













Global effects of extreme temperatures on wild bumblebees

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Abstract: Climate plays a key role in shaping population trends and determining the geographic distribution of species because of limits in species' thermal tolerance. An evaluation of species tolerance to temperature change can therefore help predict their potential spatial shifts and population trends triggered by ongoing global warming. We assessed inter- and intraspecific variations in heat resistance in relation to body mass, local mean temperatures, and evolutionary relationships in 39 bumblebee species, a major group of pollinators in temperate and cold ecosystems, across 3 continents, 6 biomes, and 20 regions (2386 male specimens). Based on experimental bioassays, we measured the time before heat stupor of bumblebee males at a heatwave temperature of 40 °C. Interspecific variability was significant, in contrast to interpopulational variability, which was consistent with heat resistance being a species-specific trait. Moreover, cold-adapted species are much more sensitive to heat stress than temperate and Mediterranean species. Relative to their sensitivity to extreme temperatures, our results help explain recent population declines and range shifts in bumblebees following climate change.

Keywords: climate change, extreme events, pollinator decline, heat resistance, inter- and intraspecific variability

Efectos Globales de las Temperaturas Extremas sobre Abejorros Silvestres

Resumen: El clima juega un papel importante en la configuración de las tendencias poblacionales y en la determinación de la distribución geográfica de las especies debido a los límites de la tolerancia térmica y al agua que tiene cada especie. Por lo tanto, una evaluación de la tolerancia de las especies al cambio térmico puede ayudar a predecir los potenciales cambios espaciales y las tendencias poblacionales detonadas por el calentamiento global en curso. Evaluamos las variaciones inter- e intraespecíficas de la resistencia al calor en relación con la masa corporal, temperaturas locales promedio y las relaciones evolutivas para 39 especies de abejorros, un grupo primordial de polinizadores en ecosistemas templados y fríos, en tres continentes, seis biomas y 20 regiones (2,386 especímenes machos). Con base en bioanálisis experimentales, medimos el tiempo previo al letargo por calor de los abejorros machos a temperaturas de 40 °C, típicas de una ola de calor. La variabilidad interespecífica fue significativa, en contraste con la variabilidad interpoblacional, lo cual fue consistente con el hecho de que la resistencia al calor sea una característica específica de la especie. Además, las especies adaptadas al frío son mucho más sensibles al estrés por calor que las especies de clima templado o Mediterráneo. En relación con

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la sensibilidad a las temperaturas extremas, nuestros resultados ayudan a explicar las recientes declinaciones poblacionales y cambios en la distribución de los abejorros posteriores al cambio climático.

Palabras Clave: cambio climático, declinación de polinizadores, eventos extremos, resistencia al calor, variabilidad inter- e intraespecífica

Introduction

Since the turn of the 20th century, human activities have triggered global warming, resulting in an average temperature increase of 0.85 °C (Hance et al. 2007). According to most scenarios of the Intergovernmental Panel on Climate Change, this situation is expected to worsen by the end of the 21st century (i.e., up to 6 °C in the worst-case scenario), especially in arctoalpine regions (Fronzek et al. 2012; IPCC 2014). The consequences of global warming are 2-fold: gradual modifications of main climatic parameters (e.g., humidity, temperature) and an increase in the frequency and intensity of extreme and localized weather events, such as heat waves (Easterling et al. 2000; Meehl & Tebaldi 2004). A heat wave corresponds to a period of 2 consecutive days with the temperature higher than 40.6 °C (Robinson 2001; Meehl & Tebaldi 2004). Cold and temperate areas are equally likely to experience heat waves, whereas extreme events are very rare in tropical biomes (Meehl & Tebladi 2004).

Current global warming is affecting ecosystems by displacing local populations, a situation that will eventually lead to extinctions (e.g., Walther et al. 2002; Parmesan 2006; Sunday et al. 2012). Although most studies to date have focused on the consequences of gradual climatic modifications, higher intensity and frequency of extreme events of temperature variation could be a more serious threat than the gradual increase in average temperatures (Hance et al. 2007; Kingsolver & Buckley 2017). Hyperthermic stress, notably induced by heat waves, is associated with physiological perturbations (Parmesan et al. 2000; Hance et al. 2007; Kingsolver & Buckley 2017), mortality (Neven 2000; Parmesan 2006; Kingsolver et al. 2013), and behavioral changes (i.e., stupor, characterized by a critical decrease in motor function and inability to escape from conditions) (Perez & Aron 2020).

