

## RESEARCH ARTICLE

# Bees display limited acclimation capacity for heat tolerance

Victor H. Gonzalez<sup>1,\*</sup>, Natalie Herbison<sup>1</sup>, Gabriela Robles Perez<sup>2</sup>, Trisha Panganiban<sup>3</sup>, Laura Haefner<sup>4</sup>, Thomas Tscheulin<sup>5</sup>, Theodora Petanidou<sup>5</sup> and John Hranitz<sup>6</sup>

## ABSTRACT

Bees are essential pollinators and understanding their ability to cope with extreme temperature changes is crucial for predicting their resilience to climate change, but studies are limited. We measured the response of the critical thermal maximum ( $CT_{Max}$ ) to short-term acclimation in foragers of six bee species from the Greek island of Lesbos, which differ in body size, nesting habit, and level of sociality. We calculated the acclimation response ratio as a metric to assess acclimation capacity and tested whether bees' acclimation capacity was influenced by body size and/or  $CT_{Max}$ . We also assessed whether  $CT_{Max}$  increases following acute heat exposure simulating a heat wave. Average estimate of  $CT_{Max}$  varied among species and increased with body size but did not significantly shift in response to acclimation treatment except in the sweat bee *Lasioglossum malachurum*. Acclimation capacity averaged 9% among species and it was not significantly associated with body size or  $CT_{Max}$ . Similarly, the average  $CT_{Max}$  did not increase following acute heat exposure. These results indicate that bees might have limited capacity to enhance heat tolerance via acclimation or in response to prior heat exposure, rendering them physiologically sensitive to rapid temperature changes during extreme weather events. These findings reinforce the idea that insects, like other ectotherms, generally express weak plasticity in  $CT_{Max}$ , underscoring the critical role of behavioral thermoregulation for avoidance of extreme temperatures. Conserving and restoring native vegetation can provide bees temporary thermal refuges during extreme weather events.

**KEY WORDS:** Critical thermal maximum, Climate change, Heat waves, Heat hardening, Heat priming

## INTRODUCTION

Climate change is considered one of the main drivers of biodiversity loss. Therefore, one of the foremost challenges in contemporary ecology and conservation is to understand the responses of organisms to climate change (e.g. Spooner et al., 2018; Kellermann and van Heerwaarden, 2019; Soroye et al., 2020;

Arneth et al., 2020; Harvey et al., 2023). Multiple aspects of the climate are expected to be altered under climate change, including temperature, CO<sub>2</sub> concentration, UV exposure, rainfall patterns, as well as the intensity, frequency, and duration of extreme weather events, such as cold snaps and heat waves (IPCC, 2021; Meehl and Tebaldi, 2004). The effects of increasingly variable and intense temperatures experienced by organisms during these extreme events are diverse and highly detrimental for most taxa (Thakur et al., 2022). For example, heat waves can be lethal if ambient temperature exceeds organisms' upper thermal limits but can also lead to behavioral or physiological changes that reduce fitness and organism roles within the ecosystem (e.g. Thakur et al., 2022; Bell et al., 2023; Campion et al., 2023).

Phenotypic plasticity, the ability of an organism to express different phenotypes in response to environmental conditions (West-Eberhard, 2003), has been recognized as one of the most important mechanism for rapid response to climate change (e.g. Matesanz et al., 2010; Bonamour et al., 2019). Plasticity in critical thermal maximum ( $CT_{Max}$ ), the maximum tolerable temperature (e.g. Lutterschmidt and Hutchison, 1997), can enhance organisms' capacity to withstand or to recover from elevated temperatures. This plasticity can be achieved via thermal acclimation following chronic or brief exposures to thermal stimuli, potentially inducing upregulation of heat-shock proteins or other molecular chaperones that mitigate cellular damage under stress (Angilletta, 2009; González-Tokman et al., 2020). Thus, as thermal plasticity might promote survival and influence patterns of selection, understanding acclimation responses is a key challenge in climate change biology (Kellermann and van Heerwaarden, 2019; González-Tokman et al., 2020; Weaving et al., 2022).

Insects are the most diverse, ecologically significant, and economically vital group of multicellular organisms on the planet, both in freshwater and terrestrial ecosystems (Grimaldi and Engel, 2005). However, they may also be the most vulnerable to climate change due to their limited capacity to regulate body temperature and water loss, given their small body size, high surface area to volume ratio, low fat storage, and relatively high metabolic rate (e.g. Angilletta, 2009; Harrison et al., 2012; Bujan et al., 2016). Consequently, the responses of insects to climate change via thermal plasticity have been an important topic of research and debate, as plastic responses might vary among populations, species, and taxonomic groups, and might also be influenced by both biotic and abiotic factors (Shah et al., 2017; Weaving et al., 2022). For example, species from more thermally variable environments are predicted to have greater capacity to increase  $CT_{Max}$  relative to species from thermally homogenous environments, as more variable climates should select for broader thermal niches ('climate variability hypothesis', Janzen, 1967; Ghalambor et al., 2006). On the other hand, plasticity in  $CT_{Max}$  is expected to be low in species with high  $CT_{Max}$  because of the energetic costs involved in maintaining a high heat tolerance ('trade-off hypothesis', Somero, 2010; Barley et al., 2021).

<sup>1</sup>Undergraduate Biology Program and Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS, 66045, USA. <sup>2</sup>Department of Agriculture, University of Puerto Rico, Mayaguez, PR, 00681-9000, USA.

<sup>3</sup>Department of Biological Sciences, California State University, Los Angeles, CA, 35229, USA. <sup>4</sup>Biology Department, Waynesburg University, PA, 47243, USA.

<sup>5</sup>Laboratory of Biogeography and Ecology, Department of Geography, University of the Aegean, University Hill, Mytilene, 81100, Greece. <sup>6</sup>Department of Biology, Commonwealth University of Pennsylvania, Bloomsburg, 17815 PA, USA.

\*Author for correspondence (vhgonza@ku.edu)

 V.H.G., 0000-0002-4146-1634

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

Recent systematic reviews indicate that thermal acclimation of critical thermal limits is relatively weak across ectotherms, particularly in insects, but with a more pronounced response in the critical thermal minimum ( $CT_{Min}$ ) when compared to the  $CT_{Max}$  (e.g. Gunderson and Stillman, 2015; Gunderson et al., 2017; Barley et al., 2021; Weaving et al., 2022). Thus, thermal plasticity in critical thermal limits might offer limited benefits to insects during extreme climatic events. However, relatively few species have been assessed (~100 species) for this incredibly diverse group, which raises concerns about whether the limited plasticity in heat tolerance is representative of plasticity for insects in general (Weaving et al., 2022).

Bees are widely recognized as key organisms due to their essential roles in plant reproduction, ecosystem maintenance, and food security (Klein et al., 2007). With over 20,000 species of bees worldwide (Michener, 2007), several studies have documented changes in community composition, population vigor, species distributions, and plant–bee interactions due to landscape-level alterations and climate change (Bartomeus et al., 2013; Kerr et al., 2015; Soroye et al., 2020; Kammerer et al., 2021; Jackson et al., 2022). Nonetheless, information of the thermal biology of bees, including the impact of extreme weather events on their heat tolerance and plasticity remains relatively poorly studied (Gonzalez et al., 2022a,b; Johnson et al., 2023; Quinlan et al., 2023; Sepúlveda and Goulson, 2023).

To improve our understanding of how bees might be impacted by extreme weather events, herein we measured the short-term acclimation ability of the  $CT_{Max}$  in foragers of six bee species from the Greek island of Lesbos. In addition, we assessed whether  $CT_{Max}$  increases following acute heat exposure simulating a heat wave. Bees occupy diverse thermal environments and vary dramatically in morphology and life history traits (Michener, 2007), which suggests potential differential responses to heat stress. Thus, we assessed species that differed in body size, nesting habit, and level of sociality, ranging from very small, social, and ground-nesting species [*Lasiglossum malachurum* (Kirby)] to large, solitary, and wood-nesting species [*Xylocopa violacea* (Linnaeus)]. Body size determines foraging distance (Greenleaf et al., 2007) and thus large bees are likely to experience a greater range of temperatures during their daily foraging trips than do small bees. Therefore, we hypothesized that larger bees would display greater plasticity in  $CT_{Max}$  when compared with small bees, an expectation consistent with the climatic variability hypothesis (Janzen, 1967; Ghalambor et al., 2006). In addition, following the trade-off hypothesis (Somero, 2010), we predicted a negative relationship between plasticity in  $CT_{Max}$  and innate  $CT_{Max}$  among bee species.

