

# The Relationship between Thermal Tolerance of Cereal Aphids and their Bacterial Symbionts <sup>†</sup>

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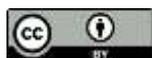
**Abstract:** This study was aimed to determine if there was any effect of chronic heat exposures and heat acclimation on the thermal thresholds and on the abundance of symbiotic bacteria of cereal aphids *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.). Aphid clones were randomly collected from the wheat fields and were reared in laboratory under controlled conditions. Thermal tolerance indices (chronic, basal and acclimated CT<sub>max</sub>) were determined for five-day old apterous female aphids. Real time quantitative-PCR was used to assess the total eubacterial (16S) and aphid-specific bacterial symbiont gene abundance in aphids. Averagely, *R. padi* were more tolerant to chronic heat exposure (to 31 °C) and its CT<sub>max</sub> values were 1.0°C higher than *S. avenae*. Aphid-specific symbiont genes abundance per aphid was almost similar for both of the species. Moreover, for both species, temperature-tolerant aphids exhibited significantly higher symbiont genes than the susceptible aphids. Likewise, thermal tolerance of both aphid species were found correlated with the gene abundance of total symbionts (16S), *Buchnera aphidicola*, *Serratia symbiotica*, *Hamiltonella defensa*, *Regiella insecticola*, *Rickettsia* spp. and *Spiroplasma* spp., suggesting their potential role in conferring thermal tolerance to these aphids.

**Keywords:** Bird cherry-oat aphid; English grain aphid; Thermal threshold; CT<sub>max</sub>; Acclimation; bacterial symbionts; *Buchnera aphidicola*; *Serratia symbiotica*

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## 1. Introduction

Temperature change has been a focal point while demonstrating the direct and indirect effects of climate change on terrestrial organisms including herbivorous insect pests [1–4]. Many studies have demonstrated the pronounced effects of chronic and acclimated exposures to high temperatures on the thermal biology and physiology of insects including aphids [5–9]. As aphids harbor a dense and diverse community of intra- and extra-cellular microbial symbionts performing various obligatory and facultative functions [10–12] and this sap-feeding insects have been a model system for investigating insect-microbial symbionts interactions [13–16], the potential impact of thermal exposures (either chronic or acclimated) on the abundance of their gut microbial symbionts has not been elucidated yet.

This study aimed to understand how extreme temperature events would alter the thermal tolerance and symbiotic bacterial abundance of cereal aphids *Rhopalosiphum padi* (Linnaeus) and *Sitobion avenae* (Fabricius). Secondary objective was to find out if there is

any correlation among the thermal tolerance traits of aphids and their respective symbiotic bacteria?

## 2. Methods

### 2.1. Insect Collection and Culture

Two cereal aphids, *i.e.* bird cherry-oat aphid *R. padi* and English grain aphid *S. avenae*, were used as model species in this study due to their differential behaviour and population performance to extreme temperature regimes [9,17]. Ninety nine wild clones of each aphid species were collected randomly from different winter wheat (*Triticum aestivum* L.) fields near Hebei and Henan provinces of China and were reared separately up to F<sub>3</sub> generation under controlled temperature conditions at 20±2°C, 65±5% RH and 16 h L: 8 h D photoperiod.

### 2.2. Thermal Exposure Experiments

Three batches were made from each of the laboratory reared aphid generation. Each batch was composed of 33 five-day old healthy and active apterous adults. One batch was exposed chronically to a temperature of 31 °C till death in an environment chamber and aphid mortality was recorded at regular intervals of 3–6 h. We divided and preserved the aphid individuals into four batches according to their tolerance to chronic temperature (31 °C). One batch was used for the determination of basal critical thermal maxima (CT<sub>max</sub>) till death, while the other batch of 33 aphids was first acclimated for 3 h to a constant temperature of 34 °C prior to the determination of CT<sub>max</sub> which was determined using a programmed glycol bath. For determining acclimation temperature, preliminary study showed that aphids can be acclimated up to 3 h without losing their fitness. After CT<sub>max</sub> experiment, dead aphid individuals were immediately preserved in 95% ethanol at -20°C for DNA extraction.