We focused on bumblebees (Hymenoptera: *Bombus*), a diversified group of crucial pollinators in temperate and cold areas (Williams 1998). Bumblebees are eusocial insects, having evolutionary origins in cold climates but comprising derived clades in Mediterranean and tropical areas (Hines 2008; Williams et al. 2017). Bumblebee distributions and declines are affected by the interaction between climate and food availability, the effects of which have resulted in negative population trends where

populations are distant from their climatic optimum, especially in warmer areas (Williams 1986; Williams et al. 2007). These endoheterothermic bees display several adaptations to cold climates but few to high temperatures (Heinrich 2005; Pimsler et al. 2020). Over the last decades, strong shifts have been reported in bumblebee populations worldwide (Goulson et al. 2004), at least partially triggered by climate change (Kerr et al. 2015).

Recent research shows that bumblebees exhibit spatial variability in their response to climate change; patterns are heterogeneous among species (Kerr et al. 2015). Bumblebees are more likely to decline where significant warming has occurred; populations living on the margins of their historical distribution are especially affected (Kerr et al. 2015). Although maximum critical temperature seems species specific (Oyen et al. 2016), high temperatures near 40 °C can trigger thermal distress (Heinrich 2005). Until now, studies on bumblebee thermal tolerance have focused on measuring maximum and minimum critical temperatures through examination of a single-species model (Oyen & Dillon 2018) or have explored the effect of elevation on these thermal limits for only 3 species (Oyen et al. 2016). Researchers used temperature-ramping methods with experimental devices set in a central location to which the specimens would be taken after field collection. These highly standardized protocols allow for precise measurements of species thermal limits but have undoubtedly restrained the number of model species and replicates that could be tested, as well as the geographical range from which the specimens can be collected. Moreover, it appears that critical thermal limits depend on ramping rates for dynamic methods (e.g., Overgaard et al. 2006; Oyen et al. 2016). The relationships between heat stress sensitivity in bumblebees, population trends, and species' range are still generally unknown. However, such information could help predict which species and communities are the most exposed to thermal stress under climate change (Ayrinhac et al. 2004; Rezende et al. 2011). Using a portable device, we evaluated the variation in heat resistance (with a static heatwave temperature of 40 °C) of a large set of bumblebee species that live in different habitats at different latitudinal and longitudinal scales and investigated the relationship between heat resistance, body mass, climatic variables, and population trends.

Methods

Sampling Sites and Species

The sampling strategy was to maximize the number of biomes, regions, species, and specimens tested to consider interindividual variability. We tested 39 bumblebee species (2386 males) across 20 regions in 6 biomes (Fig. 2) between 2013 and 2019. When necessary, we used genetic barcoding (cytochrome oxidase I [*COI*] mitochondrial sequences) or cephalic labial gland secretions or both to identify the collected specimens (Martinet et al. 2019; Valterová et al. 2019). Moreover, we tested the interpopulation heat-resistance variability of 6 widespread species for which we were able to study multiple allopatric populations: *B. cryptarum*, *B. jonellus*, *B. lapponicus*, *B. lucorum*, *B. polaris*, and *B. terrestris*. We separated specimens among 6 biotopes based on the climate of sampled stations (Mauser 2004): polar or tundra ($n = 544$), subpolar ($n = 384$), boreal ($n = 266$), mountainous ($n = 352$), temperate ($n = 679$), and Mediterranean ($n = 161$). All information about collecting sites and specimens is in Appendix S1.

We used only males because they display a constant hormonal state. Females have a changing hormonal cycle that can influence their physiology (Heinrich 2005). Males are also more exposed during their flight period (May to September depending on the species) to high air temperatures of extreme climatic events while collecting nectar and conducting their nuptial behavior in sunlight areas (Heinrich 2005). Moreover, they cannot take shelter in thermoregulated underground nests as the females do (Heinrich 2005). However, for some late summer species males may not be exposed to as warm of temperatures (depending on phenology), contrary to workers of these species.

