Mild thermal stress does not negatively affect immune gene expression in the bumblebee *Bombus terrestris*

Nuria BLASCO-LAVILLA, Andrés GARCÍA-REINA, Pilar DE LA RÚA

Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Murcia, 30100, Murcia, Spain

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Abstract – Body temperature changes can modify an insect’s ability to fight infections by altering its immune activity. This work evaluated the impact of mild cold and heat thermal stress on the expression of different immune and heat shock genes in the species *Bombus terrestris*. Additionally, a thermal treatment was repeated under starvation to analyze a possible compromise of immune gene expression in favor of a thermal stress response when energetic resources are limited. Results pointed to a role of *Hsc70* and *Aha1* genes in tolerance to moderately high temperatures. Expression of the immune genes was not negatively affected by the cold or the heat treatments, and the receptor gene *BGRP1* was upregulated with cold, possibly indicating an increase in the cellular immune activity. Under starvation, the effect of the heat and the cold treatments caused a higher upregulation in all genes analyzed, suggesting a synergistic effect of starvation and thermal stress on the activation of heat shock and immune gene expression.

bumblebee / temperature variation / heat shock genes / immunity / RT-qPCR

1. INTRODUCTION

Insects are ectothermic organisms whose basic physiological functions such as locomotion, growth, and reproduction are strongly influenced by environmental temperature (Huey and Stevenson 1979). Changes in body temperature can affect the performance of insects, altering their capacity to fight infections (Thomas and Blanford 2003). It has been proposed that temperature can directly modify the immune response in different ways: altering thermosensitive cellular and enzymatic processes that drive immune activity (Somero 1995; Le Morvan et al. 1998), eliciting

the activation of pathways and mechanisms that are shared between thermal and immune stresses (Sinclair et al. 2013), or compromising the immune response as a trade-off with the thermal response (Ferguson et al. 2018). Interaction between the immune and the thermal stress pathways (cross-talk) and activation of shared mechanisms (cross-tolerance) can confer an adaptive advantage if both stressors are encountered in nature simultaneously, e.g., an insect facing a pathogen and a cold temperature at the same time. Nonetheless, if the mechanisms activated by these two pathways are different and have a high energetic cost associated, protection against one of the stressors could be compromised with negative consequences (Sinclair et al. 2013).

Heat shock proteins (HSPs) are produced as a response mechanism for thermal stress. The transcription of this group of proteins was first found to increase dramatically under elevated temperatures (Ritossa 1962). However, they have also

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Corresponding author: N. Blasco-Lavilla,
nuria.blasco@um.es

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been found to increase under different stressful conditions such as cold, osmotic, and oxidative stress, hypoxia, exposure to toxic substances, or infections (Feder and Hofmann 1999). They help to protect other proteins from denaturation and assist in the refolding or degradation of aberrant ones. There are six major families according to their molecular mass and function: small HSP, HSP40, HSP60, HSP70, HSP90, and HSP100 (Fink 1999). In mammals, they have been proposed to act as danger signals and immunoregulatory molecules (Pockley and Henderson 2017). In insects, there is also evidence supporting their role in the immune response (Wojda and Kowalski 2013; Merklings et al. 2015). For example, the heat shock transcription factor (Hsf) has been found to be involved in the antiviral response in *Drosophila* spp. (Merklings et al. 2015) and Paim et al. (2016) found that the knockdown of Hsp70/Hsc70 compromised the immune response in the hemipteran *Rhodnius prolixus*. HSP70 and HSP90 members are the most widely studied in insects under different stresses (Zhao and Jones 2012).

Among the HSP70 family, 70-kDa heat shock cognate protein (Hsc70) isoforms are constitutively expressed, highly conserved across organisms, and involved in multiple cellular functions such as protein folding, intracellular transport, degradation, and endocytosis (Stricher et al. 2013). On the other hand, Hsp90 isoforms can be constitutive or inducible, are also very conserved, and participate in protein maturation and degradation, signal transduction, and protein homeostasis in different stress conditions (Taipale et al. 2010). In the bumblebee *Bombus terrestris*, previous studies have shown an upregulation of *Hsc70* and *Hsp90* genes in relation to diapause (Kim et al. 2008), and Riddell et al. (2011) found the gene that codifies the activator of the heat shock protein 90 ATPase (*Aha1*) to be upregulated upon infection when performing a subtractive suppression hybridization (SSH).