### 2.3. Quantification of Aphid Symbionts Gene Copy Numbers

Using TIANamp® genomic DNA Kit (Tiagen Biotech, China), total DNA from the preserved aphid individuals was extracted according to manufacturer's protocol. Diagnostic PCR assays were performed for detection and optimization of annealing temperatures of primers using thermal cycler (Bio-Rad). For qPCR, linearized recombinant plasmids were prepared and standard curves were determined from 10-fold serial dilutions of the linearized plasmids containing 10<sup>1</sup> to 10<sup>9</sup> copies of targeted bacterial genes. Three independent technical and biological replicates were determined for each sample.

### 2.4. Statistical Analysis

Statistical analysis of data was done using analytical software Statistix V. 8.1®. Data values were compared using one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) post-hoc test at standard level of significance ( $\alpha = 0.05$ ). Gene copy numbers of aphid-specific bacterial symbionts among different treatments or aphid species were compared using Student's paired *t*-test at  $p \leq 0.05$ .

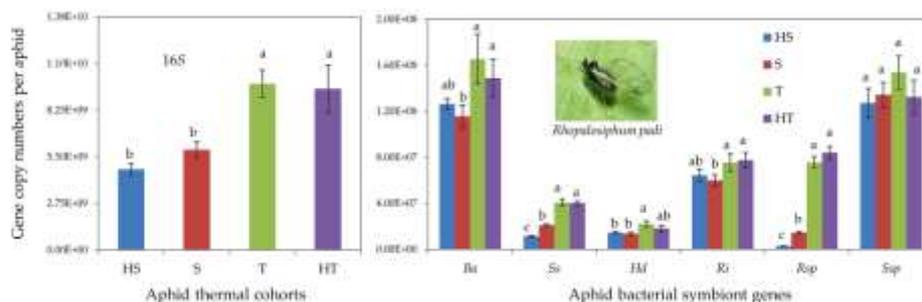
## 3. Results

### 3.1. Chronic Thermal Tolerance and Symbionts Gene Abundance

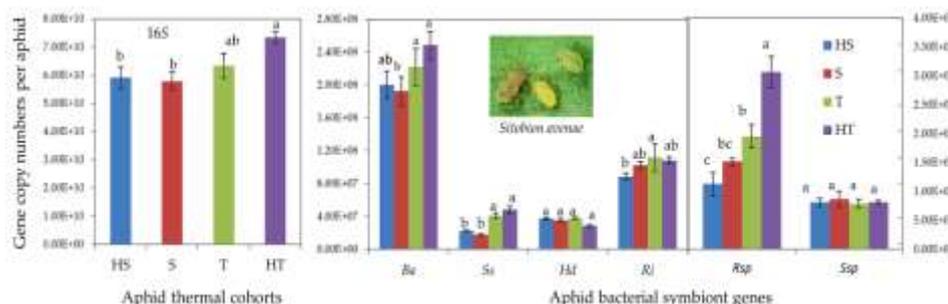
Upon chronic thermal exposure (to 31 °C) of 5 d old apterous aphid individuals, mortality was less than 5% within first 36 h and 18 h of exposure for *R. padi* and *S. avenae*, respectively, but then significantly increased and significant portion of the exposed aphid individuals (almost 75%) died between 36 to 66 h for *R. padi* and between 18 to 36 h for *S. avenae*.

Moreover, qPCR results revealed that tolerant (T) and highly tolerant (HT) aphid individuals of *R. padi* exhibited significantly higher copy numbers of total bacterial (16S

rDNA) and all of the endosymbiotic bacterial genes *i.e.* *B. aphidicola*, *S. symbiotica*, *H. defensa*, *R. insecticola* and *Rickettsia* spp. (Figure 2). However, in case of *S. avenae*, the difference was only significant for 16S rDNA, *S. symbiotica* and *Rickettsia* spp. only (Figure 3).



**Figure 2.** Gene copy numbers (mean ± SD) of aphid-specific bacterial symbionts in different thermal cohorts of *Rhopalosiphum padi* adults exposed chronically till death to 31 °C. Letters at bar tops indicate significant difference among the treatments (one-way ANOVA;  $p \leq 0.05$ ).