## RESULTS

### Acclimation capacity

After accounting for body size, we found significant differences in  $CT_{Max}$  among species (ANCOVA, Wald  $\chi^2=50.87$ ,  $DF=5$ ,  $P<0.001$ ) but not in the average estimate of  $CT_{Max}$  of bees exposed to the heat treatment (Wald  $\chi^2=2.46$ ,  $DF=1$ ,  $P=0.12$ ). The interaction between species and heat treatment was not significant (Wald  $\chi^2=5.08$ ,  $DF=5$ ,  $P=0.41$ ).

We conducted an ANCOVA test for each species individually to better understand this result. We found no significant differences between treatments for each species, except for *L. malachurum* ( $DF=1$ ,  $P<0.001$ ; Fig. 1D, Table S1), which after acclimation had a  $CT_{Max}$ , on average, 0.72°C greater than that of the control group. Across all species, variance in  $CT_{Max}$  was similar between treatments [ $F_{(175, 165)}=0.75$ ,  $P=0.06$ ]. Within species, variance of

$CT_{Max}$  was also similar between treatments, except for an increase in the  $CT_{Max}$  variance of *Apis mellifera* Linnaeus and *L. malachurum* (Table S2).

Bees' intertegular distance (ITD) in our study ranged from 1.42 mm in *L. malachurum* to 6.29 mm in *X. violacea*, while average estimates of  $CT_{Max}$  ranged from 42°C in *L. malachurum* to 47°C in *Xylocopa olivieri* Lepeletier and *X. violacea*. Across all species,  $CT_{Max}$  increased with increasing ITD ( $P<0.001$ ,  $R^2=0.435\pm0.07$ ; Fig. 2). Within species,  $CT_{Max}$  did not significantly increase with increasing ITD, except in *Bombus terrestris* (Linnaeus) ( $P=0.001$ ,  $R^2=0.163\pm0.65$ ; Table S3). Except for *A. mellifera*, the acclimation response ratio (ARR) was positive for each species, demonstrating the ability to increase  $CT_{Max}$  in response to acclimation. For every 1°C rise in acclimation temperature, ARR for  $CT_{Max}$  ranged from as low as 0.008°C in *Halictus scabiosae* (Rossi) to as high as 0.234°C in *X. olivieri*. However, ARR was not significantly associated with bees' ITD ( $P=0.548$ ,  $R^2=-0.129\pm0.03$ ; Fig. S2) or with  $CT_{Max}$  ( $P=0.356$ ,  $R^2=0.017\pm0.02$ ; Fig. S3).

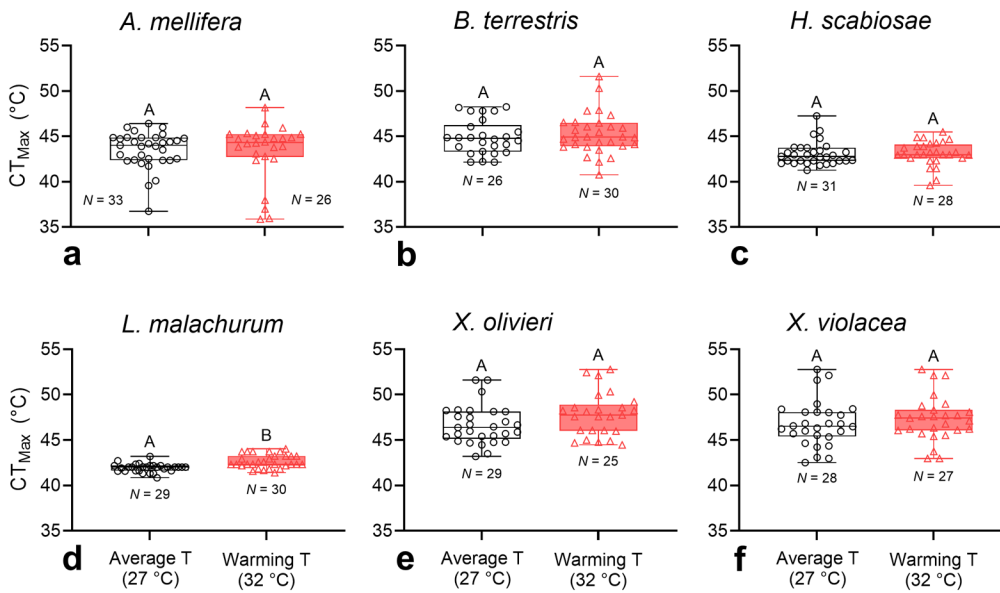
### Response to acute heat exposure

After accounting for body size,  $CT_{Max}$  differed significantly among species (Wald  $\chi^2=28.97$ ,  $DF=3$ ,  $P<0.001$ ), but not between treatments (Wald  $\chi^2=0.01$ ,  $DF=1$ ,  $P=0.93$ ) or in the time following an acute heat exposure (Wald  $\chi^2=0.01$ ,  $DF=1$ ,  $P=0.91$ ). All interactions among factors were not significant (Fig. 3A–D, Tables S4, S5). Variance in  $CT_{Max}$  was similar between treatments [ $F_{(145, 153)}=0.99$ ,  $P=0.93$ ] and timing [ $F_{(153, 145)}=1.19$ ,  $P=0.30$ ].

## DISCUSSION

Our results demonstrate that bees in our study have limited capacity to increase  $CT_{Max}$  following either short-term acclimation to warm temperatures or acute heat exposure. The average estimate of  $CT_{Max}$  did not significantly increase when bees were acclimated for 48 h to a temperature 5°C higher than the average ambient temperature. Across all species, variance in  $CT_{Max}$  was not impacted by the heat treatment, although it increased the variance of *A. mellifera* and *L. malachurum* (Table S2). Thus, these results support the idea that plasticity in  $CT_{Max}$  is relatively weak in insects (Weaving et al., 2022).

It is possible that the acclimation temperature we chose (32°C), as well as the incubation period (48 h), may have constrained bees' ability to increase their  $CT_{Max}$ . It is also possible that bees were already acclimated to the warm summer temperatures at the time of the study. However, Oyen and Dillon (2018) and Sepúlveda and Goulson (2023) used comparable or higher (36°C) temperatures in their 72-h acclimation assays for the North American bumble bee *B. impatiens* Cresson and the European bumble bee *B. terrestris*. In both cases, the average  $CT_{Max}$  did not improve with acclimation temperature. Thus, the observed limited acclimation capacity to heat tolerance in our study may be a general pattern among bees, and not a methodological artifact. However, we assessed a reduced number of species from a single location and *post-hoc* tests on individual species revealed an effect of the acclimation temperature in *L. malachurum* (Fig. 1D). Future studies should assess a larger number of bee species from several populations, as well as their responses to both constant and fluctuating acclimation temperatures. While studies often assess acclimation capacity using constant temperatures in the laboratory, organisms may respond differently to fluctuating acclimation temperatures, which better reflect the thermally variable natural environment that they experience (e.g. Sgrò et al., 2016). To date, thermal bee studies have only used constant acclimation temperatures.

