



Heat resistance variability in the Lebanese bee fauna

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Abstract – Wild bees are facing many environmental challenges that are reshaping their distributions and even causing extinctions. One of the common threats is climate change leading to a higher frequency of extreme climatic events such as heat waves. We focused on the bee fauna of the eastern Mediterranean country of Lebanon and assessed intra- and interspecific variability of the heat stress resistance in correlation with dry body weight, altitude, and collection date. We used the time before heat stupor (THS) at 40 °C in semi-controlled conditions as a proxy for heat resistance. We found significant interspecific differences in heat resistance, and a positive correlation with dry weight in some taxa. At the intraspecific level, there was a significant difference in heat resistance between sexes for some species. Also, dry/fresh weights, altitude, and collection date were correlated to a higher heat resistance in some taxa. In the context of global changes, we argue that we need heat tolerance metrics for a better understanding of bee decline and to enhance conservation measures at regional scale.

wild bees / body weight / Lebanon / heat stress / climate change / heat wave

1. INTRODUCTION

Major population shifts are currently observed at global scales for both fauna (IUCN 2023) and flora (Richner et al. 2015). Extinctions already occurred for a part of the species diversity (Parmesan 2006; Dirzo et al. 2014), while a minority of species seem able to adapt and/or migrate (Lenoir et al. 2008). Climate change is one of the main drivers of these range shifts (Jetz et al. 2007; Jenkins et al. 2011) through an increase in average temperature and frequency of short extreme events, such as heat waves (Meehl and Tebaldi 2004).

Insect populations can be highly affected by climate change (Kiritani 2013; Kingsolver et al.

2013; 2017) and many have been observed to decline in recent decades as a result (Wagner 2020). Thermal stress, notably induced by heat waves, is associated with physiological perturbations (Kingsolver and Buckley 2017), behavioral changes (i.e., heat stupor) (Perez and Aron 2020) and mortality (Neven 2000; Parmesan 2006; Kingsolver et al. 2013). Insects can adapt to heat stress through behaviours (social or individual thermoregulation such as sheltering; Sheikh et al. 2017), morphology (e.g. body size, hairs, leg length) and physiological/molecular responses such as Heat shock response (HSR) (including the production of heat shock proteins and antioxidants) and cuticular hydrocarbons (reviewed in Perez and Aron 2020). However, extreme heat can negatively affect all stages of insect development (Kierat et al. 2017) and adult survival (Sales et al. 2021),

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ultimately impacting fitness (Zizzari & Ellers 2011; Martinet et al. 2020). Heat resistance of insects is a derivative of both thermal tolerance of their body tissues and of the efficacy of their heat dissipation responses (reviewed in Perez and Aron 2020). Examining the thermal resistance across various species will enhance our comprehension of population dynamics amidst ongoing global changes.

One of the most studied insect groups regarding their population trends are pollinators (e.g. Nieto et al. 2014), especially bees, as their decline results in important economic and ecosystem services losses (Potts et al. 2016). Different threats have been identified such as habitat destruction (Kline and Joshi 2020), pesticides (Woodcock et al. 2016) and pathogens (Meeus et al. 2011). A growing body of research also shows the synergistic effects of two or more of these stressors (Goulson et al. 2015; Vanderplanck et al. 2019; Straub et al. 2022). Some groups like bumblebees seem to be more vulnerable to decline than other bees (Nieto et al. 2014; Cameron and Sadd 2020) and climate change could be an important factor explaining their population trends (Rasmont et al. 2015; Ghisbain et al. 2023). Similarly to other insect groups, effects of heat stress on bees can be detrimental by increasing extinction risk through higher adult mortality (Nacko et al. 2023) and infertility (McAfee et al. 2020).

Assessing the resistance of bee species to climate change can be conducted through static or dynamic experimental methods in field, semi-field or laboratory conditions (Martinet et al. 2015; Oyen et al. 2016, 2018; Martinet et al. 2021). While dynamic methods involve assessing CT_{min} and CT_{max} with different ramping rates, static methods used in this study involve setting the temperature and measuring the time before heat stupor (THS). Heat stupor is a physiological state reached by specimens during hyperthermic stress, which is characterized by a critical decrease in motor function with muscle spasms (Lutterschmidt and Hutchison 1997) and an inability to escape conditions (Martinet et al. 2015). These can help estimate climatic risk factors for species and can be an alternative in the absence of historic data. Existing studies on heat

resistance have mainly been centred around high latitude cold-adapted taxa (Martinet et al. 2015; Oyen et al. 2016). These studies all highlight the species-specific aspect of heat stress resistance, and consequently the need to standardise the methods of producing data across several species. The consistent interspecific variability (Martinet et al. 2015; Oyen et al. 2016; Gonzalez et al. 2020; Martinet et al. 2021) is contrasted by a lower intraspecific variability (Maebe et al. 2021). Intraspecific variability can result from diverse factors such as dry weight of individuals. However, although shown to be linked to thermoregulation (Heinrich 1975; Pereboom and Biesmeijer 2003), dry weight does not always influence the heat resistance of the individual (Maebe et al. 2021; Martinet et al. 2021). Similarly, sex can be a potential driver of intraspecific variability with females being the most resistant (Hayes and López-Martínez 2021), but this has rarely been the focus in literature on wild bees. Furthermore, exploration of the link between the heat resistance of a species and its distribution show that widespread bee species are generally more heat resistant (Martinet et al. 2021), and certain conditions such as altitude (Gonzalez et al. 2020) can be correlated with intraspecific variability.