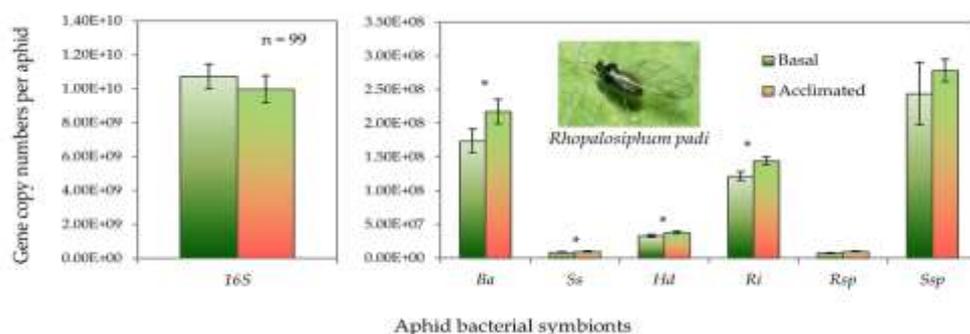


**Figure 3.** Gene copy numbers (mean ± SD) of aphid-specific bacterial symbionts in different thermal cohorts of *Sitobion avenae* adults exposed chronically till death to 31 °C. Letters at bar tops indicate significant difference among the treatments (one-way ANOVA;  $p \leq 0.05$ ).

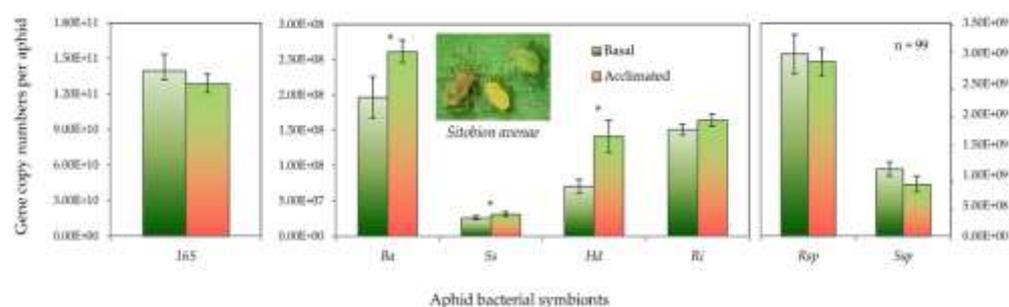
### 3.2. Critical Thermal Tolerance and Symbionts Gene Abundance

Average basal and acclimated thermal thresholds (CTmax indices) were  $37.41 \pm 0.48$  °C and  $38.82 \pm 0.44$  °C for *R. padi* and  $36.79 \pm 0.46$  °C and  $37.53 \pm 0.51$  °C for *S. avenae*. On average, *R. padi* thermal threshold values were approximately 1.0 °C higher than those of *S. avenae*. For both species, acclimated aphid individuals exhibited significantly higher thermal threshold (CTmax) than those of basal treatments ( $p < 0.001$ ). Moreover, thermal thresholds (both basal and acclimated CTmax indices) gradually increased from parental to F<sub>3</sub> generation for both aphid species, (data not shown).

In case of *R. padi*, the abundance of *B. aphidicola*, *S. symbiotica*, *H. defensa* and *R. insecticola* were significantly higher in acclimated aphid individuals than the non-acclimated (basal) ones (Figure 5). Similarly, gene copy numbers of *B. aphidicola*, *S. symbiotica* and *H. defensa* are found slightly but significantly higher in acclimated *S. avenae* aphid individuals than non-acclimated ones (Figure 6). Thermal tolerance indices were found significantly correlated with the gene abundance (copy numbers) of total symbionts, *B. aphidicola*, *S. symbiotica* and *R. insecticola* for *R. padi*, while with the gene abundance of total symbionts, *B. aphidicola*, *S. symbiotica*, *Rickettsia* spp. and *Spiroplasma* spp. for *S. avenae* (data not shown).