B. terrestris is the most common bumblebee species in Europe and an important commercial pollinator. Over the last 50 years, a decline in bumblebee populations has been documented and many stressors are thought to be implicated, including the recent spread of pathogens due to

the transport of commercial bee species (Goulson et al. 2015). This event has stimulated an interest in characterizing bumblebee immunity and its interaction with stressors such as protein limitation (Brunner et al. 2014), infection (Barribeau and Schmid-Hempel 2013), wounding (Erler et al. 2011), and exposure to pesticides (Walderdorff et al. 2018), and has turned this species into a model for studying insect immunity. However, the effect of thermal stress on bumblebee immunity has not yet been characterized. As extreme weather events are predicted to increase with climate change and expected to have a large impact on bumblebee populations (Goulson et al. 2015), it seems necessary to understand how thermal stress may affect the bumblebee's ability to fight pathogens. Additionally, the climate change might diminish the availability of preferred floral resources (Ogilvie and Forrest 2017); therefore, it is important to analyze the combined effect of thermal stress and starvation on bumblebee immune activity. Temperature shifts away from the thermal optima of the host are expected to increase their susceptibility to parasites, as the latter have shorter generation times and faster acclimation rates (Cohen et al. 2017). Palmer-Young et al. (2019) found that the infection outcomes of the parasite *Crithidia bombi* on *Bombus impatiens* decreased at moderately high temperatures even when they were optimal for the parasite growth *in vitro*. Therefore, the immune system of bumblebees could perform better under mild heat stress. Regarding cold, there are no previous studies that let us predict its effect on the immune system, but, considering that bumblebees are cold-adapted species (Heinrich 1979), we expect them to maintain or even increase immune activity at low temperatures.

The aim of this work is to provide a first insight into how mild thermal stress can affect bumblebee immunity and gain a better understanding of the interaction of these two factors on insect physiology. Our hypothesis is that thermal stress affects immune activity by increasing the expression of immune genes. To test this hypothesis, we analyzed the expression of different immune and heat shock genes under mild thermal stress treatments in commercial colonies of the species *B. terrestris*. We selected a hot (38 °C) and a cold

(9 °C) temperature that bumblebees can face in their natural habitat but are far from their ideal temperature (28 °C). The heat shock genes *Hsc70* and *Aha1* were selected as indicators of thermal stress, and immune genes codifying several components of the immune system were selected to obtain a general view of the immune activity. Expression was measured at time points ranging from 2 to 48 h to capture the response of genes with distinct temporal expression patterns.

2. MATERIALS AND METHODS

2.1. Bumblebee thermal treatments

Three young colonies of *B. terrestris* were obtained from Agrobio S.L. (Almería, Spain) for gene expression analysis. Six groups of three callow workers per colony were collected and placed in microcolonies with pollen and 50% sugar/water *ad libitum* and kept in darkness at 28 °C and 60% humidity. This microcolony-based set-up removed the thermoregulation effect of the colony and allowed us to measure the impact that environmental temperature would have upon foraging bumblebees. When workers were between 4 and 6 days of age, they were placed in an incubator and assigned to different temperature treatments: cold temperature (9 °C), hot temperature (38 °C), and control (28 °C). For each colony and treatment, one group of three individuals was snap-frozen in liquid nitrogen after 2 h, 6 h, 12 h, 24 h, and 48 h of treatment. They were always fed with pollen and sugar/water *ad libitum* except for the 6 h treatment that was replicated without feeding the bees to analyze the response under resource limitation (starvation) and test a possible trade-off between thermal stress and the immune activity. The cold treatment in starving bees (9 °C) could not be tested in one of the colonies, as it did not have enough callow workers. Individuals were kept at − 80 °C until they were analyzed.

2.2. RNA extraction and cDNA synthesis

Workers were dissected and the abdomen and thorax kept to ensure the presence of both *hsp* and immune gene transcripts. Homogenization was performed with a TissueRuptor (Qiagen) in lysis

RT solution (Invitrap kit, Stratec), and each group of three individuals per colony and treatment was pooled. RNA extractions were performed following the manufacturer's instructions (Invitrap kit, Stratec). Isolated RNA was quantified with NanoDrop 1000 (Thermo Fisher Scientific®), diluted to a concentration of 200 ng/μl, and treated with a TURBO DNA-free kit (Ambion, Life Technologies) to remove genomic DNA contamination. cDNA was synthesized using PrimeScript™ RT Reagent Kit (Takara Bio Inc.) and stored at − 20 °C.