Assessment of Hyperthermic Stress Resistance and Related Factors

Following the protocol established by Martinet et al. (2015), specimens were placed in controlled and constant conditions in an incubator (Herp Nursery II, Lucky Reptile, Waldkirch, Germany) at 40 °C and humidity of 50–60%. The temperature of 40 °C was chosen to simulate a realistic, average heatwave temperature, as recorded in natural environments across more than 90 countries (Russo et al. 2015; Ragone et al. 2018). Moreover, 40 °C is below the CT_{max} (maximum critical temperature) for species in the *Bombus* genus (e.g., mean [SD] = 45 °C [1] for *B. huntii*; 44 °C [0] for *B. bifarius*, and 40 °C [4] for *B. sylvicola* [Oyen et al. 2016]).

The time before heat stupor (THS) was measured for each specimen with a chronometer as an estimator of the heat stress resistance (10 individual tubes at the same time to minimize thermal load effect). The

THS corresponds to the time from the insertion in the incubator after an initiation period of 5 min until the heat stupor. When the specimen was unable to return to normal position even after the vial was turned, it was assumed to be in heat stupor. Once in heat stupor, THS was recorded and the insect was removed from the enclosure to recover at room temperature. We additionally recorded mortality after the experiment. We also tested whether body mass influenced THS because it could be an important predictor associated with heat tolerance. Given that larger individuals could be expected to be more prone to overheating (Heinrich 1975, 2005), we measured both fresh mass (i.e., mass of the living specimen) and dry mass (i.e., mass of the specimen after complete dehydration) (Hagen & Dupont 2013). All technical details and experimental limitations associated with the methods are in Appendix S2.

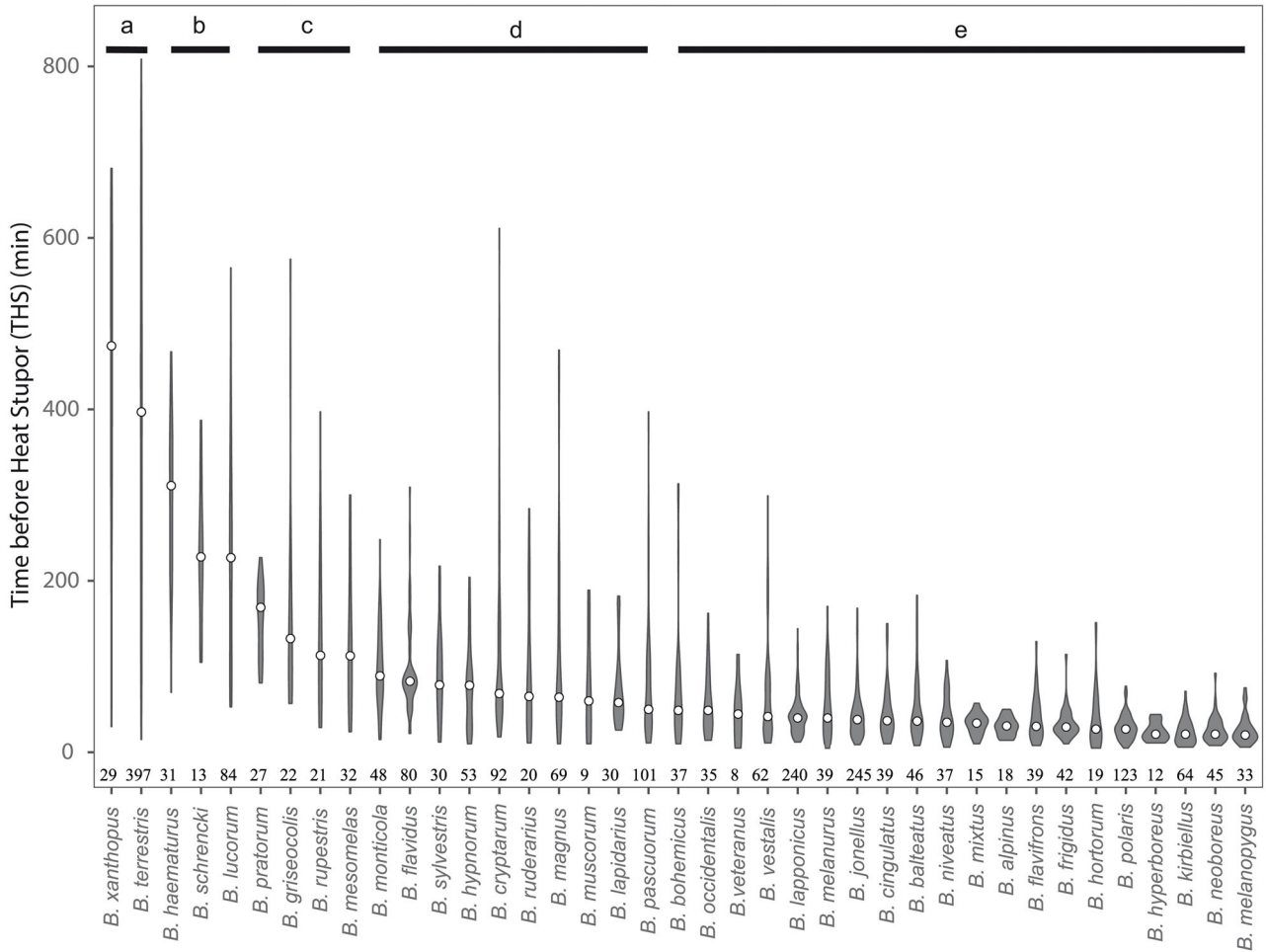
Evolutionary Patterns and Phylogenetic Signals Underlying Heat Stress Resistance