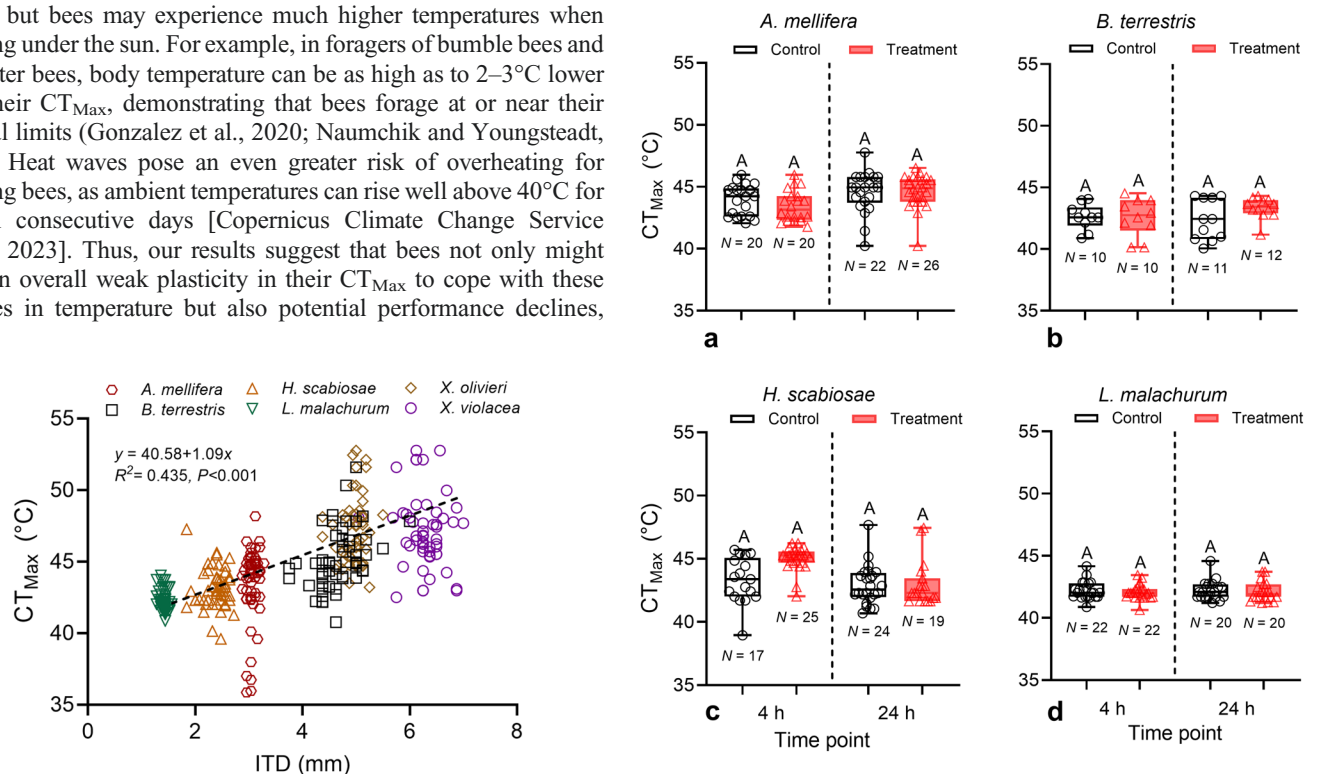


**Fig. 1.** Boxplots showing critical thermal maxima ( $CT_{Max}$ ) of bee foragers from six species acclimated for 48 h at either 27°C (average ambient temperature) or 32°C (warming temperature). To better understand the results, we conducted *post-hoc* ANCOVA tests for each species individually. Boxplots display median, quartiles, and extreme values. For each species, different letters above boxplots indicate significant mean differences in the *post-hoc* tests ( $P < 0.05$ ).

The weak plasticity in  $CT_{Max}$  displayed among bees is reflected in the small values of ARR we calculated from the acclimation assays (Table 1). On average, for every 1°C rise in acclimation temperature, bees'  $CT_{Max}$  increased by 0.09°C, which is comparable to the average ARR calculated for all insects in the recent meta-analysis by Weaving et al. (2022). The negative ARR value displayed by *A. mellifera* (−0.028) indicates the opposite trend observed for all other bees, that is, a reduction in the  $CT_{Max}$  with an increase in the acclimation temperature. At our study sites, mean hourly air temperature ranges from 16°C to 38°C in the shade (Gonzalez et al., 2020), but bees may experience much higher temperatures when foraging under the sun. For example, in foragers of bumble bees and carpenter bees, body temperature can be as high as to 2–3°C lower than their  $CT_{Max}$ , demonstrating that bees forage at or near their thermal limits (Gonzalez et al., 2020; Naumchik and Youngsteadt, 2023). Heat waves pose an even greater risk of overheating for foraging bees, as ambient temperatures can rise well above 40°C for several consecutive days [Copernicus Climate Change Service (C3S), 2023]. Thus, our results suggest that bees not only might have an overall weak plasticity in their  $CT_{Max}$  to cope with these changes in temperature but also potential performance declines,

although the latter could have resulted from the stress of holding bees in vials during the experiment.

We predicted that large bees would display greater plasticity in  $CT_{Max}$  when compared with small bees and that species with high  $CT_{Max}$  will display low plasticity in their  $CT_{Max}$ . Contrary to our expectations, we found no association between ARR and body size or  $CT_{Max}$ . Thus, our observations do not support these predictions based on the climatic variability hypothesis or the trade-off hypothesis, but we only assessed a reduced number of species.



**Fig. 2.** Relationship between intertegular distance (ITD) and critical thermal maxima ( $CT_{Max}$ ) across all six species of bees assessed in this study.  $CT_{Max}$  values are for bees kept at average ambient temperature (27°C) without prior acclimation.

**Fig. 3.** Boxplots showing critical thermal maxima ( $CT_{Max}$ ) of bees following a 4-h acute heat exposure (38°C) and 24 h after heat exposure (treatment). As controls, bees were kept at 27°C. Boxplots display median, quartiles, and extreme values. For each figure, different letters above boxplots indicate significant mean differences ( $P < 0.05$ ).



**Table 1. Life-history traits, intertegular distance (ITD), critical thermal maxima (CT<sub>Max</sub>), and acclimation response ratio (ARR) of bee species used in the acclimation experiment**

Species	Sociality	Nesting	ITD (mm)	CT <sub>Max</sub> (°C) Average T	CT <sub>Max</sub> (°C) Warming T	ARR
<i>Apis mellifera</i> (Linnaeus)	Eusocial	Cavity	3.06±0.01, n=20	43.52±0.35, n=33 <sup>a</sup>	43.38±0.62, n=26 <sup>a</sup>	−0.028
<i>Bombus terrestris</i> (Linnaeus)	Eusocial	Cavity	4.66±0.05, n=56	44.92±0.37, n=26 <sup>a</sup>	45.28±0.41, n=30 <sup>a</sup>	0.074
<i>Halictus scabiosae</i> (Rossi)	Primitively eusocial	Ground	2.38±0.03, n=59	43.08±0.23, n=31 <sup>a</sup>	43.12±0.25, n=28 <sup>a</sup>	0.008
<i>Lasioglossum malachurum</i> (Kirby)	Primitively eusocial	Ground	1.42±0.01, n=59	41.94±0.84, n=29 <sup>a</sup>	42.66±0.14, n=30 <sup>b</sup>	0.144
<i>Xylocopa olivieri</i> (Lepeletier)	Solitary†	Ground	4.96±0.03, n=54	46.65±0.39, n=29 <sup>a</sup>	47.82±0.48, n=25 <sup>a</sup>	0.234
<i>Xylocopa violacea</i> (Linnaeus)	Solitary	Wood	6.29±0.05, n=55	46.81±0.48, n=28 <sup>a</sup>	47.34±0.47, n=27 <sup>a</sup>	0.106

ARR ranges from 0 to 1, with 0 indicating no change in CT<sub>Max</sub> (no acclimation) and 1 indicating an increase of 1°C in CT<sub>Max</sub> to every 1°C increase in acclimation temperature (100% of acclimation). Bee foragers were incubated for 48 h at either 27°C (average ambient temperature) or 32°C (warming temperature). Standard error (±) followed by sample size. †=Crepuscular species. For each species, different lowercase letters (a,b) next to CT<sub>Max</sub> value indicate significant mean differences ( $P<0.05$ ) between control and treatment (see Table S1).

