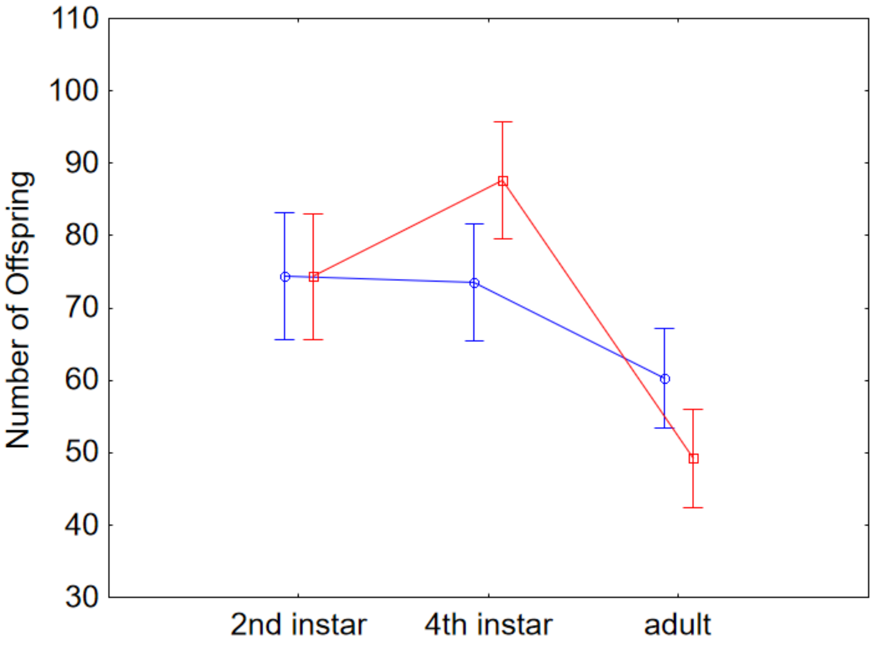

Fig. 4. Total reproduction across age groups of exposure to heat stress. Number of offspring produced by the control (blue) and treatment groups (red)**,** pooling all three different symbiont lines: LSR1-01 (primary symbiont only), LSR1-Ui, and LSR1-CO21 (primary and secondary symbionts). Analysis using F-test yielded p=.00489.

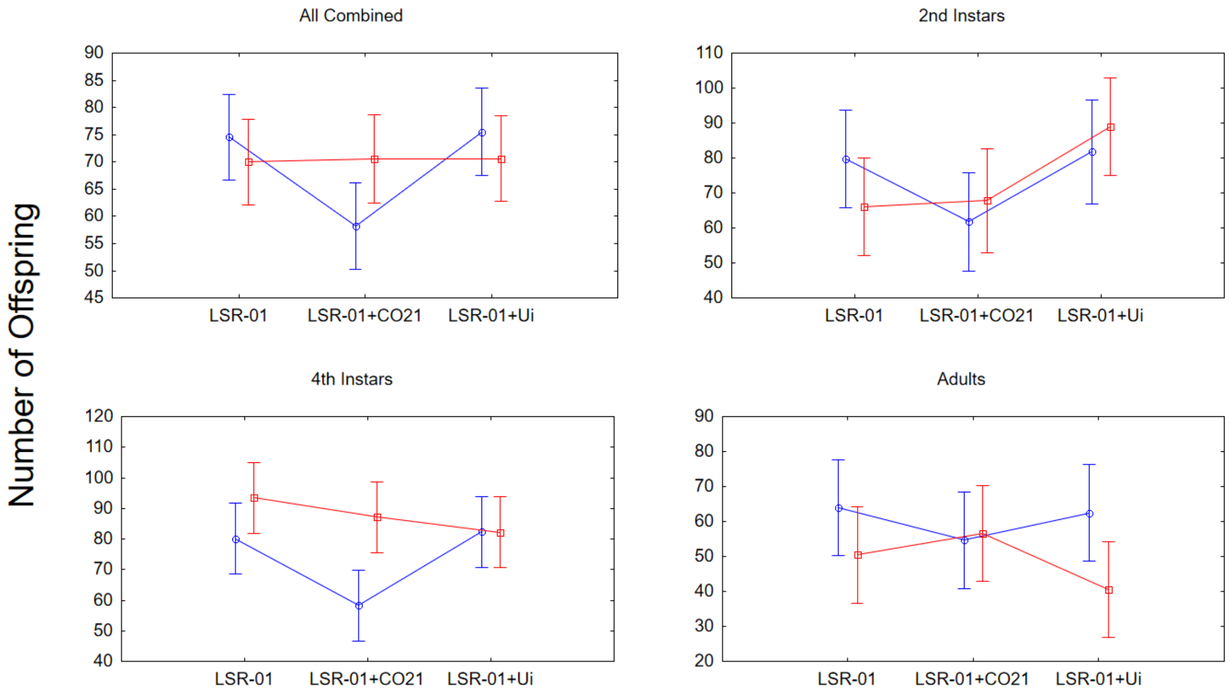

Fig. 5. Total reproduction across symbiont lines by treatment groups. Comparison of the total number of offspring across symbiont lines and treatment, with ages of exposure to heat stress pooled as well as individual age groups. Controls are in blue while the heat shock treatment groups are in red.