**Figure 5.** Gene copy numbers (mean ± SD) of aphid-specific bacterial symbionts in basal and acclimated *Rhopalosiphum padi* adults. Asterisk (\*) symbol indicates significant difference among both basal and acclimated treatments (Student’s paired *t*-test;  $p \leq 0.05$ ).



**Figure 6.** Gene copy numbers (mean ± SD) of aphid-specific bacterial symbionts in basal and acclimated *Sitobion avenae* adults. Asterisk (\*) symbol indicates significant difference among both basal and acclimated treatments (Student’s paired *t*-test;  $p \leq 0.05$ ).

#### 4. Discussion

This study was aimed to determine the impact of different heat treatments or high temperature exposures on the thermal thresholds of cereal aphids *R. padi* and *S. avenae* and on the abundance of their primary (obligate) endosymbiotic bacterium *B. aphidicola* and secondary (facultative) endosymbiotic bacterial symbionts (i.e. *S. symbiotica*, *H. defensa*, *R. insecticola*, *Rickettsia* spp. and *Spiroplasma* spp. [16,18].

Results revealed that *R. padi* individuals were more tolerant to chronic thermal exposure than *S. avenae* and averagely exhibited 1.0 °C higher thermal threshold (basal and acclimated CTmax) values than those of *S. avenae*. These results regarding the species-specific differential heat tolerance and/or thermal traits are consistent with the findings of Zhu et al. [17] who demonstrated that *R. padi* aphid species had higher evolutionary potential when exposed to extreme high-temperature events while *S. avenae* had less fitness under thermal extremes. Our results are in line with previous studies showing that *S. avenae* is more heat sensitive than *R. padi* [7,19].

For both species, acclimated aphid individuals exhibited significantly higher thermal threshold (CTmax) than those of basal treatments. These results are line with Zhu et al. [17] revealing that acclimated *R. padi* had a higher heat tolerance (CTmax) and fitness than *S. avenae*. Enhanced thermal threshold and plasticity induced by the acclimation to elevated temperatures has been documented in other organism as well including mites [20] and aquatic holobiont (hydra/algae) systems [21].

Moreover, it was found that as compared to susceptible ones, the cohorts of tolerant aphid individuals of both species harbored significantly higher bacterial symbiont genes particularly of *B. aphidicola*, *S. symbiotica*, *R. insecticola* and *Rickettsia* spp. Similarly, *B. aphidicola*, *S. symbiotica* and *H. defensa* gene abundance were significantly higher in acclimated aphid individuals than non-acclimated (basal) ones. Moreover, thermal tolerance indices correlated significantly with the abundance (gene copy numbers) of total symbionts, *B.*

*aphidicola*, *S. symbiotica* and *R. insecticola* for *R. padi*, while with the gene abundance of total symbionts, *B. aphidicola*, *S. symbiotica*, *Rickettsia* spp. and *Spiroplasma* spp. for *S. avenae*. These findings substantiate the putative role of these aphid specific bacterial symbionts in mediating host's thermal tolerance [22,23]. Russell and Moran [23] demonstrated that aphid symbionts *S. symbiotica* and *H. defensa* confer tolerance to high temperature exposures. Overall, our results suggest that aphid-bacterial symbiont interactions may play a crucial role in the thermal adaptation of hosts to extreme temperature events or exposures.

## 5. Conclusions

In brief, study revealed that *R. padi* and *S. avenae* individuals tolerant to chronic thermal exposures harbored significantly higher bacterial symbiont abundance than the susceptible ones. Acclimation to 34 °C for 3 h significantly increased the thermal tolerance (CT<sub>max</sub>) and bacterial symbiotic gene abundance for both aphid species. On average, *R. padi* thermal threshold values were approximately 1.0 °C higher than those of *S. avenae*. For both species, the abundance of *B. aphidicola*, *S. symbiotica*, *H. defensa* and *R. insecticola* were significantly higher in acclimated aphid individuals than basal (non-acclimated ones) and were found positively correlated with the basal and acclimated thermal indices.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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