



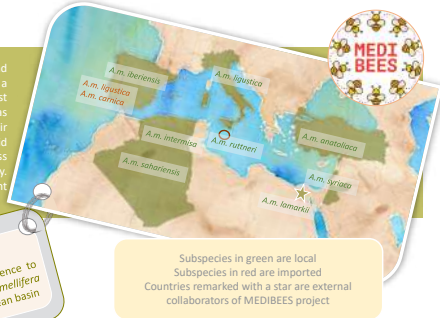
Studying dehydration as an adaptive trait in honey bees: differences among nine *Apis mellifera* subspecies living in Mediterranean basin

Soledad Sagastume¹, Giovanni Cilia², Banan Al Shagour³, Asmaa Anwer Eissa⁴, Dalida Darazy⁵, Dilek Muz⁶, Sami Alarsan³, Fernando Doblado¹, Marion Zammit Mangion⁷, Thomas Galea⁸, Nouredine Adjlane⁹, Mustafa Necati Muz⁶, Chadi Hosri⁵, Nizar Haddad³, Antonio Nanetti², Mariano Higes¹, Raquel Martín-Hernández¹

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INTRODUCTION

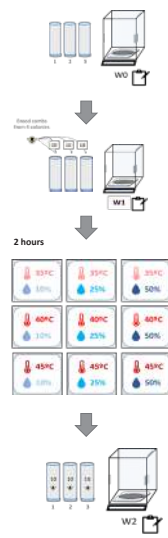
Adaptability is defined as the ability of an organism to adapt to new situations. Nowadays, climate change is leading to hotter and drier environments, and one of the main concerns for honey bees is how they can withstand and adapt to these new conditions. In particular, the Mediterranean region is a recognised hotspot for climate change [1,2] and house several subspecies of *Apis mellifera*. The increasingly high temperatures suppose one of the most important abiotic pressures for bees, but changes in precipitation patterns are also key. Both variables are closely related and, when get combined, act as new independent stressor. Honey bees are able to regulate both temperature and humidity inside the hive in two ways: (i) fanning bees increase air exchange rates from inside to outside the hive by orienting themselves at the entrance, and (ii) bees evaporate water from nectar and regurgitate liquid droplets within the hive to restore favourable conditions [3]. The dehydration capacity of the bees has been defined as an adaptive trait under heat stress that can be study calculating the percentage of body water loss (BWL) per bee after the exposure to a specific combination of temperature and humidity. This work is part of MEDIBEEs project, and studied the BWL of nine *Apis mellifera* subspecies from the Mediterranean basin, which were exposed to different temperature and humidity conditions. The results presented here aim to shed light on the different adaptability of the Mediterranean honey bees.



MATERIAL & METHODS

Within MEDIBEEs project, BWL assays were performed with nine subspecies living in Mediterranean basin: *Apis mellifera ligustica* in Italy, *A.m. syriaca* in Jordan and Lebanon, *A.m. iberiensis* in Spain, *A.m. anatolica* in Turkey, *A.m. lamarkii* in Egypt, *A.m. ruttneri* in Malta, and *A.m. intermissa* and *A.m. sahariensis* in Algeria. Three cages were weighed first when empty and then when containing 10 bees each. The bees were exposed to nine different conditions (combining temperatures of 35°C, 40°C and 45°C with relative humidities of 10%, 25% and 50%) for two hours. They were then weighed again and %BWL was calculated [4]. An additional study was carried out in Spain to analyse the adaptation of 3 different subspecies in the same apary: *A.m. iberiensis* (local subspecies) was compared with two introduced subspecies stabilised during 1 year (*A.m. ligustica* and *A.m. carnica*).

Histograms were created using average data. Statistical analyses of variance (ANOVA) and means were compared by using JASP 0.95.1 program.



DISCUSSION

Several subspecies of *Apis mellifera* live in the Mediterranean basin, each adapted to specific environments. For example, to arid and dry environments in Jordan, Egypt and Algeria; temperate environment in Malta; and some live adapted to several different environments, such as in Spain, Italy, Turkey or Lebanon. *Apis mellifera ligustica* (native to Italy) as well as *A.m. carnica* (native from Balkans), are used commercially beyond their native range because of their supposed adaptability. However, this practice endangers the survival of local subspecies, as they are negatively selected in beekeeping, and the risk of hybridisation with introduced subspecies increases. Given the worrying scenario of climate change in the Mediterranean region, the value of local subspecies adapted to their specific environments is key.

This work showed different dehydration ability (% BWL) within and inter-subspecies under different warm and humid environments, supporting the different adaptation degree of all of them: subspecies in Spain, Italy, Turkey, Egypt, and Malta showed significant higher dehydration at 45°C than 35°C. Otherwise, humidity seems to play an interesting role at 45°C, with significance in *A.m. anatolica*, but no effects are evident at 35°C and 40°C. Finally, Algerian and Jordanian subspecies did not show significant effect of heat on BWL. On the other hand, an experiment comparing local (*A.m. iberiensis*) and imported subspecies (*A.m. ligustica* and *A.m. carnica*) was carried out in Spain after one year of adaptation of the new subspecies. No significant differences were found between Italian bees in 2023 and *A.m. ligustica* in Spain in 2024. In the three subspecies showed a protective effect of heat stress when humidity increased, as well as no significant differences at 40°C. *A.m. ligustica* showed significant lower % BWL at 45°C 10% of RH.