2.3. Quantitative PCR analysis

To select the optimal genes for normalization, we analyzed the stability of a set of reference genes: arginine kinase (*AK*), elongation factor 1α (*Ef1α*), phospholipase A2 (*PLA2*), α-tubulin (*TUB*), β-actin (*ACTB*), ribosomal protein L13 (*RPL13*), ribosomal protein L23 (*RPL23*), and inositol 1,4,5-triphosphate (*ITPR*), using the thorax and the abdomen of bumblebees in our experimental temperature conditions (38 °C, 28 °C, and 9 °C).

Five genes from different classes were chosen to quantify the immune response: the receptor *BGRP1* involved in pathogen recognition, the signaling genes *Pelle* and *Relish* from the Toll and Imd pathways, respectively, the antimicrobial peptide gene *Abaecin* and the gene *Vitellogenin* that codifies a protein with antimicrobial activity and is involved in metabolism, pathogen recognition, and transgenerational immune priming (Barribeau and Schmid-Hempel 2013; Salmela et al. 2015; Park et al. 2018). The heat shock genes *Hsc70* and *Aha1* were analyzed to quantify the thermal stress response. Primers used have been previously published (Table SI) except for *Hsc70* primers which were designed with Primer Express 3 software (Applied Biosystems). Efficiencies and specific amplifications for all genes were checked using serial dilutions of cDNA with the standard curve method. Quantitative PCR was performed with NZYSpeedy qPCR Green Master (NZYTech) with primers at 0.1 μM and following a program consisting of an initial denaturation step at 95 °C for 10 min and 40 amplification cycles of denaturation at 95 °C for 15 s and

annealing/elongation at 60 °C for 1 min. Non-template samples were used as negative controls and a final melt-curve step was added to check for non-specific amplifications. Every reaction was run in triplicate on a StepOnePlus™ Real-Time PCR system (Applied Biosystems).

2.4. Expression data analyses

Reference gene stability was checked with the 6 h treatment samples, following the procedures described above. Raw C_T data were obtained with StepOne Software v2.3, transformed to logarithmic scale, and corrected using efficiencies. The most stable reference genes and the optimal number of needed genes were obtained with geNorm (Vandesompele et al. 2002) and NormFinder (Andersen et al. 2004) algorithms.

The selected reference genes were used as internal controls for the expression experiment with target genes. Raw data were obtained with the previous qPCR conditions and expression was analyzed using the delta-delta- C_T ($\Delta\Delta C_T$) method (Livak and Schmittgen 2001) considering efficiencies correction. Every pool of three individuals per colony was treated as a biological replicate, and three biological and three technical replicates were used. GraphPad Prism version 5.00 software (GraphPad Software, San Diego, CA, USA, www.graphpad.com) was used for statistical analyses. An unpaired t test was performed with delta C_T mean values for every time point and gene to detect significant effects ($p < 0.05$) of the cold (9 °C) and heat (38 °C) treatments against the control (28 °C) treatment. Welch's correction was applied when variances were significantly ($p < 0.05$) different.

3. RESULTS

3.1. Reference genes validation for thermal treatments

Three out of eight candidate reference genes were discarded from the validation test after showing unspecific amplifications or low efficiencies (Table SI). The five remaining genes (*PLA2*, *TUB*, *ACTB*, *RPL23*, and *ITPR*) showed low and similar M values with both geNorm and

NormFinder algorithms, indicating a high stability (Table I). Since the optimal number of reference genes necessary for normalization was two according to geNorm (Fig. S1), the genes *RPL23* and *TUB* were chosen for expression analyses.