Nonetheless, support for two these hypotheses is mixed in the literature, with some studies favoring either hypothesis. Such a lack of consensus among studies might be due to the lack of broad-scale comparative empirical studies, the lack of a phylogenetic framework to account for potential phylogenetic effects, or by not considering the effect of behavior in regulating species exposure to the environment (Gunderson and Stillman, 2015; Kingsolver and Buckley, 2017; Kellermann et al., 2018). Future studies should test these hypotheses using a higher number of species at a greater spatial scale.

Our findings revealed a significant increase in CT<sub>Max</sub> across all species as ITD increased (Fig. 2). This indicates that large-bodied species, such as the bumble bee *B. terrestris* and the carpenter bees *X. olivieri* and *X. violacea*, can tolerate higher temperatures than small-bodied species like *L. malachurum*. Such a positive relationship between heat tolerance and body size is expected, given that small bees heat up more quickly than large bees due to their high surface area to volume ratio, which increases convective heat transfer (Johnson et al., 2023; Oyen et al., 2016; Gonzalez et al., 2022c). However, such a relationship between CT<sub>Max</sub> and body size is not always straightforward, as it seems to vary among bee communities, lineages, and species. For example, while some studies provide support for this relationship (Oyen et al., 2016; Gonzalez et al., 2022c), others find no effect of body size on this thermal trait (Hamblin et al., 2017; Oyen and Dillon, 2018; Gonzalez et al., 2020, 2022a; Jones et al., 2024). Similarly, the influence of body size on CT<sub>Max</sub> appears to be species specific, even varying within species of the same genus as in the case of bumble bees (Oyen et al., 2016; Oyen and Dillon, 2018; Gonzalez et al., 2022a). Unlike the study by Sepúlveda and Goulson (2023), where CT<sub>Max</sub> of *B. terrestris* remained unaffected by body size, we found that *B. terrestris* was the only species in which CT<sub>Max</sub> increased with increasing ITD. Considering that several distinct populations or subspecies of *B. terrestris* have been recognized across its distributional range (Rasmont et al., 2008), our work indicates varied responses in this thermal trait at the population level.

Our study also revealed that bees' average CT<sub>Max</sub> did not increase following acute heat exposure or 24 h after the exposure (Fig. 3). These results suggest that the prior heat exposure in our experiments did not prepare bees to tolerate further heat exposure. An improvement of heat tolerance due to the upregulation of heat shock proteins has been documented in other insects (González-Tokman et al., 2020), and honey bees and bumble bees are known to upregulate these proteins when exposed to thermal stress (Koo et al., 2015; Pimsler et al., 2020; Al-Ghzawi et al., 2022). While unanticipated, our results agree with those of a recent study by Quinlan et al. (2023) in which *B. impatiens* did not show improvement on its heat tolerance, as measured as the time to heat

stupor, after brief or longer exposures to heat stress. It is likely that inducible heat shock proteins are variable in their expression within bee species and populations (González-Tokman et al., 2020). If climate change may result in bees operating more closely to their upper thermal limits, it will be important to know how heat-shock proteins could mediate local adaptations to heat. Unfortunately, this aspect remains largely unexplored in bees.

CT<sub>Max</sub> has been assessed in a relatively small number of bee species, most of them from North America (Gonzalez et al., 2022a,b; Johnson et al., 2023). However, our estimates of CT<sub>Max</sub> fall within the range reported for other species, with the highest values recorded for bumble bees and carpenter bees. Except for *L. malachurum* and *H. scabiosae*, CT<sub>Max</sub> has previously been assessed in the remaining species we studied. Maebe et al. (2021) and Sepúlveda and Goulson (2023) estimated CT<sub>Max</sub> in lab-reared subspecies of *B. terrestris*, and several authors (e.g. Burdine and McCluney, 2019; Sánchez-Echeverría et al., 2019; Gonzalez et al., 2022b; Benito et al., 2024) have assessed various subspecies as well as populations of *A. mellifera*, including feral and managed Africanized honey bees. Gonzalez et al. (2020) assessed CT<sub>Max</sub> for *X. violacea* and *X. olivieri*, both from the island of Lesvos. There are differences in the average estimates of CT<sub>Max</sub> between those studies and the values we obtained, which are likely due to methodological variations that include the rate of temperature change in dynamic protocols, the type of equipment used, and the origin of bees (lab-reared versus wild individuals). See Gonzalez et al. (2022a,b) and references therein for a discussion.

### Implications for conservation and future studies

Our results have significant implications for understanding bees' heat tolerance mechanisms and their potential responses to climate change. The weak plasticity in bees' CT<sub>Max</sub> after short-term acclimation, as well as their inadequate ability to improve heat tolerance after prior acute heat exposure, suggest physiological limitations in forager bees to cope with increasingly variable and intense temperatures during extreme climatic events. Consequently, other mechanisms, such as behavioral thermoregulation or nest selection, might be even more important for foraging bees to cope with these extreme climatic events. Indeed, bees can restrict the range of temperatures they experience by adjusting their foraging and nesting activities. For example, bees are known to shorten or shift their foraging schedule (Willmer and Stone, 2004; de Farias-Silva and Freitas, 2021), reduce the number or duration of pollen-foraging trips (Maia-Silva et al., 2015), or regurgitate nectar or water for evaporative cooling (Portman et al., 2021). In addition, social insects are expected to display greater behavioral plasticity to tolerate environmental changes when compared to solitary species (Parr and Bishop, 2022). Social species are not only able to

thermoregulate their nest (Jones and Oldroyd, 2007), but they may also display within-group variation in the thermal tolerance across colony members, as documented in ants (Baudier and O'Donnell, 2017). For solitary species or species unable to thermoregulate the nests, optimal nest selection or structural adjustments to their nests are critical to influence the thermal environment they experience (Richards et al., 2020; Gonzalez et al., 2023). For example, among our focal species, *H. scabiosae* ceases foraging at the heat of the day and seals its nest entrance, which suggests heat-avoidant behavior. However, at least for an Austrian population, such behavior appears to be more related to an increase of nest-infecting parasites than in response to elevated ambient temperatures (Lienhard et al., 2010). The limited physiological plasticity of foraging bees also underlines the significance of conserving and restoring native vegetation to provide them with temporary thermal refuges.

To obtain a more comprehensive understanding of bees' responses to climate change, future studies should not only attempt to evaluate the acclimation capacity of other bee species at various locations, but also investigate the thermal tolerance and acclimation potential of their immature stages. While adult bees can employ behavioral thermoregulation to cope with temperature fluctuations, this ability is absent in their immobile immature stages. However, greater thermal tolerance and plasticity might be anticipated in these developmental stages compared to adults due to the limited opportunities for behavioral thermoregulation, a prediction consistent with the observed pattern known as the Bogert effect (Gunderson and Stillman, 2015). This could be the case for immature stages of solitary bee species or thermoconform social species, such as stingless bees. A recent study shows that stingless bees' larvae and pupae are indeed more heat tolerant than foragers (Nacko et al., 2023), which agrees with our prediction.

## MATERIALS AND METHODS

### Study site and species

We captured bees for this study during June and July of 2023 at two locations along Kalloni Bay on the Greek island of Lesbos: a coastal semi-natural low scrub (phrygana) habitat in Achladeri, near Ancient Pyrrha (39° 09' 17.40" N, 26° 16' 32.74" E, 1.3 ma.s.l.) and an unmanaged pasture near Skala Kallonis (39° 12' 36" N, 26° 12' 12" E, 1.0 ma.s.l.), approximately 9 km northwest of Achladeri. Except for honey bees, which we captured from two experimental Langstroth hives each trained to scented feeders, we collected bees at flowers between 6:00 and 11:00 h with a net and transferred them individually to plastic vials, which we then capped with fabric (with holes ~1 mm in diameter). We kept bees inside a cooler with an ice pack covered in a piece of paper towel (16–19°C) until we completed fieldwork. We selected the following six species based on their local abundance and differences in life history traits, which included ground and wood nesting habits, solitary and social behaviors, diurnal and crepuscular activity, and varying sizes (Table 1): *Apis mellifera*, *Bombus terrestris*, *Halictus scabiosae*, *Lasioglossum malachurum*, *Xylocopa violacea*, and *X. olivieri*. The senior author identified these species by comparing specimens with the reference bee collection of the Melissotheque of the Aegean at the University of the Aegean, Mytilene, Lesbos, Greece. We attempted to capture at least 30 individuals per species per treatment, but because most bees were collected opportunistically, sample sizes varied among species and treatments. We brought bees to the laboratory and fed them *ad libitum* with a small piece of commercial honey bee food paste (Viepol, Kirka®, Greece) placed on a moistened cotton ball with a 50% sucrose solution. Then, we transferred bees to a 25 L reptile incubator (Vevor®) where we used them for either the acclimation or acute heat exposure assays, as described below (Fig. S1). Bees were transferred to incubators within 1–2 h after being captured in the field.