These results support the use of local bee breeds in Mediterranean beekeeping, as rising temperatures and changing rainfall patterns are making environmental conditions harsher, being the imported subspecies even more vulnerable.

CONCLUSIONS

- Significant differences within and inter- subspecies
- Heat and humidity seems to affect differently to subspecies depending on their local adaptation
- In some cases, 50% of humidity decreases the heat stress degree
- Comparing local and imported subspecies, significant differences in % of BWL, specially at 45°C 10% RH



MEDIBEEs
Adaptation, resilience and
climate change of *Apis mellifera*
subspecies living in Mediterranean basin

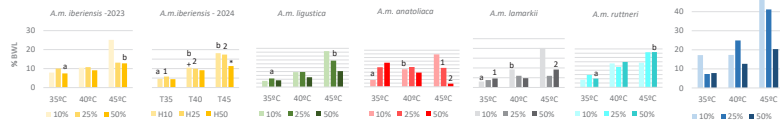
Subspecies in green are local
Subspecies in red are imported
Countries remarked with a star are external
collaborators of MEDIBEEs project

RESULTS

Significant differences within subspecies

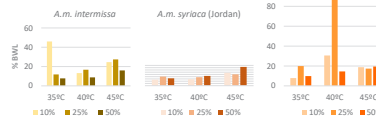
Subspecies showing increasing % BWL with higher temperature

The histograms show average data



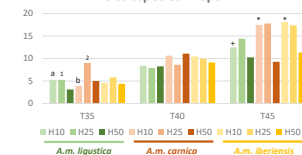
Subspecies showing no effect of T (°C) and HR (%) in % BWL

Algerian and
Jordanian bees



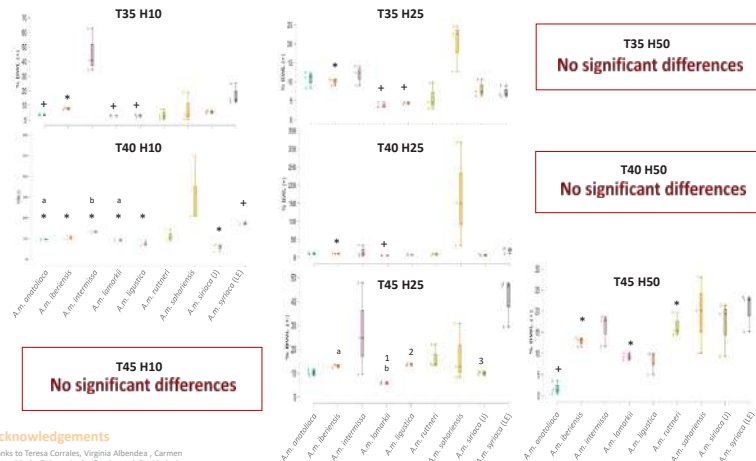
Comparative analysis of native and imported subspecies in Spain (1 year later)

3 subspecies in Spain



Significant differences between subspecies

The raincloud plots show data from biological replicates



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[1] Giorgi F. Climate change hot-spots. Geophysical research letters. 2006 Apr;33(8).

[2] Lionello P, Scarascia L. The relation between climate change in the Mediterranean region and global warming. Regional Environmental Change. 2018 Jun;18:1481-93.

[3] Stonick & Heidinger (1987). Temperature control in honey bee colonies. BioScience, Vol. 37 No. 6, pp. 395-399

[4] Abou-Shareh, H.F., Al-Ghamdi, A.A., Mohamed, A.A., 2012. Tolerance of two honey bee races to various temperature and relative humidity gradients. Environmental and Experimental Biology (2012) 10: 133-138.



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Adaptability is defined as the ability of an organism to adapt to new situations. Nowadays, climate change is creating new environments to which all living organisms must respond and adapt. The increasingly high temperatures suppose one of the most important abiotic pressures, but changes in precipitation patterns are also key. Both variables are closely related and, when get combined, act as new independent stressor. In bees, the dehydration capacity is defined as an adaptive trait under heat stress. One way to study this characteristic is calculating the body water loss (BWL) per bee before and after the exposure to a specific combination of temperature and humidity. Within MEDIBEES project, BWL assays were performed with nine subspecies living in Mediterranean basin: *Apis mellifera ligustica* in Italy, *A.m. syriaca* in Jordan and Lebanon, *A.m. iberiensis* in Spain, *A.m. anatoliaca* in Turkey, *A.m. lamarkii* in Egypt, *A.m. ruttneri* in Malta, and *A.m. intermissa* and *A.m. sahariensis* in Algeria. In this study, we tested 9 different conditions which combined 35°C, 40°C and 45°C of temperature with 10%, 25% and 50% of RH. Using standard protocols, the same experiment has been carried out at different laboratories of each country with their corresponding subspecies. The results showed differences in the dehydration ability between subspecies which point to a different adaptability to hot environments. An additional study was carried out in Spain to analyse the adaptation of 3 different subspecies in the same apiary: *A.m. iberiensis* (local subspecies) was compared with two introduced subspecies stablished during 1 year (*A.m. ligustica* and *A.m. carnica*). Our results show no signs of adaptation of the Italian bees to the Spanish environment, and a similar behaviour between *A.m. iberiensis* and *A.m. carnica*. Additionally, we demonstrate the repeatability of our tests comparing *A.m. iberiensis* to the previous year. This knowledge of the adaptability of bees is key to understanding the challenges facing beekeeping today and in the future. These results are under the project 2011-MEDIBEES, which is part of the PRIMA programme supported by the European Union.