Little is known about a large part of bee diversity, particularly from Asia and Africa, where temperature increases are also observed and predicted (Cui et al. 2021). The Mediterranean region is one of the least studied areas in this regard. Moreover, most previous studies focused on one genus at a time or genera from the same family. The present study focuses on 15 bee species from Lebanon, a country located in the little-studied East Mediterranean strip, at the crossing point between Europe, Asia and Africa. For the first time, 7 genera from two different families are evaluated simultaneously, using the same protocol. We adopt a species-centric approach to measure the heat stress resistance of the target species. Moreover, we consider factors potentially associated with the expected interspecific variation: (i) taxon, (ii) dry weight. We also consider factors that could explain intraspecific variation: (i) fresh weight, (ii) sex, (iii) altitude, and (iv) collection date (i.e. Julian day).

We hypothesise that taxon will influence interspecific variability of heat stress resistance (i.e. that dry body weight is positively correlated with heat resistance), and that sex, dry weight, fresh weight, altitude, and Julian day can influence the intraspecific variability of heat stress resistance.

2. MATERIALS AND METHODS

2.1. Sampling

Sampling was carried out throughout 2018 and 2019 spring and summer in Lebanon (Localities in Supplementary Material A). Sampling sites were chosen based on the abundance of the target bee species and their commonly foraged flowers; in addition each target species had at least two sampling localities 3 km apart as previously observed in (Boustani et al. 2020, 2021). The sites included coastal areas and intermediate altitudes, but we mainly collected at sites at 900 m of altitude and higher.

In total, we sampled 60 species from 11 genera, for a total of 618 specimens (Supplementary material A). The identification of individuals was carried out by taxonomic experts of each group and was listed in Boustani et al. 2021. However due to a small sample size for many (less than 10 individuals) we were only able to consider seven different bee genera in the analyses for a total of 500 specimens (*Anthidium* Fabricius 1804, *Anthophora* Latreille 1803, *Bombus* Latreille 1802, *Eucera* Scopoli 1770, *Habropoda* Smith 1854, *Megachile* Latreille 1802 and *Xylocopa* Latreille 1802). Sampling includes social (all species of the genus *Bombus* and the facultatively social species *Xylocopa*) and solitary taxa (all the remaining species) displaying different regional distributions. *Xylocopa pubescens* Spinola, 1838 is restricted to coastal and semi-arid areas. *Anthidium manicatum* (L., 1758), *Anthophora plumipes* (Pallas, 1772), *A. nigriceps* (Morawitz, 1886), *Bombus argillaceus* (Scopoli, 1763), *B. terrestris* (L., 1758), *Eucera plumigera* Kohl 1905, *Eucera* sp. a, *Megachile montenegrensis* Dours, 1873, *M. parietina*

(Geoffroy, 1785), *Xylocopa valga* Gerstaecker, 1872 and *X. violacea* (L., 1758) are widespread. While *Bombus melanurus* Lepelletier 1835, *B. niveatus* Kriechbaumer, 1870 and *Habropoda hakkariensis* Schwarz and Gusenleitner 2001 are restricted to higher altitudes (> 1500 m) (Boustani et al. 2021; Table I).

Specimens were collected between 8:00 and 17:00 on sunny days with no or little wind. After being caught by hand net, specimens were placed in individual plastic containers with a cardboard support inside and placed in a portable cooler (Dometic CoolFreeze CF11 10.5 L) at 12 °C to avoid any overheating in the containers. Collection date (i.e. Julian Day) was considered in our analyses, given that thermal tolerance could vary along the phenology (Leclair et al. 2020; Oliveira et al. 2021).

2.2. Assessment of hyperthermic stress resistance

We measured time before heat stupor (THS) following a protocol adapted from that of Martinet et al. (2015, 2021). In total, we measured the THS of 500 wild bee specimens (Table I). The protocol has two main phases, a first phase of cooling and feeding the specimens to standardise their physiological state, and a second phase where they are submitted to heat stress in an incubator (Figure 1).

For the first step, we placed the specimens in a cool chamber (Dometic CoolFreeze CF11 10.5L), directly after their collection in the field. We selected a temperature between 10–12 °C. This was chosen above the 8 °C (standby and non-stressful temperature for cold-adapted bees like bumblebees; Heinrich 1975; Owen et al. 2013) developed in Martinet et al. protocol (2015; 2021) but below the 16–19 °C proposed for solitary species like *Xylocopa* (Gonzalez et al. 2020). This adaptation aims to extend the protocol to a wider range of targeted genera. After two hours of cooling, we fed the specimens with sugar syrup and let them re-activate for one hour at room temperature (24 °C) before the heat exposure.

For the second step, the specimens were placed in an incubator (Herp Nursery II) at 40 °C,

coupled with a thermostat (Lucky Reptile TC2-PRO Thermo Control Pro II). The temperature of 40 °C with a maximum duration of 10 h (600 min) was chosen, as it is the maximum temperature recorded in Beirut (Rafic Hariri International Airport, Meteorological Department) and is also the average heat wave temperature recorded in more than 90 countries (Ragone et al. 2018). The 10-h duration represents the maximum length of high temperature in the course of one day. Since the specimens are not fed during the test, the 10-hour period is also not exceeded to avoid the bias from starvation (Martinet et al. 2015). Humidity was monitored (Lucky Reptile Thermometer Hygrometer PRO) and kept at approximately 50%. The relative humidity rate has also been chosen to correspond to what is observed in nature during a heatwave (see Martinet et al. 2021). Bees were placed in individual empty plastic tubes with perforated top, bottom, and sides to allow for air flow and temperature homogenisation. Specimens were tested in batches up to 20 and placed only in the upper section of the incubator (i.e. place with the most stable temperature according to preliminary measurements). Specimens were then monitored through the transparent door of the incubator. The Time before Heat Stupor (THS) was measured using a chronometer that is started once the specimens are in the incubator. THS corresponds to the time from the insertion into the incubator until the heat stupor. Specimens are considered to have reached heat stupor when they are lying on their back and unable to return to normal position when the tube is rotated. This state is defined by the onset of muscle spasms (Lutterschmidt and Hutchison 1997). Once this is verified, the insect is removed from the incubator to recover at room temperature (24 °C), although many specimens died right after reaching THS. After field collection, specimens in bad conditions (i.e. dying) were not considered for the experiments.