We used a Bayesian approach to investigate the phylogenetic signal associated with THS measures averaged for each species. We started from the Bayesian phylogenetic analysis performed by Cameron et al. (2007) on the *Bombus* genus. We sampled 1000 post-burn-in trees from 3 independent posterior distributions inferred by Cameron et al. (2007). We then used the R package phytools to estimate Blomberg's K statistic, which measures the phylogenetic signal of a trait by comparing the observed signal of this trait with the signal under a Brownian motion model of trait evolution on a phylogeny (Blomberg et al. 2003; Revell 2012). For each tree sampled from posterior distributions, we estimated 2 K values: the K value based on the original mean thermal resistance values and the K value based on mean thermal resistance values permuted among species producing a posterior ($K_{posterior}$) and a null distribution (K_{null}) of K values, respectively. The statistical support associated with the posterior distribution of K was evaluated by comparing it with its corresponding null of distribution and formalized by approximating a Bayes factor (BF) value. The BF support associated with K was approximated by the posterior odds that $K_{posterior} > K_{null}$ divided by the equivalent prior odds (the prior probability for $K_{posterior} > K_{null}$ is 0.5):

$$BF_K = \frac{\left(\frac{p_K}{1-p_K}\right)}{\left(\frac{0.5}{1-0.5}\right)}, \quad (1)$$

where p_K is the posterior probability that $K_{posterior} > K_{null}$ (i.e., the frequency at which $K_{posterior} > K_{null}$ in the samples from the posterior distribution). The prior odds are 1 because we assumed an equal prior expectation for $K_{posterior}$ and K_{null} (Suchard et al. 2005; Dellicour et al. 2017). For the visualization, the maximum clade