In Experiment 2, across all aphid instar stages, aphids exposed to heat stress at the 2nd and 4th instar stages had greater fecundity (Fig. 2) in comparison to adult aphids, with marginally non-significant differences due to *Regiella* presence (Fig. 5). There was a significant interaction (p = 0.00489) between heat-shock treatment and age of exposure (Fig. 4). When examining the impact of heat-shock across individual age groups, the reproduction of aphids exposed to heat-shock at the 2nd instar stage was similar to that of their control counterparts. Aphids exposed to heat-shock at the 4th instar stage had greater reproduction than the control group. For the adults who were exposed to heat stress, heat-shocked aphids had lower reproduction than the control group (Fig. 4).

Upon closer examination of heat treatment and individual symbiont lines, we noted that LSR1-CO21 controls across age groups had lower reproduction compared to the other lines (Fig. 5). In addition, those exposed to heat shock generally had equal or higher production compared to controls when considering all the symbiont lines and age groups. However, for the adults, the LSR1-Ui heat-shocked group had significantly lower fecundity than its control counterpart (Fig 5).

Survival

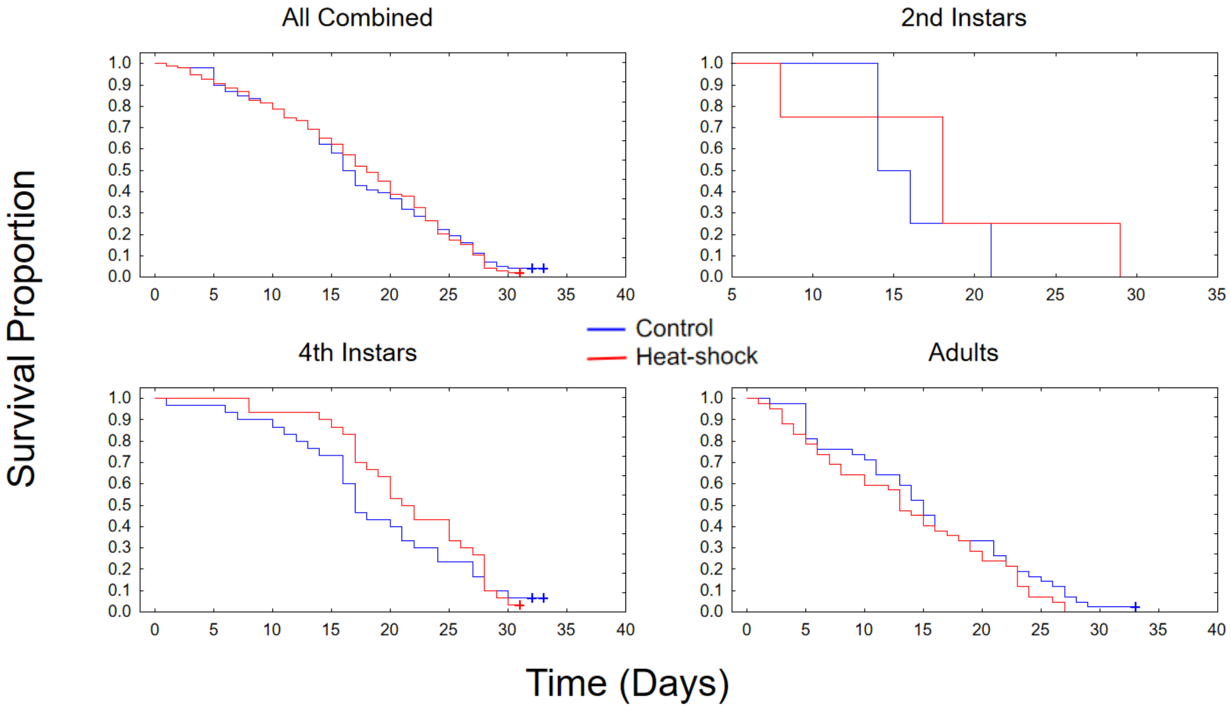

Fig. 6. Survival plots of pooled and individual age groups by treatment. Survival rates between control (blue) and treatment groups (red) across all ages of heat shock exposure, pooling

all three different symbiont lines: LSR1-01 (primary symbiont only), LSR1-Ui, and LSR1-CO21 (primary and secondary symbionts).

Regarding survival among the different age groups and their treatment, aphids exposed to heat shock as 4th instars, had the greatest survival rates in the 30-day period. Aphids exposed to heat shock at the 2nd and 4th instar stages performed better than their control counterparts. In contrast, when the adults were exposed to heat stress, they had worse survival rates than the controls, with the entire treatment population dead by day 27 while the controls survived to day 30 and even beyond (Fig. 6).

