



GENE EXPRESSION RESPONSES TO THERMAL STRESS IN MEDITERRANEAN *APIS MELLIFERA* SUBSPECIES

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INTRODUCTION & AIM

Thermal stress poses a serious ecological threat to honey bees (*Apis mellifera* L.), affecting their physiology, behaviour, and colony viability under global warming. Transcriptomic analyses, particularly through RNA-sequencing (RNA-Seq), have become indispensable for identifying genes and regulatory networks activated in response to high temperature, revealing mechanisms of tolerance at molecular resolution.

The aim was to determine gene expression after RNA-Seq in bees exposed to comfort, stress and hyperthermia temperatures and to understand the effect of humidity as a way to study cellular stress. The tests were survival rate assay and body water loss (BWL) using native subspecies from Italy, Spain, Malta, Jordan, Lebanon and Turkey

MATERIAL & METHODS

Survival rate assay: Bees were exposed to six different temperature and humidity conditions in order to study the different survival rates. Here, two additional cages with 33 bees each were also included, one for be exposed 2 hours (acute stress) and the other for 2 days (chronic stress). This design was intended to study cellular stress using molecular tools such as enzymatic activity assays

Acute Stress- 2 hours

T 35°C H 15% (n=10)	T 35°C H 30% (n=10)	T 35°C H 50% (n=10)	T 35°C H 75% (n=10)
E	E	E	E
E	E	E	E
E	E	E	E

Chronic Stress- 2 days^g

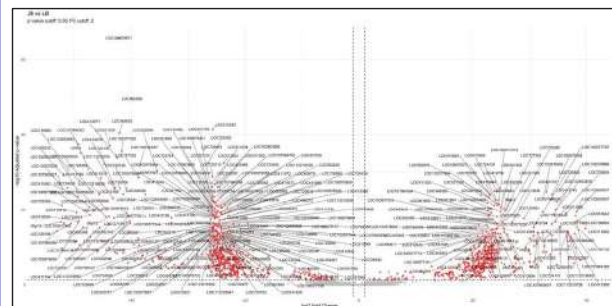
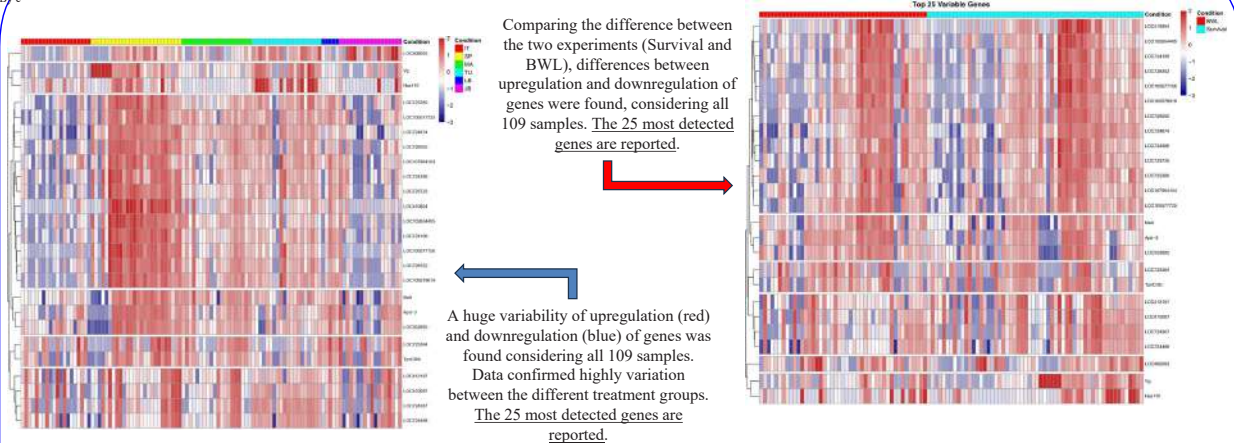
T 35°C H 15% (n=10)	T 35°C H 30% (n=10)	T 35°C H 50% (n=10)	T 35°C H 75% (n=10)
E	E	E	E
E	E	E	E
E	E	E	E