(a) Interspecific variability in THS



(b) Intraspecific variability in THS

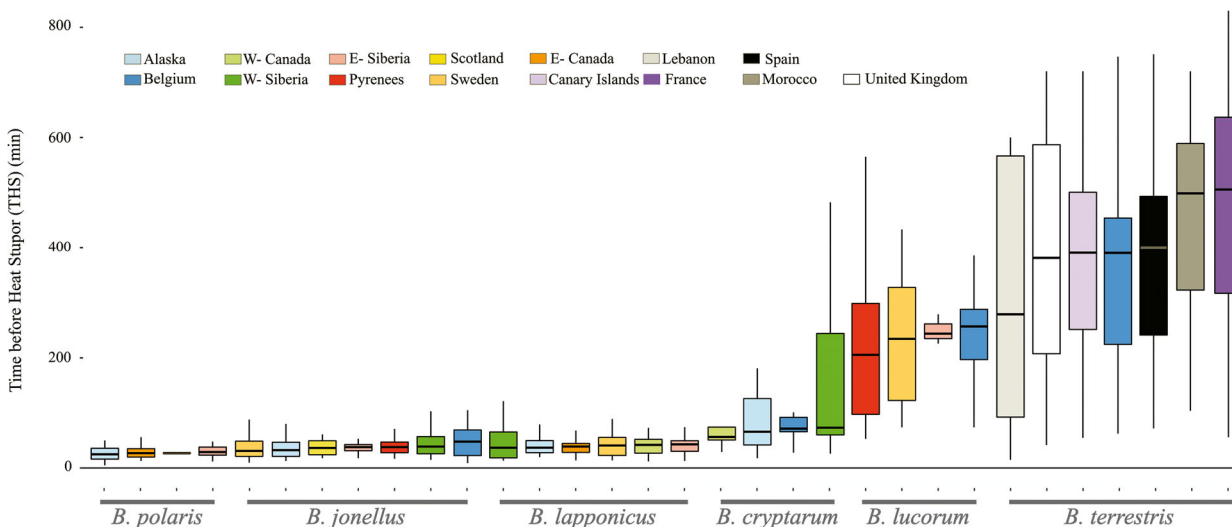


Figure 1. Interspecific variability of heat stress resistance (a) among 39 bumblebee species (letters a, b, c, d, and e, species group supported by a post hoc multiple comparison test [Kruskal-Wallis, $p < 0.05$]; numbers above x-axis line, number of specimens per species; white circles, median) and (b) among 6 bumblebee species at different sampling sites (horizontal lines in boxes, median values; whiskers, minimum, first quartile, third quartile, and maximum).