3.2. Effect of thermal treatments on gene expression

Results from the analyzed immune and heat shock gene expression dynamics are shown in Figure 1. A general upregulation was observed under cold and hot temperatures with respect to the control treatment, except for the genes *Vitellogenin* and *Abaecin*, which were downregulated at several time points. Fold change values were usually below 0.5, but higher values were obtained for the genes *BGRP1*, *Abaecin*, *Hsc70*, and *Aha1*. T tests carried out with delta C_T values showed a significant change in expression in six out of the seven genes analyzed: the cold treatment caused a significant upregulation in *BGRP1* (24 h log₂ fold change = 1.02*), *Relish* (2 h log₂ fold change = 0.48*), and *Hsc70* (24 h log₂ fold change = 0.40***) and a significant downregulation in *Abaecin* (6 h log₂ fold change = -0.79*), whereas the heat treatment caused a significant upregulation in *Pelle* (6 h log₂ fold change = 0.44**), *Hsc70* (2 h log₂ fold change = 1.81**; 6 h log₂ fold change = 0.75*; 24 h log₂ fold change = 0.31**), and *Aha1* (2 h log₂ fold change = 1.01*; 24 h log₂ fold change = 0.79*; 48 h log₂ fold change = 0.94**). No clear expression patterns through time were observed except for *Hsc70*, which showed a peak of upregulation at 2 h followed by a decrease. The cold treatment apparently decreased the expression of *Vitellogenin* and *Aha1* progressively but no significant downregulation was obtained. A tendency for downregulation and upregulation of *Abaecin* was observed for the heat and cold treatments at 6 h, respectively, but there was a high variability between biological replicates.

3.3. Effect of thermal treatments on immune and heat shock genes under starvation

Results from the expression of immune and heat shock genes after 6 h of starvation and

Table 1. Stability values of reference genes (*M*) calculated with the algorithms geNorm and NormFinder. All genes were considered valid for normalization as their stability values were under the cut-off value ($M = 1.5$) established by geNorm

Algorithm	Gene				
	<i>ACTB</i>	<i>ITPR</i>	<i>PLA2</i>	<i>RPL23</i>	<i>TUB</i>
geNorm	0.240	0.464	0.326	0.272	0.270
NormFinder	0.177	0.082	0.095	0.144	0.128

thermal treatments are shown in Figure 2. Starvation caused a higher expression of all immune and heat shock genes measured when bumblebees were exposed to cold and hot temperatures. With respect to the treatment where bumblebees were fed *ad libitum*, *Pelle* upregulation was higher but

not significant after the heat treatment, *Hsc70* was significant for both treatments showing higher upregulations (heat log2 fold change = 0.89*; cold log2 fold change = 0.94*), and *Aha1* had a significantly higher upregulation with heat (log2 fold change = 1.12*). In general, the increase in

Figure 1. Temporal expression of immune and heat shock genes. Log2 fold change values are shown for the cold (9 °C) and heat (38 °C) treatments with respect to the control temperature (28 °C). The x-axis indicates the time points at which the samples were taken. Dashed lines mark the values 1 and -1, corresponding to doubled and halved gene expression, respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

expression was more notable with the cold treatment as the immune genes tended to be downregulated or did not change much when bumblebees were fed *ad libitum*.

4. DISCUSSION

4.1. Effect of thermal treatments on heat shock gene expression

In our study, the heat treatment caused a significant upregulation of both *Hsc70* and *Aha1* at many time points, which also points to an increment in *Hsp90* activity. *Hsc70* and *Hsp90* have been shown to rise under high temperatures in different insects like the mirid bug *Apolygus lucorum* (Sun et al. 2014, 2016) and other hymenoptera species (Xu et al. 2010; Nguyen et al. 2016). We found the highest expression after 2 h with *Hsc70* declining later. The downregulation of heat shock proteins during acclimation to heat stress may be a mechanism to reduce the costs of a heat response in favor of fecundity and development when temperatures are high but not extreme (Hoffmann et al. 2003). Another explanation is that *Hsc70* could be replaced by other heat shock proteins involved in heat acclimation, as was observed by Luo et al. (2015), who analyzed two *Hsc70* genes during heat shock recovery in the butterfly *Glanville fritillary* and found that one of them reduced its expression with time while the other did not. Even at the time points when the expression of heat shock genes was maximum, the upregulation was much lower than that observed for heat shock genes in *Apis mellifera* (McKinstry et al. 2017). This probably occurred because we chose a temperature well below the critical thermal limits reported for ubiquitous bumblebee species: *B. lucorum* enters the heat stupor when maintained at 40 °C (Martinet et al. 2015), and *B. impatiens* loses its postural control at 46 °C (Hamblin et al. 2017) and starts having muscular spasms at an average of 53 °C (Oyen and Dillon 2018). Possibly, expression of heat shock genes is stronger at critical temperatures for survival, although it may also occur that the genes we chose are not particularly heat responsive since *B. terrestris* has more heat shock genes belonging to the HSP70 and HSP90 families and heat shock

genes can be differently induced by heat (Nguyen et al. 2016, McKinstry et al. 2017).