### Acclimation assays

We acclimated bees for 48 h in the dark at one of two constant temperature treatments, 'average' and 'warming'. We used 27°C as the average temperature, which represents the mean ambient daily temperature recorded at both study locations the previous year during the summer months. At each site, we used an iButton data logger (DS1923 Hygrochron; Maxim Integrated, San Jose, CA, USA), which we protected from solar radiation with aluminum foil and hung 1 m above ground from tree branches (see Gonzalez et al., 2020). We recorded temperature and humidity every 30 min for seven consecutive weeks from mid-June to late July. We used 32°C as the warming temperature. As in Shah et al. (2017), we used a 5°C increase from the average temperature as the warming temperature, which is within the range of natural variation recorded at the location sites. We conducted experiments in the dark to minimize the effect of potential physiological and behavioral stressors associated with light. We chose the acclimation period of 48 h because pilot studies indicated higher mortality at longer incubation periods. We replenished food every 24 h and kept humidity inside the incubators between 40–60% by adding water to the bottom tray. We used a different set of bees for each acclimation temperature treatment and measured their CT<sub>Max</sub> as indicated below.

### Acute heat exposure assays

To assess the impact of acute heat exposure on CT<sub>Max</sub>, we placed bees in an incubator at 38°C (40–60% RH) for a 4-h period. We measured CT<sub>Max</sub> following the acute heat exposure and assessed post-heat exposure recovery after 24 h in an incubator at 27°C. Bees incubated at 27°C for 4 and 24 h served as control groups. For this experiment, we focused on the social species, as individuals of both solitary carpenter bees (*Xylocopa olivieri* and *X. violacea*) became scarcer and difficult to capture towards the end of our field season at the study site. We chose 38°C, which is 11°C higher than the average ambient temperature at our study locations, and an exposure period of 4 h for several reasons. First, this temperature is, on average, about 3°C lower than the CT<sub>Max</sub> of *L. malachurum*, the species in our study with the lowest CT<sub>Max</sub>. Second, even large bees, such as some bumble bees, may reach heat stupor and eventually die when exposed to 40°C in an incubator for periods as short as 30 min (Martinet et al., 2015). Third, 4-h acute exposures are ecologically relevant simulation of afternoon heat stress that increases *Hsc70* expression in life stages of insects (Feder et al., 1997; Barthell et al., 2002; Hranitz et al., 2009). Blasco-Lavilla et al. (2021) showed significant expression of heat shock genes *Hsc70* in *B. terrestris*, a species with a high CT<sub>Max</sub> (as shown in Table 1), between 2 h and 6 h after exposure to 38°C. Similarly, some subspecies of the European honey bees are known to upregulate heat shock proteins after 4 h of exposure to 40°C or 45°C (Alqarni et al., 2019). In addition, some solitary bee species, such as leaf cutter bees, expressed heat shock proteins even when exposed to 35°C for 3 h (Barthell et al., 2002). Fourth, studies demonstrate that foraging in at least some bumble bees, including *B. terrestris*, is significantly reduced at temperatures higher than 32°C (e.g. Kwon and Saeed, 2003; Kenna et al., 2021; Hemberger et al., 2023). Thus, we considered 38°C to be a moderately high temperature that would allow us to compare all species, and 4 h treatment as a long enough period for bees to potentially display changes in the expression of heat shock proteins while reducing mortality.

### CT<sub>Max</sub> assays

We measured bees' CT<sub>Max</sub> using a dynamic (ramping temperature) protocol with the Elara 2.0 (IoTherm, Laramie, WY, USA). We placed bees individually inside glass shell vials (either 9×30 mm, 0.92 cm<sup>3</sup> for small bees or 12×35 mm, 1.80 cm<sup>3</sup> for large bees) and plugged them with a moistened cotton ball to ensure consistent humidity during the assays. We used an initial temperature of 27°C and held bees for 10 min at this temperature before increasing it at a rate of 0.5°C min<sup>-1</sup>. We chose this rate of temperature change to reduce the time required for each assay and to minimize the effect of confounding physiological stressors, such as dehydration or starvation (Gonzalez et al., 2022b). We placed vials horizontally on the stage to prevent bees from climbing the sides of the vial. To estimate the temperature inside the vials, we placed a K-type thermocouple inside two empty glass vials plugged with a cotton ball. We individually tracked these vial temperatures using a TC-08 thermocouple

data logger (Pico Technology, Tyler, TX, USA).  $CT_{Max}$  was taken as the temperature at which bees lost muscular control, spontaneously flipping over onto their dorsa and spasming (Lutterschmidt and Hutchison, 1997; García-Robledo et al., 2016, 2018). See Gonzalez et al. (2022b) for details of the thermal apparatus and setting used. Once experiments concluded, we euthanized bees and estimated their body size as indicated below.

### Body size

We estimated body size by measuring the minimum distance between the tegulae (ITD) of each specimen. However, for honey bees, which display little variation in body size, we used an average taken from 20 individuals. ITD is a robust estimator of bee dry mass (Cane, 1987) and it is commonly used in ecological studies as a proxy for body size (Kendall et al., 2019). We measured bees using an ocular micrometer on S6E stereomicroscope (Leica Microsystems, Wetzlar, Germany). Voucher specimens have been deposited in the Melissotheque of the Aegean.

### Statistical analyses

We conducted statistical analyses in R (R Core Team, 2018). We used an ANCOVA test to compare  $CT_{Max}$  between bees exposed to average and warming temperature scenarios while controlling for the effects of body size. We implemented a linear mixed-effect model (LMM) using the lmer function in the lme4 package (Bates et al., 2015) with species and treatment (average and warming temperatures) as fixed factors, date of collection as a random factor, and ITD as a covariate. To evaluate the relationship between  $CT_{Max}$  and ITD among and within species, we implemented a linear regression analysis using the lm function. To assess the level of acclimation among species, we calculated the acclimation response ratio (ARR) as  $\Delta CT_{Max}/\Delta$  Acclimation (Claussen, 1977), where  $\Delta CT_{Max}$  is the average  $CT_{Max}$  after acclimation to the warming temperature (32°C) minus the average  $CT_{Max}$  after acclimation to the average temperature (27°C) and  $\Delta$  acclimation is the difference between the warming and average acclimation temperatures (5°C). This ARR ranges from 0 to 1, with 0 indicating no change in  $CT_{Max}$  (no acclimation) and 1 indicating an increase of 1°C in  $CT_{Max}$  to every 1°C increase in acclimation temperature (100% of acclimation). To assess whether bees' acclimation capacity is associated with their body size or  $CT_{Max}$ , we used a linear regression analysis to assess the relationship between ARR and bees' average ITD and  $CT_{Max}$ . For this analysis, we used the average  $CT_{Max}$  estimated for bees kept at average ambient temperature, without prior acclimation. To assess changes in  $CT_{Max}$  after exposure to heat stress, while controlling for the effects of body size, we used a mixed-model ANCOVA with species, heat treatment (27°C and 38°C) and timing (4 h and 24 h) as fixed factors, date of collection as a random factor, and ITD as a covariate. We assessed the significance of fixed effects using a Type II Wald  $\chi^2$  test with the car package (Fox and Weisberg, 2019). When factors and factor interactions were significant, we used the lsmeans package (Lenth, 2016) to conduct multiple pairwise comparisons with Bonferroni adjustment to assess for differences among groups. We compared variance in  $CT_{Max}$  using  $F$ -tests with the var.test function from the car package.