Specimens were weighed right before the test (fresh weight) and after removing them from the incubator using Ace CT-50 Portable Milligram Scale, precision ± 0.001 g, and weighed after complete dehydration (dry weight) using Lyovac GT2 LEYBOLD-HERAEUS for two

days at 70 °C. We used dry weight as a proxy of body size which could influence thermal tolerance (Gérard et al. 2020) and fresh weight to assess the amount of water inside the body during the heat test which could also influence heat tolerance.

2.3. Experimental limitations

While much information is available for (semi-)domesticated species of the genera *Apis*, *Bombus* and *Osmia*, very little is known about the potential effects of captivity on strictly wild bees. For this reason, the effect of fresh weight was not tested at the interspecific level as the consumption of the offered food in the first phase may not be homogenous between genera (i.e. some specimens eating but others not). Furthermore, the behavioural cues indicating heat stress (e.g. muscle contraction) in these species may be less visible or manifest differently. Another inherent difficulty in targeting uncommon wild species is having a good sample size for both sexes.

2.4. Statistical analyses

In order to include as many species as possible in the analyses, a minimum sample size of 11 was set for the variability between sexes, 7 for interspecific variability, and 30 for correlations of heat stress resistance with dry weight, fresh weight, altitude, and collection date.

2.5. Interspecific variation of heat stress resistance

As we collected both male and females of the same species when possible, we use Kaplan–Meier survival curves using the “Survminer” R package (Alboukadel et al. 2021) to assess differences between different taxa (interspecific variability) separately for males and females. To investigate interspecific variability, correlation between THS and dry weight on the entire dataset was performed

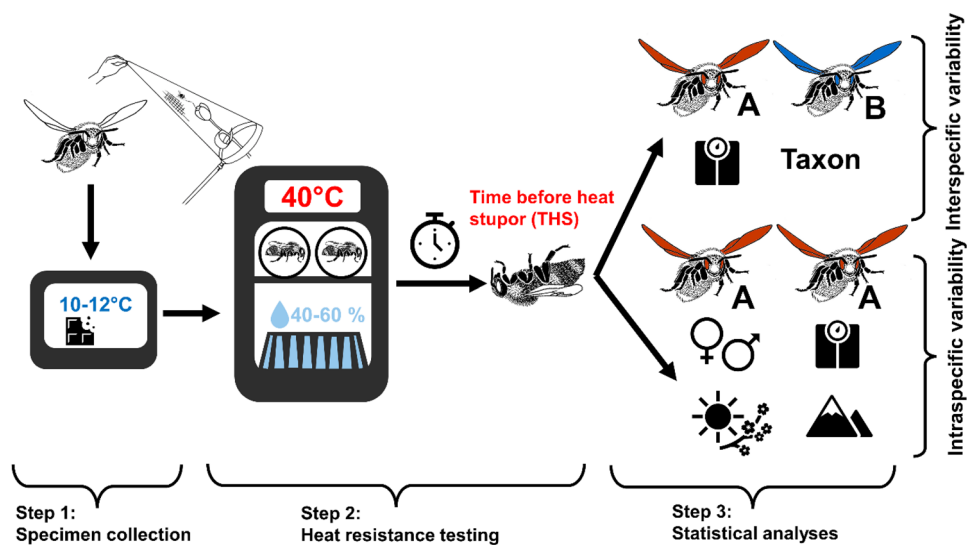


Figure 1. Methodology for the measurement of heat resistance and data analyses.

using non-parametric Spearman tests after checking whether or not data was normally distributed (Shapiro test).

2.6. Intraspecific variation of heat stress resistance

Correlation with sex We compared heat resistance between males and females with Kaplan-Meier survival curves using the “Survminer” R package (Alboukadel et al. 2021). The test was performed for all species with a large enough sample number ($N > 11$ for each sex, see Table I). The medians are not estimated in Kaplan-Meier when the percentage of survivors (in this case specimens exiting the test without THS) is above 50% (Zwiener et al. 2011); therefore, male and female heat stress resistance of *Xylocopa pubescens* could not be compared.

Correlation with dry weight, fresh weight, altitude, and collection date We first used Shapiro to test whether the data were normally distributed. As data were not normally distributed, we performed non-parametric Spearman tests to investigate correlations between THS

and explaining factors on the entire dataset to test for interspecific variability (dry and fresh weights, altitude and collection date). These correlations were calculated for the taxa which had a large sample size ($n > 30$): *Bombus terrestris*, *B. niveatus*, *B. melanurus*, *Xylocopa violacea*, *X. valga*, *Anthophora plumipes* and *A. nigriceps*.