Discussion

In this study, we utilized the pea aphid as a model organism to assess whether the age at which aphids are exposed to stress (i.e., heat shock) impacts host response and defense trade-offs. We hypothesized that the effects of heat stress would negatively affect younger aphids, particularly at the 2nd instar stage, resulting in lower lifespan and fecundity because they are not fully developed. Furthermore, for Experiment 2 particularly, we hypothesized that the heat stress would have the greatest impact on 2nd instars who harbor Ui *Regiella*, which have negative fitness consequences for aphids in the absence of stress, resulting in the shortest lifespans and poorest reproductive rates.

Contrary to our hypotheses, Experiments 1 and 2 did not find negative fitness consequences in younger aphids exposed of heat stress. Experiment 1, which only focused on the LSR1-CO21 line across the three age groups, observed a beneficial heat stress effect in fecundity and survival. Those exposed to heat shock as 2nd instars had higher fecundity than their control counterparts. However, for those exposed as 4th instars and adults, there were no significant differences (Fig. 2). In terms of survival, all respective heat-shock groups had higher survival rates than their control counterparts, with aphids exposed to heat as 2nd instars having the highest among the ages of exposure (Fig. 3).

In Experiment 2, there were no differences in reproduction rates between the control and treatment groups when they were exposed to heat shock at the 2nd instar stage. Among the population exposed to heat stress as 4th instars, a significant difference was observed in which the heat-shocked group had greater fecundity than the controls. On the other hand, with consideration of the adult aphids from previous work, the treatment group had lower reproduction than the control group (Fig. 3). These patterns were also reflected in survival in which the heat-shocked groups had higher survival rates than their control counterparts among those exposed to heat shock at the 2nd and 4th instar stages. Adults displayed a different trend in which controls performed better than the treatment group (Fig. 5). Our results suggest that heat stress at younger life stages in a pea aphid may neutralize negative fitness consequences and possibly improve fitness by adulthood.

Stress in early developmental stages is significant, such that juvenile aphids have fewer advantages in terms of survival. The young instars have limited mobility, unlike adults who can escape from threats and stressful environments, but it is possible for the them to exhibit an offset effect during development that can dilute or buffer the negative effects of stress before maturation. This particular window of development can be observed in other species. A study on the consequences of a limited diet in juvenile stickleback fish observed compensatory growth responses in which the youngest groups of nutrient-deprived sticklebacks matched growth rates and lipid regeneration levels equivalent to controls while the oldest deprived sticklebacks showed partial body growth and less recovery of lipid levels (Reyes and Baker 2016).

The higher fecundities and survival of aphids exposed to heat shock at the 2nd and 4th instar stages could be a result of the heat shock interacting with *Buchnera aphidicola*, the pea aphid's primary symbiont. Studies have shown that a single nucleotide deletion within the transcriptional promoter for *ibpA*, a small heat-shock protein, eliminates its transcriptional response to heat stress and lowers the protein's expression at cool or moderate temperatures (Dunbar et al., 2007). This symbiont mutation has significant, direct impact on host fitness in which it can govern the thermal tolerance of aphid hosts. Researchers observed that, following a short heat exposure as juveniles, aphids with short-allele symbionts produced few or no progeny and contained almost no *Buchnera* compared to those bearing symbionts without the deletion. They also saw that lowering *ibpA* expression can improve host fitness under some conditions. Under constant cool conditions, aphids containing symbionts with the short-allele reproduced earlier and maintained higher productive rates in contrast to those bearing *Buchnera* without the deletion (Dunbar et al., 2007). Therefore, heat stress could impact *Buchnera* in a way that lower the costs from this host-symbiont relationship, resulting in higher host fitness.

In regard to the relationship between heat stress and symbiosis, Experiment 2 did not find LSR1-Ui *Regiella* with the greatest negative fitness consequences as we hypothesized. Instead, the non-protective *Regiella* strain, LSR1-CO21 was observed with the worst survival rates and fecundities out of the three symbiont lines when examining the pooled ages of exposure data or among the 2nd and 4th instars. The adult data from previous work did not illustrate significant differences in fecundity between the different symbiont lines but found LSR1-Ui with the lowest survival rates (Fig. 2 and 3). Therefore, our results suggest that there are underlying variables that may not be accounted for in these experiments or the previous work.

There are many factors that could contribute to the large variation between the previous work and the two experiments performed in this study. Experiment 2 utilized adult aphid data from previous work which was conducted at a different time of the year by different researchers, allowing for more variations in the execution of the experiments despite attempts to use the same protocols. In addition, the seasonal differences may be a confounding factor that could affect plant health or the aphids themselves. The quality of the stock populations from which we bred our experimental samples may have an effect as well. In the future, more studies can be done, such as a large-scale experiment that encompasses the same three symbiont lines and age groups of heat shock exposure to control for temporal differences as well as experimental execution. In addition, microbiological and molecular studies can be conducted to observe how heat stress interacts with the symbionts directly, whether there are mutations at play or a possible decrease of *Buchnera* in the host, as a result of heat stress, that could potentially alleviate symbiotic costs at an early age and result in higher fitness.

In conclusion, this study expands on previous work and provides some insights particularly into the effects of heat stress on early life stages, suggesting a possible window during development that buffers these effects until an organism matures and reaches adulthood.

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