The cold treatment caused a slight upregulation of *Hsc70* that was significant only after 24 h, and a non-significant increase of *Aha1* that was reduced during acclimation. It is possible that these heat shock proteins are not induced during cold stress but while insects are recovering as was observed in *Drosophila* (Colinet et al. 2010) and *Pyrrhocoris apterus* (Košťál and Tollarová-Borovanská 2009). This hypothesis might be supported by the increase of *Hsc70* and *Hsp90* expression in muscle observed in late and post diapause stages in *B. terrestris* (Kim et al. 2008). However, heat shock protein genes have been shown to be upregulated only in a certain range of cold temperatures and not when they are too extreme (Wang et al. 2012; Sun et al. 2014, 2016). Therefore, cold treatments with other temperatures might activate these genes in *B. terrestris*.

4.2. Effect of thermal treatments on immune gene expression

According to previous literature (Wojda 2017), an upregulation of immune genes in insects has been observed under thermal stress as a consequence of an interaction between immune and heat shock responses. However, McKinstry et al. (2017) found evidence for an antagonistic relationship between thermal stress and immune genes in the honey bee *Apis mellifera*.

In our study, the heat treatment caused a general upregulation of the receptor *BGRP1* and the signaling genes *Pelle* and *Relish* across all time points. However, it was very small and only significant in the case of *Pelle* at 6 h. This increase in transcription could be a consequence of a higher metabolic rate only (Somero 1995) or part of an enhanced immune response under heat stress as has been shown in other insects (Xu and James 2012; Wojda and Taszłow 2013). Other studies in *B. terrestris* have found signaling gene expression to be more stable compared with receptor or effector genes that had a strong variation even between individuals (Erler et al. 2011; Barribeau and Schmid-Hempel 2013). In this study, the elevated variance in *Abaecin* expression suggests a predominant effect of biological variation over

Figure 2. Expression of immune and heat shock genes after 6 h of treatment in bumblebees fed *ad libitum* (left) or starved (right). Log2 fold change values are shown for the cold (9 °C) and heat (38 °C) treatments with respect to the control temperature (28 °C). Only two biological replicates were obtained for the cold treatment under starvation. Dashed lines mark the values 1 and -1, corresponding to doubled and halved gene expression, respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

thermal treatments. This maintenance of immune activity under moderately high temperatures could be explained by the ubiquitous distribution of *B. terrestris*, which may correlate with a higher resistance to heat than species restricted to colder climatic regions (Martinet et al. 2015).

The cold treatment also caused a small upregulation of *Pelle* and *Relish* at most time points, with a significant increase in *Relish* after 2 h. An alteration in metabolic rate causing this overexpression would be ruled out, because in that case, a downregulation would be expected with cold temperatures, suggesting a direct link between cold stress and an increase in their expression. On the other hand, the weak upregulation observed does not follow a clear pattern so it is also probable that it is being affected by other factors

than cold temperature (e.g., biological variation). *BGRP1* remained high throughout the cold treatment and the increase was significant after 24 h, whereas *Abaecin* showed a significant downregulation after 6 h but progressively rose throughout the rest of the treatment. As a beta-1,3-glucan receptor, *BGRP1* is likely linked with the activation of the proPO system and the cellular response (Soltanian et al. 2009) in addition to the activation of the Toll pathway (Barribeau and Schmid-Hempel 2013), while *Abaecin* is an effector regulated by the Imd pathway (Schlüns and Crozier 2007). The upregulation of *BGRP1* in contrast to *Abaecin* is consistent with previous studies reporting a higher cellular response at low temperatures (Murdock et al. 2012; Franke and Fischer 2013) that may compensate for an

impaired humoral activity to maintain some level of defenses while conserving energy and investing in thermal tolerance during winter (Ferguson and Sinclair 2017; Ferguson et al. 2018). In bumblebees, an adaptive pressure to keep immune activity in the cold is expected due to the vertical transmission of bumblebee pathogens through queens in diapause during winter (Imhoof and Schmid-Hempel 1999; Rutrecht and Brown 2008). Another possible explanation for an activation of the cellular immune response is that it could be simply induced by the tissue damage caused by thermal stress (Salehipour-Shirazi et al. 2017).