3. RESULTS

3.1. Interspecific variation of heat stress resistance

There is a significant difference between the heat stress resistance of males and females across the different taxa ($p < 0.05$). Therefore, interspecific variability is tested separately for males and females. In both males and females, there is an interspecific variability between taxa. The paragraphs below list the species from the highest to the lowest values of THS. Females with the highest values are the *Xylocopa* species (*Xylocopa pubescens* (median > 600 min) $>$ *X. valga* (median = 590 min) $>$ *X. violacea*

Table I Summary table for sampling

| Taxon | Number of sites | THS Median Males (mins) | N Males | THS Median Females (min) | N Females | Global Distribution | Altitude range (m) |
|---------------------------------|-----------------|-------------------------|---------|--------------------------|-----------|---------------------|--------------------|
| <i>Anthidium manicatum</i> | 10 | 147 | 13 | 190 | 12 | Palaearctic | 0-3000 |
| <i>Anthophora nigriceps</i> | 14 | 33 | 7 | 59 | 43 | West Palaearctic | 0-3000 |
| <i>Anthophora plumipes</i> | 14 | 20 | 22 | 49 | 26 | Palaearctic | 0-3000 |
| <i>Bombus argillaceus</i> | 9 | 132 | 13 | NA | 2 | Palaearctic | 0-3000 |
| <i>Bombus melanurus</i> | 8 | 46 | 39 | NA | 3 | Palaearctic | 1800-3000 |
| <i>Bombus niveatus</i> | 9 | 34 | 49 | NA | 3 | Palaearctic | 1800-3000 |
| <i>Bombus terrestris</i> | 13 | 253 | 44 | NA | 2 | Palaearctic | 0-3000 |
| <i>Eucera</i> sp. 1 | 3 | 100 | 10 | NA | 5 | Levant | 0-3000 |
| <i>Eucera plumigera</i> | 4 | 66 | 7 | NA | 2 | West Palaearctic | 0-1800 |
| <i>Habropoda hakkariensis</i> | 3 | 55 | 8 | NA | 3 | East Mediterranean | 1500-1750 |
| <i>Megachile montenegrensis</i> | 2 | NA | 3 | 162 | 14 | Palaearctic | 900 |
| <i>Megachile parietina</i> | 4 | NA | 1 | 95 | 16 | West Palaearctic | 0-3000 |
| <i>Xylocopa parviceps</i> | 1 | NA | 0 | NA | 1 | East Mediterranean | 1500-3000 |
| <i>Xylocopa pubescens</i> | 3 | > 600 | 25 | > 600 | 15 | West Palaearctic | 0-1000 |
| <i>Xylocopa valga</i> | 18 | 216 | 25 | 590 | 34 | West Palaearctic | 0-3000 |
| <i>Xylocopa violacea</i> | 15 | 177 | 32 | 538 | 21 | Palaearctic | 0-3000 |

THS medians for males and females, species distribution and body size category (n=number of tested specimens). The global distributions are taken from Rasmont et al. (2015) for bumblebees and Ascher and Pickering (2020) for the other genera. Local distribution (altitude range) taken from Boustani et al. (2021). NA=not available due to low sample number

(median = 538 min)) followed by *Anthidium*, *Megachile*, and *Anthophora* species (*Anthidium manicatum* (median = 189 min) > *Megachile montenegrensis* (median = 162 min) > *M. parietina* (median = 95 min) > *Anthophora nigriceps* (median = 59 min) > *A. plumipes* (median = 49 min)) (Figure 2, Table I).

Xylocopa males show a strong resistance to heat stress (Figures 3, 4, Table I) as well as *Bombus terrestris* males [*X. pubescens* (median > 600) > *Bombus terrestris* (median = 252 min) > *Xylocopa valga* (median = 216 min) > *X. violacea* (median = 177 min)]. The genera *Anthidium*,

Eucera, *Habropoda*, *Anthophora* and the other species of *Bombus* show lower survival [*Anthidium manicatum* (median = 147 min) > *Bombus argillaceus* (median = 132 min) > *Eucera* sp. 1 (median = 100 min) > *E. plumigera* (median = 66 min) > *Habropoda hakkariensis* (median = 54.5 min), *Bombus melanurus* (median = 46 min), *B. niveatus* (median = 34 min), *Anthophora nigriceps* (median = 33 min), *A. plumipes* (median = 19.5 min)].

Considering species as replicates, there is a positive correlation between dry weight and THS ($\rho = 0.684$; $p < 0.05$). In other words, the higher the dry weight is, the higher is the THS.