### Acknowledgements

We are indebted to Amy Comfort, Karl Roeder, Michael Dillon, and anonymous reviewers for comments and suggestions that improved this manuscript. The Kapsalis family at the hotel Malemi provided constant logistic support during the fieldwork on Lesvos.

### Competing interests

The authors declare no competing or financial interests.

### Author contributions

Conceptualization: V.H.G., J.H.; Methodology: V.H.G., N.H., G.R.P., T. Panganiban, L.H., T.T., T. Petanidou, J.H.; Validation: V.H.G.; Formal analysis: V.H.G.; Investigation: V.H.G., N.H., G.R.P., T. Panganiban, L.H., T.T., T. Petanidou, J.H.; Resources: V.H.G., T.T., T. Petanidou, J.H.; Data curation: V.H.G.; Writing - original draft: V.H.G., T.T., T. Petanidou, J.H.; Writing - review & editing: V.H.G., N.H., G.R.P., T. Panganiban, L.H., T.T., T. Petanidou, J.H.; Visualization: V.H.G.; Supervision: V.H.G.; Project administration: V.H.G.; Funding acquisition: V.H.G., T.T., T. Petanidou, J.H.

### Funding

This work was supported by the National Science Foundation's REU program (EAR 1950805). Open Access funding provided by The University of Kansas. Deposited in PMC for immediate release.

### Data availability

All relevant data can be found within the article and its supplementary information.

### References

- Al-Ghazawi, A. A.-M. A., Al-Zghoul, M. B., Zaitoun, S., Al-Omary, I. M. and Alahmad, N. A. (2022). Dynamics of heat shock proteins and heat shock factor expression during heat stress in daughter workers in pre-heat-treated (rapid heat hardening) *Apis mellifera* mother queens. *J. Therm. Biol.* **104**, 103194. doi:10.1016/j.jtherbio.2022.103194
- Alqarni, A. S., Ali, H., Iqbal, J., Owayss, A. A. and Smith, B. H. (2019). Expression of heat shock proteins in adult honey bee (*Apis mellifera* L.) workers under hot-arid subtropical ecosystems. *Saudi. J. Biol. Sci.* **26**, 1372-1376. doi:10.1016/j.sjbs.2019.08.017
- Angilletta, M. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford: Oxford University Press.
- Arnett, A., Shin, Y. J., Leadley, P., Rondinini, C., Bukvareva, E., Kolb, M., Midgley, G. F., Oberdorff, T., Palomo, I. and Saito, O. (2020). Post-2020 biodiversity targets need to embrace climate change. *Proc. Natl. Acad. Sci. USA* **117**, 30882-30891. doi:10.1073/pnas.2009584117
- Barley, J. M., Cheng, B. S., Sasaki, M., Gignoux-Wolfsohn, S., Hays, C. G., Putnam, A. B., Sheth, S., Villeneuve, A. R. and Kelly, M. (2021). Limited plasticity in thermally tolerant ectotherm populations: evidence for a trade-off. *Proc. R. Soc. B* **288**, 20210765. doi:10.1098/rspb.2021.0765
- Barthell, J. F., Hranitz, J. M., Thorp, R. W. and Shue, M. K. (2002). High temperature responses in two exotic leafcutting bee species: *Megachile apicalis* and *M. rotundata* (Hymenoptera: Megachilidae). *Pan-Pac. Entomol.* **78**, 235-246.
- Bartomeus, I., Ascher, J. S., Gibbs, J., Danforth, B. N., Wagner, D. L., Hedtkke, S. M. and Winfree, R. (2013). Historical changes in northeastern US bee pollinators related to shared ecological traits. *Proc. Natl. Acad. Sci. USA* **110**, 4656-4660. doi:10.1073/pnas.1218503110
- Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1-48. doi:10.18637/jss.v067.i01
- Baudier, K. M. and O'Donnell, S. (2017). Weak links: How colonies counter the social costs of individual variation in thermal physiology. *Curr. Opin. Insect Sci.* **22**, 85-91. doi:10.1016/j.cois.2017.06.004
- Bell, J. J., Smith, R. O., Micaroni, V., Strano, F., Balemi, C. A., Caiger, P. E., Miller, K. I., Spykma, A. J. P. and Shears, N. T. (2023). Marine heat waves drive bleaching and necrosis of temperate sponges. *Curr. Biol.* **33**, 158-163. doi:10.1016/j.cub.2022.11.013
- Benito, J. B., Raton, B., Baena-Díaz, F., González-Tokman, D. and Dáttilo, W. (2024). Thermal tolerance of honeybees (*Apis mellifera* L.) changes across an elevation gradient in the Mexican Transition Zone. *Sociobiology* **71**, e10155. doi:10.13102/sociobiology.v71i1.10155
- Blasco-Lavilla, N., García-Reina, A. and De la Rúa, P. (2021). Mild thermal stress does not negatively affect immune gene expression in the bumblebee *Bombus terrestris*. *Apidologie* **52**, 163-173. doi:10.1007/s13592-020-00806-w
- Bonamour, S., Chevin, L.-M., Charmanier, A. and Teplitsky, C. (2019). Phenotypic plasticity in response to climate change: the importance of cue variation. *Phil. Trans. R. Soc. B* **374**, 2018017820180178. doi:10.1098/rstb.2018.0178
- Bujan, J., Yanoviak, S. P. and Kaspari, M. (2016). Desiccation resistance in tropical insects: causes and mechanisms underlying variability in a Panama ant community. *Ecol. Evol.* **6**, 6282-6291. doi:10.1002/ece3.2355
- Burdine, J. D. and McCluney, K. E. (2019). Differential sensitivity of bees to urbanization-driven changes in body temperature and water content. *Sci. Rep.* **9**, 1643. doi:10.1038/s41598-018-38338-0
- Campion, C., Rajamohan, A. and Dillon, M. E. (2023). Sperm can't take the heat: short-term temperature exposures compromise fertility of male bumble bees (*Bombus impatiens*). *J. Insect Physiol.* **146**, 104491. doi:10.1016/j.jinsphys.2023.104491
- Cane, J. H. (1987). Estimation of bee size using intertegular span (Apoidea). *J. Kansas Entomol. Soc.* **60**, 145-147.
- Claussen, D. L. (1977). Thermal acclimation in ambystomatid salamanders. *Comp. Biochem. Physiol.* **58A**, 333-340. doi:10.1016/0300-9629(77)90150-5
- Copernicus Climate Change Service (C3S) (2023). European State of the Climate 2022. Full report: climate.copernicus.eu/ESOTC/2022.
- de Farias-Silva, F. J. and Freitas, B. M. (2021). Thermoregulation in the large carpenter bee *Xylocopa frontalis* in the face of climate change in the Neotropics. *Apidologie* **52**, 341-357. doi:10.1007/s13592-020-00824-8
- Feder, M. E., Blair, N. and Figeuras, H. (1997). Natural thermal stress and heat-shock protein expression in *Drosophila* larvae and pupae. *Funct. Ecol.* **11**, 90-100. doi:10.1046/j.1365-2435.1997.00060.x
- Fox, J. and Weisberg, S. (2019). *An R Companion to Applied Regression*. Thousand Oaks: Sage.