An upregulation of *Vitellogenin* was expected as the protein participates in protection against oxidative stress (Seehuus et al. 2006) and this gene has been shown to be upregulated with several stressors including heat (Bordier et al. 2017; Zhang et al. 2017) and cold (Zhang et al. 2017) in the honeybee. In opposition to these studies, we observed a relatively stable expression with a tendency for downregulation after 24 h with both the heat and cold treatments. Although the temperatures used in this study did not trigger the expression of *Vitellogenin*, it is likely that other temperatures might do it as it was observed in the case of the silkworm *Bombyx mori* (Paul and Keshan 2016).

4.3. Effect of thermal treatments on immune and heat shock genes in starved bees

The thermal treatment of 6 h was repeated with individuals under starvation to study how gene expression varies under resource limitation. If compensation were to occur, one would expect to see a decrease in the expression of immune genes in favor of energy saving and improved thermal tolerance, whereas if shared pathways or mechanisms exist between the thermal stress and the immune responses, one would expect to see a maintenance or increase in the expression of immune genes. We considered 6 h of starvation to be enough to cause an energetic stress, since at this time some individuals had lost their postural control and showed an increased risk of death.

McKinstry et al. (2017) found evidence for a trade-off between the thermal and the immune responses in *A. mellifera*, congruent with the high costs of the activation of the immune response in *B. terrestris* (Moret and Schmid-Hempel 2000). Contrarily, we found that all genes were more upregulated at 6 h in starving bees than in non-starving ones, especially with the cold treatment. Previous studies have related heat shock gene expression with starvation, including *Hsc70* and *Hsp90* (Wang et al. 2012; Paim et al. 2016). It is possible that stresses derived from temperature, starvation, or even desiccation have a synergistic effect on activating immune activity. Several studies support this positive regulation: in *Drosophila*, starvation caused an upregulation of AMPs through the FOXO transcription factor (Becker et al. 2010) and heat shock factor (*Hsf*)-deficient adults were hypersensitive to viral infection (Merkling et al. 2015); in *Rhodnius prolixus*, *Hsp70* knockdown compromised immune gene upregulation in addition to affecting tolerance to starvation (Paim et al. 2016). Remarkably, Riddell et al. (2011) found that *Aha1* was upregulated when performing an SSH in *B. terrestris* infected with a natural parasite. Although this study could not confirm the results when performing a qPCR, it is possible that it constitutes a link between the immune response and thermal or even starvation stresses, similar to what has been observed for the activator of *hsp90* and other heat shock protein genes in *A. mellifera* (McMenamin et al. 2020) or the *Hsp90* gene in the frog *Quasipaa spinosa* (Miao-An et al. 2017).

To sum up, the heat but not the cold treatment caused a significant upregulation of heat shock genes *Hsc70* and *Aha1*, indicating a role of these proteins on tolerance to moderately high temperatures. Also, only the receptor gene *BGRP1* was clearly upregulated with cold, possibly reflecting an activation of immune cell activity. These results suggest that there is no negative effect of moderately high and low temperatures on *B. terrestris*' expression of immune-related genes. Further experimental infections would be necessary to elucidate if these temperatures do not affect the bumblebee's ability to fight pathogens. Finally, starvation produced a higher upregulation in all genes when bees were under heat and cold

thermal treatments, suggesting a synergistic effect of both stressors.

This work provides a first insight into understanding the impact of temperature on the immune activity of *B. terrestris* and highlights the importance of studying the interaction of different factors. However, more studies are needed involving infection and the analysis of multiple immune components in species from different climatic regions to gain a better knowledge on how temperature affects the adaptability and survivability of bumblebees.

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CONTRIBUTION

NBL, AGR, and PDIR conceived this research and designed experiments; NBL and AGR participated in the design and interpretation of the data; NBL performed experiments and analysis; NBL, AGR, and PDIR wrote the paper. All authors read and approved the final manuscript.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest The authors declare that they have no conflict of interest.

Un léger stress thermique n'affecte pas négativement l'expression des gènes immunitaires chez le bourdon *Bombus terrestris*.

bourdon / variation de température / gènes du choc thermique / immunité / RT-qPCR.

Ein milder Hitzestress hat keine negative Auswirkung auf die Expression von Immungenen bei der Hummel *Bombus terrestris*.

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