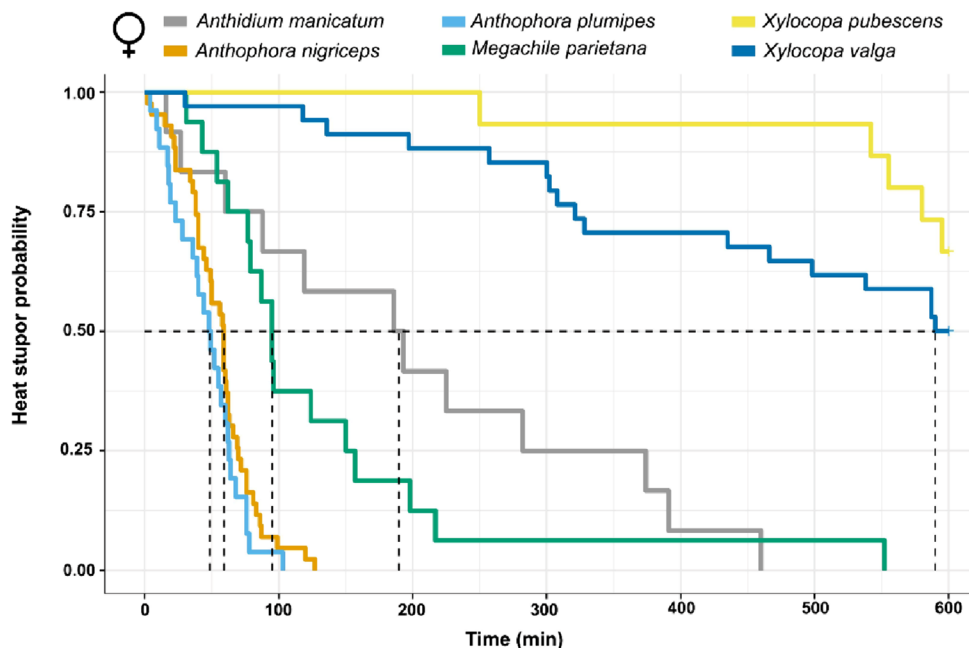


Figure 2 Time before heat stuper of females of *Anthophora nigriceps*, *A. plumipes*, *Megachile parietina*, *Anthidium manicatum*, *Xylocopa valga*, *X. pubescens*. The black dotted lines represent the medians. The time is in minutes.

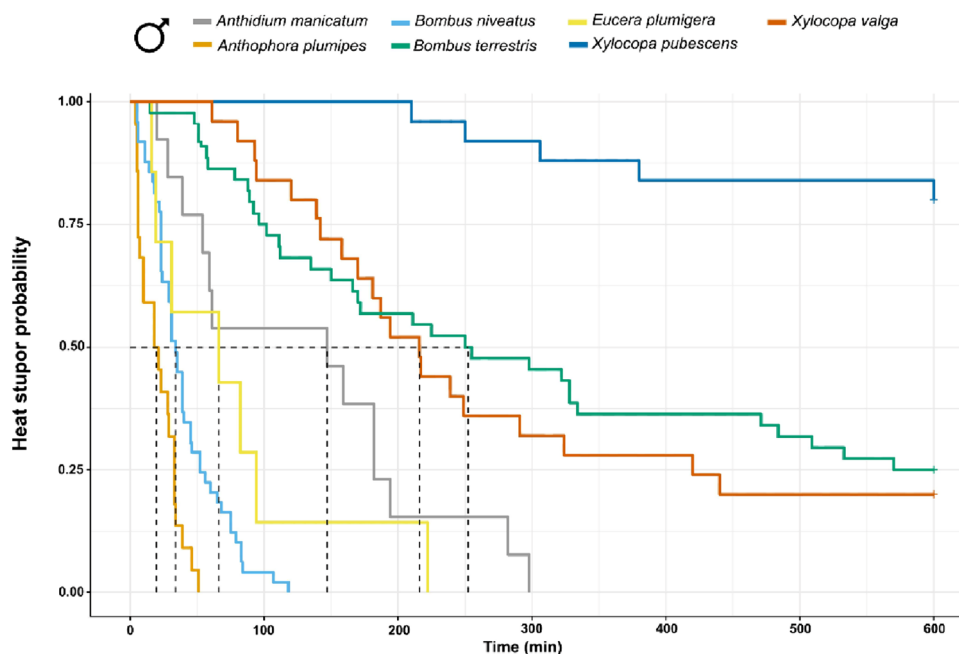


Figure 3. Time before heat stuper of males of *Anthophora plumipes*, *Bombus niveatus*, *Eucera plumigera*, *Anthidium manicatum*, *Bombus terrestris*, *Xylocopa valga*, and *X. pubescens*. The black dotted lines represent the medians. The time is in minutes.

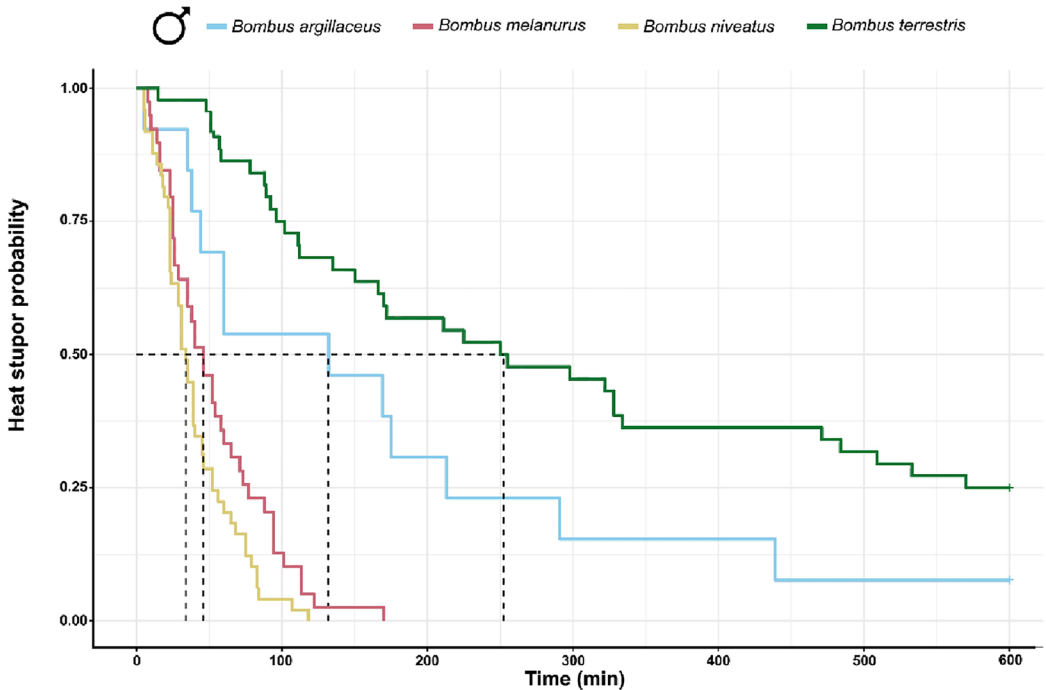


Figure 4. Time before heat stupor of males of *Bombus niveatus*, *Bombus melanurus*, *Bombus argillaceus*, and *Bombus terrestris*. The black dotted lines represent the medians. The time is in minutes.