- García-Robledo, C., Kuprewicz, E. R., Staines, C. L., Erwin, T. L. and Kress, W. J. (2016). Limited tolerance by insects to high temperatures across tropical elevational gradients and the implications of global warming for extinction. *Proc. Natl. Acad. Sci. USA* **113**, 680-685. doi:10.1073/pnas.1507681113
- García-Robledo, C., Charlotten-Silva, M., Cruz, C., Kuprewicz, E. K., (2018) Low quality diet and challenging temperatures affect vital rates, but not thermal tolerance in a tropical insect expanding its diet to an exotic plant. *Journal of Thermal Biology* **77**, 7-13. doi:10.1016/j.jtherbio.2018.07.018
- Ghalambor, C. K., Huey, R. B., Martin, P. R., Tewksbury, J. J. and Wang, G. (2006). Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Int. Comp. Biol.* **46**, 5-17. doi:10.1093/icb/icj003
- Gonzalez, V. H., Hranitz, J. M., Percival, C. R., Pulley, K. L., Tapsak, S. T., Tscheulin, T., Petanidou, T. and Barthell, J. B. (2020). Thermal tolerance varies with dim-light foraging and elevation in large carpenter bees (Hymenoptera: Apidae: Xylocopini). *Ecol. Entomol.* **45**, 688-696. doi:10.1111/een.12842
- Gonzalez, V. H., Oyen, K., Aguilar, M., Herrera, A., Martin, R. D. and Ospina, R. (2022a). High thermal tolerance in high-elevation species and laboratory-reared colonies of tropical bumble bees. *Ecol. Evol.* **12**, e9560. doi:10.1002/ece3.9560
- Gonzalez, V. H., Oyen, K., Ávila, O. and Ospina, R. (2022b). Thermal limits of Africanized honey bees are influenced by temperature ramping rate but not by other experimental conditions. *J. Therm. Biol.* **110**, 103369. doi:10.1016/j.jtherbio.2022.103369
- Gonzalez, V. H., Oyen, K., Vitale, N. and Ospina, R. (2022c). Neotropical stingless bees display a strong response in cold tolerance with changes in elevation. *Conserv. Physiol.* **10**, coac073. doi:10.1093/conphys/coac073
- Gonzalez, V. H., Manweiler, R., Smith, A. R., Oyen, K., Cardona, D. and Wcislo, W. T. (2023). Low heat tolerance and high desiccation resistance in nocturnal bees and the implications for nocturnal pollination under climate change. *Sci. Rep.* **13**, 22320. doi:10.1038/s41598-023-49815-6
- González-Tokman, D., Córdoba-Aguilar, A., Dáttilo, W., Lira-Noriega, A., Sánchez-Guillén, R. A. and Villalobos, F. (2020). Insect responses to heat: physiological mechanisms, evolution and ecological implications in a warming world. *Biol. Rev.* **95**, 802-821. doi:10.1111/brev.12588
- Greenleaf, S. S., Williams, N. M., Winfree, R. and Kremen, C. (2007). Bee foraging ranges and their relationships to body size. *Oecologia* **153**, 589-596. doi:10.1007/s00442-007-0752-9
- Grimaldi, D. and Engel, M. S. (2005). *Evolution of the Insects*. New York & Cambridge: Cambridge University Press.
- Gunderson, A. R. and Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc. R. Soc. B* **282**, 20150401. doi:10.1098/rspb.2015.0401
- Gunderson, A. R., Dillon, M. E. and Stillman, J. H. (2017). Estimating the benefits of plasticity in ectotherm heat tolerance under natural thermal variability. *Funct. Ecol.* **31**, 1529-1539. doi:10.1111/1365-2435.12874
- Hamblin, A. L., Youngsteadt, E., López-Urbe, M. M. and Frank, S. D. (2017). Physiological thermal limits predict differential responses of bees to urban heat-island effects. *Biol. Lett.* **13**, 20170125. doi:10.1098/rsbl.2017.0125
- Harrison, J. F., Woods, H. A. and Roberts, S. P. (2012). *Ecological and Environmental Physiology of Insects*. New York: Oxford University Press.
- Harvey, J. A., Tougeron, K., Gols, R., Heinen, M., Abarca, P. K. Abram, Y. Basset, M. Berg, C. Boggs, J. Brodeur, et al. (2023). Scientists' warning on climate change and insects. *Ecol. Monogr.* **93**, e1553. doi:10.1002/ecm.1553
- Hemberger, J. A., Rosenberger, N. M. and Williams, N. M. (2023). Experimental heatwaves disrupt bumblebee foraging through direct heat effects and reduced nectar production. *Funct. Ecol.* **37**, 591-601. doi:10.1111/1365-2435.14241
- Hranitz, J. M., Barthell, J. F., Thorp, R. W., Overall, L. M. and Griffith, J. L. (2009). Nest site selection influences mortality and stress responses in developmental stages of *Megachile apicalis* Spinola (Hymenoptera: Megachilidae). *Environ. Entomol.* **38**, 484-492. doi:10.1603/022.038.0223
- IPCC (2021). Climate change 2021: The physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change. [V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, and R. B. Zhou (eds.)]. Cambridge, NY, USA: Cambridge University Press.
- Jackson, H. M., Johnson, S. A., Morandin, L. A., Richardson, L. L., Guzman, L. M. and McGonigle, L. K. (2022). Climate change winners and losers among North American bumblebees. *Biol. Lett.* **18**, 20210551. doi:10.1098/rsbl.2021.0551
- Janzen, D. H. (1967). Why mountain passes are higher in the tropics. *Am. Nat.* **101**, 233-249. doi:10.1086/282487
- Johnson, M. G., Glass, J. R., Dillon, M. E. and Harrison, J. F. (2023). Chapter One – How will climatic warming affect insect pollinators? *Adv. Insect Physiol.* **64**, 1-115. doi:10.1016/bs.aip.2023.01.001
- Jones, J. C. and Oldroyd, B. P. (2007). Nest thermoregulation in social insects. *Adv. Insect Physiol.* **33**, 153-191. doi:10.1016/S0065-2806(06)33003-2
- Jones, L. J., Miller, D. A., Schilder, R. J. and López-Urbe, M. M. (2024). Body mass, temperature, and pathogen intensity differentially affect critical thermal maxima and their population-level variation in a solitary bee. *Ecol. Evol.* **14**, e10945. doi:10.1002/ece3.10945
- Kammerer, M., Goslee, S. C., Douglas, M. R., Tooker, J. F. and Grozinger, C. M. (2021). Wild bees as winners and losers: Relative impacts of landscape composition, quality, and climate. *Glob. Change Biol.* **27**, 1250-1265. doi:10.1111/gcb.15485
- Kellermann, V. and van Heerwaarden, B. (2019). Terrestrial insects and climate change: adaptive responses in key traits. *Physiol. Entomol.* **44**, 99-115. doi:10.1111/phen.12282
- Kellermann, V., Hoffmann, A. A., Overgaard, J., Loeschcke, V. and Sgrò, C. M. (2018). Plasticity for desiccation tolerance across *Drosophila* species is affected by phylogeny and climate in complex ways. *Proc. R. Soc. B* **285**, 20180048. doi:10.1098/rspb.2018.0048
- Kendall, L. K., Rader, R., Gagic, V., Cariveau, D. P., Albrecht, M., Baldock, K. C. R., Freitas, B. M., Hall, M., Holzschuh, A., Molina, F. P., et al. (2019). Pollinator size and its consequences: Robust estimates of body size in pollinating insects. *Ecol. Evol.* **9**, 1702-1714. doi:10.1002/ece3.4835
- Kenna, D., Pawar, S. and Gill, R. J. (2021). Thermal flight performance reveals impact of warming on bumblebee foraging potential. *Funct. Ecol.* **35**, 2508-2522. doi:10.1111/1365-2435.13887
- Kerr, J. T., Pindar, A., Galpern, P., Packer, L., Potts, S. G., Roberts, S. M., Rasmont, P., Schweiger, O., Colla, S. R., Richardson, L. L. et al. (2015). Climate change impacts on bumblebees converge across continents. *Science* **349**, 177-180. doi:10.1126/science.aaa7031
- Kingsolver, J. D. and Buckley, L. B. (2017). Quantifying thermal extremes and biological variation to predict evolutionary responses to changing climate. *Phil. Trans. R. Soc.* **372**, 20160147. doi:10.1098/rstb.2016.0147
- Klein, A. M., Vaisière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C. and Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *P. Roy. Soc. B-Biol. Sci.* **274**, 303-313. doi:10.1098/rspb.2006.3721
- Koo, J., Son, T.-G., Kim, S.-Y. and Lee, K.-Y. (2015). Differential response of *Apis mellifera* heat shock protein genes to heat shock, flower-thinning formulations, and imidacloprid. *J. Asia-Pac. Entomol.* **18**, 583-589. doi:10.1016/j.aspen.2015.06.011
- Kwon, Y. J. and Saeed, S. (2003). Effect of temperature on the foraging activity of *Bombus terrestris* L. (Hymenoptera, Apidae) on greenhouse hot pepper (*Capsicum annum* L.). *Appl. Entomol. Zool.* **38**, 275-280. doi:10.1303/aez.2003.275
- Lenth, R. V. (2016). Least-squares means: the R package lsmeans. *J. Stat. Softw.* **69**, 1-33. doi:10.18637/jss.v069.i01
- Lienhard, A., Mirwald, L., Hötzel, T., Kranner, I. and Kastberger, G. (2010). Trade-off between foraging activity and infestation by nest parasites in the primitively eusocial bee *Halictus scabiosae*. *Psyche* **13**, 707501.
- Lutterschmidt, W. I. and Hutchison, V. H. (1997). The critical thermal maximum: data support the onset of spasms as the definitive end point. *Can. J. Zool.* **75**, 1553-1560. doi:10.1139/z97-782
- Maebé, K., De Baets, A., Vandamme, P., Vereecken, N. J., Michez, D. and Smagghe, G. (2021). Impact of intraspecific variation on measurements of thermal tolerance in bumble bees. *J. Therm. Biol.* **99**, 103002. doi:10.1016/j.jtherbio.2021.103002
- Maia-Silva, C., Hrnčir, M., da Silva, C. I. and Imperatriz-Fonseca, V. L. (2015). Survival strategies of stingless bees (*Melipona subnitida*) in an unpredictable environment, the Brazilian tropical dry forest. *Apidologie* **46**, 631-643. doi:10.1007/s13592-015-0354-1
- Martinet, B., Lecocq, T., Smet, J. and Rasmont, P. (2015). A protocol to assess insect resistance to heat waves, applied to bumblebees (*Bombus Latreille*, 1802). *PLoS ONE* **10**, e0118591. doi:10.1371/journal.pone.0118591
- Matesanz, S., Gianoli, E. and Valladares, F. (2010). Global change and the evolution of phenotypic plasticity in plants. *Annu. N. Y. Acad. Sci.* **1206**, 35-55. doi:10.1111/j.1749-6632.2010.05704.x
- Meehl, G. A. and Tebaldi, C. (2004). More intense, more frequent, and longer lasting heat waves in the 21st century. *Science* **305**, 994-997. doi:10.1126/science.1098704
- Michener, C. D. (2007). *The Bees of the World*, 2nd edn Baltimore: Johns Hopkins University Press.
- Nacko, S., Hall, A. M., Gloag, R., Lynch, K., Spooner-Hart, R., Cook, J. and Riegler, M. (2023). Heat stress survival and thermal tolerance of Australian stingless bees. *J. Therm. Biol.* **117**, 103671. doi:10.1016/j.jtherbio.2023.103671
- Naumchik, M. and Youngsteadt, E. (2023). Larger pollen loads increase risk of heat stress in foraging bumblebees. *Biol. Lett.* **19**, 20220581. doi:10.1098/rsbl.2022.0581
- Oyen, K. J. and Dillon, M. E. (2018). Critical thermal limits of bumblebees (*Bombus impatiens*) are marked by stereotypical behaviors and are unchanged by acclimation, age or feeding status. *J. Exp. Biol.* **221**, jeb165589. doi:10.1242/jeb.165589
- Oyen, K. J., Giri, S. and Dillon, M. E. (2016). Altitudinal variation in bumble bee (*Bombus*) critical thermal limits. *J. Therm. Biol.* **59**, 52-57. doi:10.1016/j.jtherbio.2016.04.015
- Parr, C. L. and Bishop, T. R. (2022). The response of ants to climate change. *Glob. Chang. Biol.* **28**, 3188-3205. doi:10.1111/gcb.16140