T 35°C H 15% (n=10)	T 35°C H 30% (n=10)	T 35°C H 50% (n=10)	T 35°C H 75% (n=10)
E	E	E	E
E	E	E	E
E	E	E	E

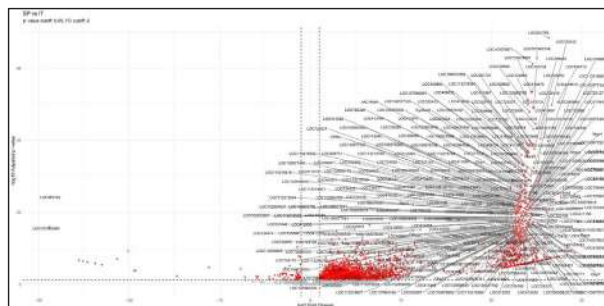
Body water loss assay (BWL): Bees were exposed to nine different temperature and humidity conditions for 2 hours to determine the dehydration effect. In addition to the cages designated for BWL measure, an independent one was included to provide bees exposed to those conditions for molecular studies, including the enzymatic assays.

RNA was extracted by each sample pool (5 bees) and RNAseq analysis was designed to deliver high quality. Sequencing was performed using Illumina, and raw sequenced reads were analyzed, using NovaSeq Control Software.

RESULTS



Considering *A. m. syriaca* investigated in Jordan (JB) and Lebanon (LB), a very similar proportion of up-regulate (right) and downregulated (left) genes was found related to thermal stress



Local subspecies from Turkey, Malta, and Spain highlighted opposite trends, with a high number of upregulated gene related to thermal stress compared to *A. m. ligustica*. Specifically, *A. m. iberiensis* (figure) and *A. m. rutneri* samples showed a higher upregulation (right) of investigated genes

CONCLUSION

This study revealed that the transcriptomic analyses demonstrated clear subspecies-specific molecular responses to thermal and humidity stress, highlighting distinct regulatory pathways involved in stress adaptation and offering valuable insights into the genetic mechanisms underpinning honey bee subspecies (*Apis mellifera* *ligustica*, *A. m. iberiensis*, *A. m. rutneri*, *A. m. syriaca*, *A. m. anatoliaca*) resilience in Mediterranean environments

The analyses are still in progress, especially for specific genes ... Stay tuned!

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Climate change poses a significant threat to the welfare of honey bees (*Apis mellifera*), both by exacerbating challenges such as nutritional stress (increasing susceptibility to pathogens and shortened flowering periods), and by directly altering honey bee physiology and thereby, affecting their lifespan. One of the most worrying abiotic stressors resulting from climate change is rising temperatures, which induce heat stress and lead to molecular changes such as gene expression and enzyme activity. The PRIMA project MEDIBEES (Monitoring the Mediterranean Honey Bee Subspecies and Their Resilience to Climate Change for Sustainable Agro-Ecosystems) aims to understand the impact of climate change on different *Apis mellifera* subspecies in the Mediterranean region. As part of the project, the effects of temperature and relative humidity (RH) on survival and adaptability were studied in five honey bee subspecies: *A. m. ligustica* (Italy), *A. m. iberiensis* (Spain), *A. m. syriaca* (Jordan), *A. m. ruttneri* (Malta), and *A. m. anatoliaca* (Turkey). Newly emerged workers were used in survival trials under six different conditions: 35°C, 40°C, and 45°C at 75% RH, as well as 35°C at 15%, 30%, and 50% RH. Bees were sampled from each condition after 2 hours and 2 days to compare acute and chronic stress, respectively. In addition, the dehydration capacity of newly emerged workers was also assessed by analysing body water loss (BWL) during 2 hours under nine conditions, which combined temperatures of 35°C, 40°C and 45°C with RH levels of 10%, 25% and 50%. Twenty-one samples were obtained (12 and 9 from survival and BWL, respectively) from each subspecies. Finally, a pool of nine bees from each sample was selected for the total RNA extraction. Whole transcriptome analysis by total RNA sequencing (RNA-Seq) identified specific genes associated with heat stress in the subspecies studied, and the gene expression was further evaluated using quantitative PCR assays. These findings provide a foundation for identifying specific stressors affecting *A. mellifera* health and welfare in the context of climate change, offering crucial insights for conservation strategies and sustainable beekeeping practices.

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