3.2. Intraspecific variation of heat stress resistance

Correlation with sex We collected enough specimens to test the effect of sex on THS for five species (See Table II). There is no difference between females and males of *Xylocopa violacea* and *Anthidium manicatum* ($p > 0.05$; Figure 5a, d). In contrast, results show that females are significantly more heat resistant than males for *Xylocopa valga*, *Anthophora plumipes* and *A. nigriceps* (Figure 5b, c, e, f; Tables I, II).

Correlation with dry weight, fresh weight, altitude, and collection date There is a positive correlation between dry weight and THS for *Bombus terrestris* ($\rho = 0.352$; $p < 0.05$), *Xylocopa violacea* ($\rho = 0.575$; $p < 0.05$), and *X. valga* ($\rho = 0.528$; $p < 0.05$). In contrast, no correlation is observed for *Bombus niveatus*, *Anthophora plumipes*, and *A. nigriceps*. Concerning the

fresh weight, there is a positive correlation with THS for *Bombus terrestris* ($\rho = 0.729$; $p < 0.05$), *B. melanurus* ($\rho = 0.336$; $p < 0.05$), *Xylocopa violacea* ($\rho = 0.604$; $p < 0.05$), *X. valga* ($\rho = 0.672$; $p < 0.05$), *Anthophora plumipes* ($\rho = 0.564$; $p < 0.05$), and *A. nigriceps* ($\rho = 0.312$; $p < 0.05$). In contrast, no correlation is observed for *Bombus niveatus* (Table II).

Bombus niveatus and *B. melanurus* THS have no correlation with altitude. *Bombus terrestris* ($\rho = -0.478$; $p < 0.05$) THS has a negative correlation with altitude. While *Xylocopa valga* ($\rho = 0.324$; $p < 0.05$) as well as *X. violacea* ($\rho = 0.578$; $p < 0.05$) both have a positive correlation with altitude (Table II).

Xylocopa violacea ($\rho = 0.765$; $p < 0.05$) and *X. valga* ($\rho = 0.460$; $p < 0.05$) THS have a positive correlation with the increasing value of Julian Day. In other words, specimens collected later during the season display a higher THS. In contrast, *Bombus terrestris*, *B. melanurus*,

Table II Intraspecific variation test results per species

| Taxon | Male vs Female THS | Cor. DW and THS | Cor. FW and THS | Cor. Alt. and THS | Cor. JD and THS |
|-----------------------------|--------------------|-----------------|-----------------|-------------------|-----------------|
| <i>Anthidium manicatum</i> | 0.12 | \ | \ | \ | \ |
| <i>Anthophora nigriceps</i> | 0.0027** | 0.2937 | 0.0273* | \ | 0.2342 |
| <i>A. plumipes</i> | <0.001*** | 0.06333 | <0.001*** | \ | 0.4507 |
| <i>Bombus argillaceus</i> | \ | \ | \ | \ | \ |
| <i>B. melanurus</i> | \ | 0.9324 | 0.02912* | 0.051 | 0.9093 |
| <i>B. niveatus</i> | \ | 0.8041 | 0.06562 | 0.5455 | 0.4216 |
| <i>B. terrestris</i> | \ | 0.01756* | <0.001*** | <0.001*** | 0.7008 |
| <i>Xylocopa valga</i> | <0.001*** | <0.001*** | <0.001*** | 0.01215* | <0.001*** |
| <i>X. violacea</i> | 0.19 | <0.001*** | <0.001*** | <0.001*** | <0.001*** |

Cor Correlation, DW Dry weight, FW Fresh weight, Alt altitude, JD Julian day, THS Time before heat stupor

Anthophora nigriceps, and *A. plumipes* THS have no correlation with Julian day (Table II).

4. DISCUSSION

Our results show that heat resistance predominantly varies between species and is also correlated with dry weight at an interspecific level. At the intraspecific level, sex, dry weight, fresh weight, and seasonality were associated with variation in heat stress in some taxa only.

Our results on interspecific variability in heat resistance are in line with recent studies (Martinet et al. 2015; Oyen et al. 2016, 2018; Gonzalez et al. 2020; Martinet et al. 2021). These differences could be explained by variation of species abilities to cope with variation in temperature, with widespread species displaying higher plasticity and adaptability to different habitats (Ayrinhac et al. 2004). Furthermore, similarities in the heat resistance between species of the same genus (i.e. high for *Xylocopa* species and low for *Anthophora* species) could indicate a phylogenetic signal in bees as observed for upper thermal limits in ants (Bujan et al. 2020) and flies (Kellermann et al. 2012). Additionally, differences in body size can also explain specific heat resistance differences. Indeed, it can be argued that large bees are more

hydrated than smaller bees and lose heat at a lower rate than smaller bees because of their volume to surface area ratio. However, our results of THS tests do not seem to confirm this hypothesis, as already suggested in bumblebees by Martinet et al. (2021). We also confirm the positive correlation between dry weight and heat resistance at the interspecific level. Studies comparing different genera from the same family already showed that species from the larger-bodied genus thermoregulate more efficiently, i.e. larger *Bombus* versus smaller bee taxa (Bishop and Armbruster 1999), and species from larger Euglossini genera versus smaller ones (May and Casey 1983). Similarly, in our results the larger-bodied *Bombus* and *Xylocopa* include the most heat resistant species and seem to be driving this overall trend.