- Pimsler, M. L., Oyen, K. J., Herndon, J. D., Jackson, J. M., Strange, J. P., Dillon, M. E. and Lozier, J. D. (2020). Biogeographic parallels in thermal tolerance and gene expression variation under temperature stress in a widespread bumble bee. *Sci. Rep.* **10**, 17063. doi:10.1038/s41598-020-73391-8
- Portman, Z. M., Ascher, J. and Cariveau, D. P. (2021). Nectar concentrating behavior by bees (Hymenoptera: Anthophila). *Apidologie* **52**, 1169–1194. doi:10.1007/s13592-021-00895-1
- Quinlan, G. M., Feuerborn, C., Hines, H. M. and Grozinger, C. M. (2023). Beat the heat: Thermal respites and access to food associated with increased bumble bee heat tolerance. *J. Exp. Biol.* **226**, jeb245924. doi:10.1242/jeb.245924
- Richards, M. H., Cardama, G. A., Shehata, M., Groom, D., Tattersall, G. J. and Welch, K. C. (2020). Effects of nest microclimate temperatures on metabolic rates of small carpenter bees, *Ceratina calcarata* (Hymenoptera: Apidae). *Can. Entomol.* **152**, 772–782. doi:10.4039/tce.2020.50
- R Core Team (2018). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <https://www.R-project.org>.
- Rasmont, P., Coppée, A., Michez, D. and De Meulemeester, T. (2008). An overview of the *Bombus terrestris* (L. 1758) subspecies (Hymenoptera: Apidae). *Ann. Soc. Entomol. Fr.* **44**, 243–250. doi:10.1080/00379271.2008.10697559
- Sánchez-Echeverría, K., Castellanos, I., Mendoza-Cuenca, L., Zuria, I. and Sánchez-Rojas, G. (2019). Reduced thermal variability in cities and its impact on honey bee thermal tolerance. *PeerJ* **7**, e7060. doi:10.7717/peerj.7060
- Sepúlveda, Y. and Goulson, D. (2023). Feeling the heat: Bumblebee workers show no acclimation capacity of upper thermal tolerance to simulated heatwaves. *J. Therm. Biol.* **116**, 103672. doi:10.1016/j.jtherbio.2023.103672
- Sgrò, C. M., Terblanche, J. S. and Hoffmann, A. A. (2016). What can plasticity contribute to insect responses to climate change? *Annu. Rev. Entomol.* **61**, 433–451. doi:10.1146/annurev-ento-010715-023859
- Shah, A. A., Funk, W. C. and Ghalambor, C. K. (2017). Thermal acclimation ability varies in temperate and tropical aquatic insects from different elevations. *Integr. Comp. Biol.* **57**, 977–987. doi:10.1093/icb/ix101
- Somero, G. N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J. Exp. Biol.* **213**, 912–920. doi:10.1242/jeb.037473
- Soroye, P., Newbold, T. and Kerr, J. (2020). Climate change contributes to widespread declines among bumble bees across continents. *Science* **367**, 685–688. DOI:10.1126/science.aax8591
- Spooner, F. E. B., Pearson, R. G. and Freeman, R. (2018). Rapid warming is associated with population decline among terrestrial birds and mammals globally. *Glob. Change Biol.* **24**, 4521–4531. doi:10.1111/gcb.14361
- Thakur, M. P., Risch, A. C. and van der Putten, W. H. (2022). Biotic responses to climate extremes in terrestrial ecosystems. *Isience* **25**, 104559. doi:10.1016/j.isci.2022.104559
- Weaving, H., Terblanche, J. S., Pottier, P. and English, S. (2022). Meta-analysis reveals weak but pervasive plasticity in insect thermal limits. *Nat. Commun.* **13**, 592. doi:10.1038/s41467-022-32953-2
- West-Eberhard, M. J. (2003). *Developmental Plasticity and Evolution*. New York: Oxford University Press.
- Willmer, P. G. and Stone, G. N. (2004). Behavioral, ecological, and physiological determinants of the activity patterns of bees. In *Advances in the Study of Behavior*, Vol. 34, pp. 347–466. San Diego, CA: Academic Press Inc.