At the intraspecific level, males and females have differences in their heat stress resistance for some species (three out of five tested), with females being systematically more resistant than males in these cases. A recent study on *Megachile rotundata* also shows that females have higher heat stress resistance (Hayes and López-Martínez 2021). Although females can spend more time in thermoregulated nests (e.g. social species (Vogt 1986)), they also emerge later in the season and fly under higher temperatures (Danforth et al. 2019). In contrast, males spend

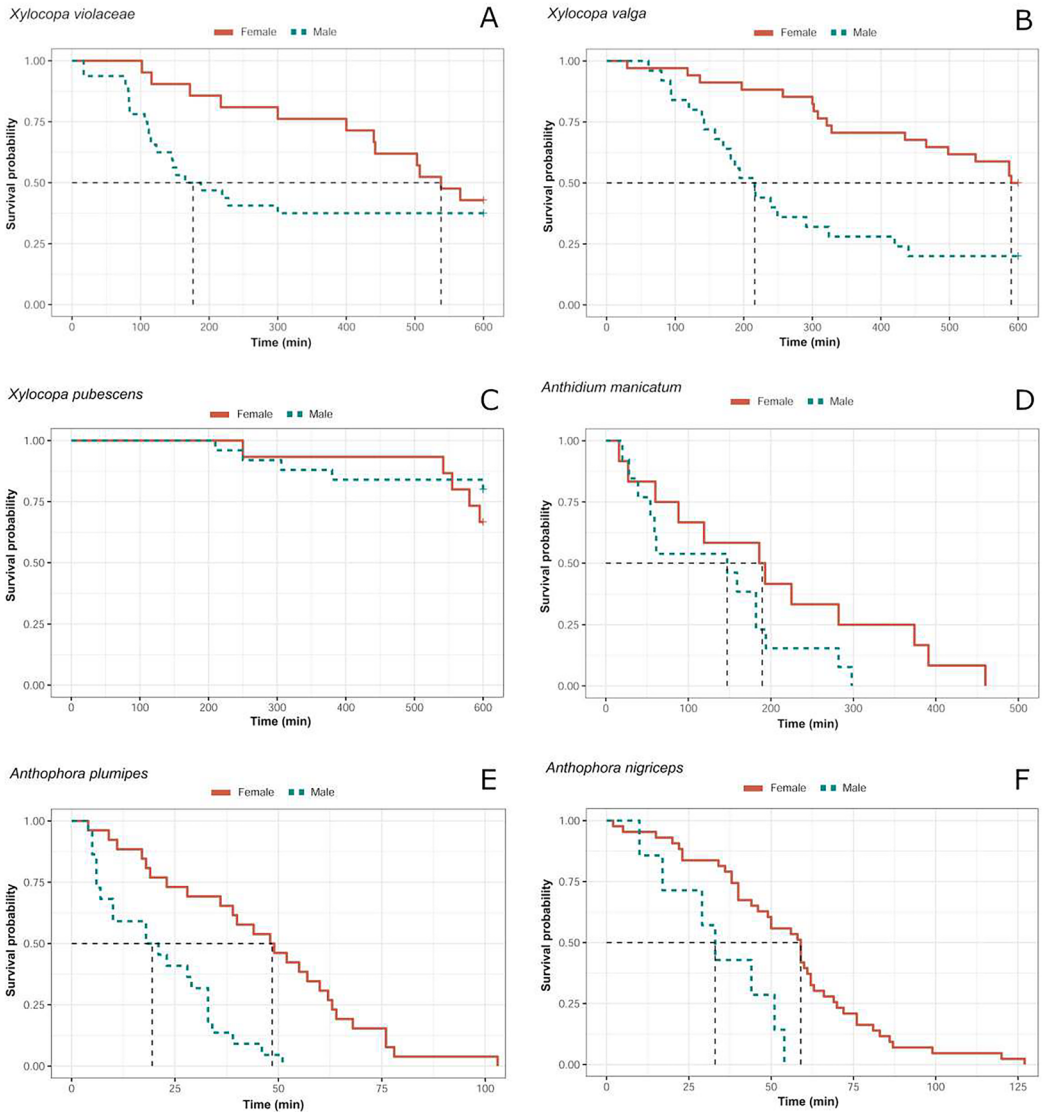


Figure 5. Time before heat stupor of males versus females for (a) *Xylocopa violacea*, (b) *Xylocopa valga*, (c) *Xylocopa pubescens*, (d) *Anthidium manicatum* (e) *Anthophora plumipes* and (f) *A. nigriceps*. The black dotted lines represent the medians. The time is in minutes.

their adult lives outside the nest where they can be exposed to more extreme temperature variations (Danforth et al. 2019). Consequently, they might be more exposed to the effects of a heat wave and the resulting compromised male fertility would still have damaging effects on the species fitness (Martinet et al. 2020). Withstanding higher temperatures by females may be more

efficient through the production of heat shock proteins (Zhao et al. 2021). These proteins are produced in several tissues including fat bodies (McKinstry et al. 2017), and it could be argued that bee females that are mostly larger than males have larger fat bodies that increase the capacity to produce these proteins. Furthermore, the haploid male – diploid female system of bees (Cook

1993) could be responsible for more regulating genes being expressed in females. This difference has been observed between haploid and diploid males of *Apis* (Yan et al. 2016) but is yet to be tested between male and female solitary bees to our knowledge.

Similarly, we show a positive intraspecific variability of heat resistance in relation to dry body weight for *Bombus terrestris*, in contrast with Martinet et al. (2021), and Maebe et al. (2021) who found no correlation, as well as Heinrich and Heinrich (1983) who found the opposite trend. While low intraspecific variability in heat resistance has been found in bumblebees (Martinet et al. 2021) this difference could be explained by the fact that we sampled *Bombus terrestris* at the very margin of its distribution, where higher plasticity can be observed in more challenging eco-climatic conditions and a potentially poorer diet (affecting hydration rate). We found the same positive correlation with dry body weight for *Xylocopa violacea* and *X. valga*. The heat resistance variability for these species could be explained by sexual dimorphism as we included results from males and females in our analyses. However, fresh weight seems to better explain the intraspecific variability of THS across different genera than dry weight as suggested for bumblebees (Martinet et al. 2021). Indeed, this metric can reflect the nutritional intake of the specimens (Wright et al. 2018) or the hydration stage of the bees. Bees with higher content of water might be able to handle longer exposure to temperature. Although it cannot give us information on how the nutrition quality can enhance overall bee health (Di Pasquale et al. 2013), it can reflect the specimen's water intake (Willmer 1986). The nutritional status of the specimen seems to influence its capacity to resist heat as shown in our case by a significant effect of fresh weight on all but one of the tested species (i.e. *Bombus niveatus*) (IUCN 2023).

Altitude was correlated to heat resistance only for the large-bodied taxa with wide altitudinal range. As altitudinal distribution of species is influenced notably by their thermoregulation capacity (Hodkinson 2005), we would expect that specimens from higher altitude, subjected

to more extreme temperatures, have low thermoregulation abilities at high temperatures. For instance, Gonzalez et al. (2020) show that thermal maximum of *X. violacea* decreased with altitude. In contrast, we found a positive correlation between altitude and heat stress resistance of both *Xylocopa violacea* and *X. valga*. As these two taxa are the only ones with a variability of heat stress resistance in correlation with collection date, the positive relationship can be explained by an enhanced heat shock response as summer temperatures rise. This could be driven by a high plasticity when facing heat stress related to enhanced production of heat shock proteins (Cahan et al. 2017), or the availability of better quality of nutrition later during the season (Vanderplanck et al. 2019; Iltis et al. 2021). Good quality nutrition could allow bees from higher elevation at cooler and more humid environments to be more hydrated than those from low elevations and therefore be more resistant to heat. Bumblebees, which are adapted to cold and temperate eco-climatic conditions, have a range of physiological and molecular adaptations to low temperatures but few to high temperatures with a low variability in heat stress resistance among conspecific populations (Martinet et al. 2021). Moreover, no significant regional differentiation in gene expression at intraspecific level under high temperatures exposure was observed in bumblebees (CT_{max}) (Pimsler et al. 2020). However, considering that the genus *Xylocopa* is a group of bees adapted to warm Mediterranean climates, we can hypothesize different response patterns in gene expression to high temperatures in this group with variations with altitudes and seasonality.

Most of the species we tested had no individuals surviving the 10-h heat test without undergoing THS, making them likely to undergo substantial mortality in case of a heat wave. This highlights that insect populations from warm temperate climates can still be vulnerable to heat stress (Andrew et al. 2013). Widespread species could have a heightened sensitivity of their populations in the southern range of their distribution, associated or not to a higher intra-population variability (Soroye et al. 2020). Indeed, *Bombus*

melanurus and *Habropoda hakkariensis* that ranked among the lowest in heat resistance are at their southernmost distribution limit in Lebanon, and *B. niveatus* being very close to its limit (Boustani et al. 2020). Therefore, more research on the vulnerability of Lebanese taxa and Mediterranean taxa in general is needed to understand the effects of extreme climate on the bee fauna of the region. Indeed, 15 species were tested for heat resistance among the 573 recorded bee species for Lebanon in this work (Boustani et al. 2021). In addition, different measurement methods (Terblanche et al. 2007) and chosen temperature ramping rate (Oyen and Dillon 2018) can cause discrepancies between observations, stressing the importance of using several methods to constitute a representative image of species heat stress resistance.

Finally, conservation plans should consider all aspects allowing for the species survival. This includes heat resistance metrics, the wide distribution buffering the effects of climatic stress, the morphological and physiological traits involved in responses to heat stress, and the abiotic factors such as higher temperatures that could be stimulating the enhanced response to heat stress. It is necessary to acquire thermal tolerance data on a wide range of scales, both continental and regional, to identify the most sensitive species and attract the attention of policymakers/stakeholders to develop specific and adequate conservation plans aimed at protecting these species of interest.

SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTIONS

MB: Conceptualization, Data curation, Formal analysis, Writing—Original Draft. BM: Conceptualization, Formal analysis, Writing- Reviewing and Editing. DM: Funding acquisition, Writing- Reviewing and Editing. NN: Supervision, Writing- Reviewing and Editing. PR: Conceptualization, Funding acquisition, Supervision.

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DATA AVAILABILITY

Measured variables of tested specimens with geographic coordinates of the collecting sites are available in Supplementary Material A .

CODE AVAILABILITY

R scripts related to the analysis of hyperthermic resistance of bees are all available on request at corresponding author.

DECLARATIONS

Consent to participate Not Applicable.

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