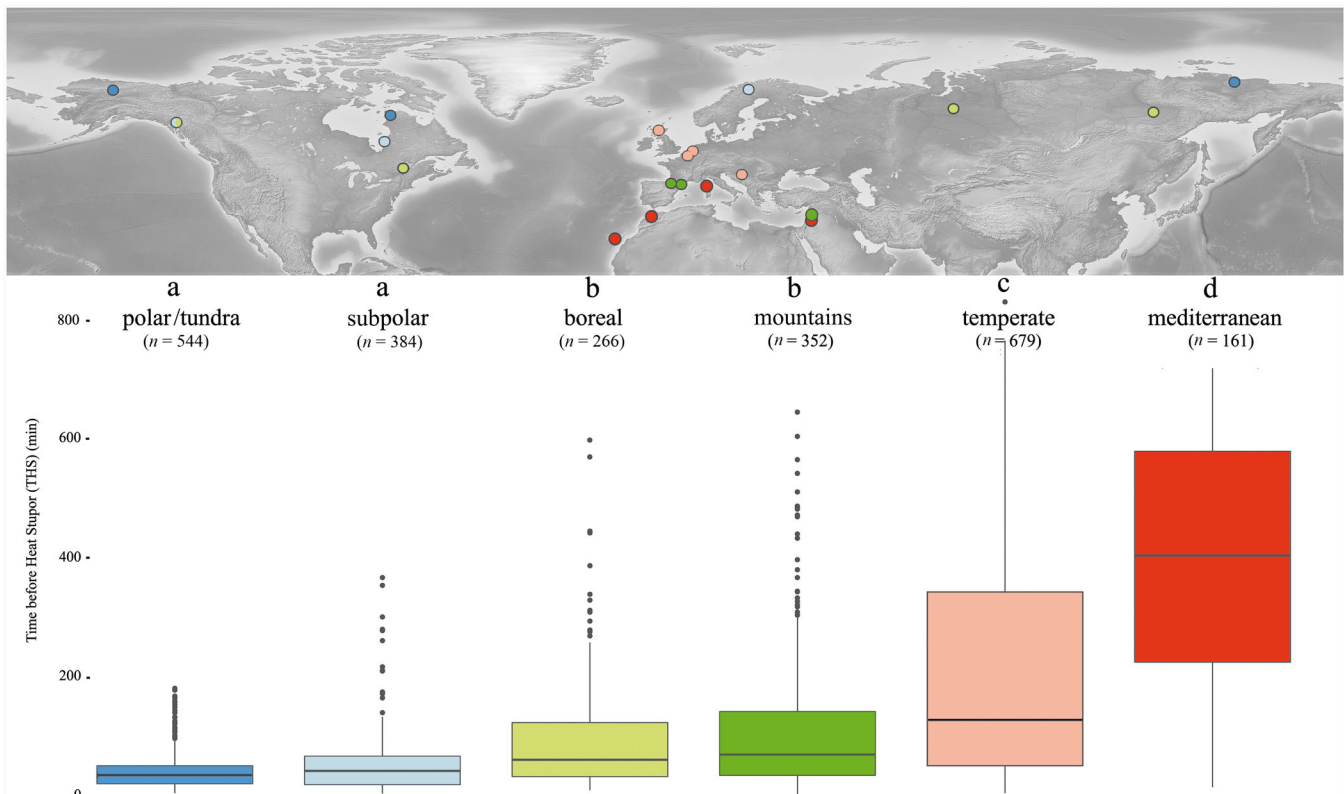


Figure 2. Variation of heat stress resistance in bumblebee individuals across biotopes: polar or tundra (dark blue), subpolar (light blue), boreal (light green), mountains (dark green), temperate (light red), and Mediterranean (dark red). Letters a, b, c, and d represent groups supported by a post hoc multiple comparison test (Kruskal-Wallis, $p < 0.05$).

credibility tree was obtained with TreeAnnotator 1.10 (Rambaut & Drummond 2020).

Statistical Analyses

We performed Kruskal-Wallis tests to compare THS among species and biotopes (pgirmess R package [R Development Core Team 4.0.3 2020]). We then tested the correlation between THS and different response variables: mean annual temperature (mean T°), mean temperature of the warmest quarter (mTWQ), mean temperature of the coolest quarter (mTCQ) at collecting sites, dry weight of specimens, and fresh weight of specimens. We extracted the climatic variables from the WorldClim 2 database (Fick & Hijmans 2017). We first performed a visual exploration of these correlations with a principal component analysis (PCA) and then formally investigated these correlations by performing a linear regression (LR) coupled with a commonality analysis (CA). The LR-CA approach allowed us to estimate both the unique and combined contributions of predictors to the explained variance in the response variable. Unique and combined effects, respectively, represent the amount of explained variance in the response variable (i.e., here

THS). Finally, to investigate the relation between THS and the conservation status of the analyzed species (Nieto et al. 2014), we used the population trend estimation proposed by the International Union for Conservation of Nature (IUCN) to sort sampled species according to their IUCN population trend categories: unknown, decreasing, stable, and increasing.

Results

Variability in Heat Stress Resistance of Bumblebees

We measured THS for 2386 bumblebee males belonging to 39 species (Fig. 1; Appendix S1). In each species, heat stress caused mortality in approximately 50% of the specimens after experimentation. The THS differed significantly among species ($p < 0.01$) (Fig. 1). Five groups exhibited decreasing THS (post hoc Kruskal-Wallis multiple comparison test) (Fig. 1a): *Bombus xanthopus* and *B. terrestris* ($p < 0.01$); *B. haematurus*, *B. schrencki*, and *B. lucorum* ($p < 0.01$); *B. pratorum*, *B. griseocollis*, *B. rupestris*, and *B. mesomelas* ($p < 0.01$); *B. monticola*, *B. flavidus*, *B. sylvestris*, *B. hypnorum*, *B. cryptarum*, *B. ruderarius*, *B. magnus*, *B. muscorum*,

and *B. lapidarius* ($p < 0.01$); and all other species ($p < 0.01$). The species with a distribution range centered on Mediterranean areas, such as *B. terrestris* (median = 397 min), or restricted to this region, such as *B. xanthopus* (median = 474 min), had the highest heat resistance among sampled species. Arctoalpine species, such as *B. polaris* (median = 27 min), *B. hyperboreus* (median = 21 min), and *B. frigidus* (median = 30 min), had the lowest heat resistance, including values up to 10 times lower than *B. terrestris*.

More resistant species also exhibited higher variation in their THS (Fig. 1b). However, none of the 6 tested species showed a significant interpopulation difference ($p > 0.05$) in THS (Kruskal–Wallis test): *B. cryptarum* ($p = 0.1$), *B. jonellus* ($p = 0.25$), *B. lapponicus* ($p = 0.82$), *B. lucorum* ($p = 0.71$), *B. polaris* ($p = 0.39$), and *B. terrestris* ($p = 0.06$). In other words, despite living in different ecoclimates, conspecific populations did not differ from each other in their THS (Fig. 1b).

Factors Related to the Variability of Heat Stress Resistance in Bumblebees

Analyses of aggregate data related to the biotopes showed some significant differences in THS ($p < 0.01$) (Fig. 2). Post hoc Kruskal–Wallis tests revealed 4 groups ($p < 0.05$) along a latitudinal and elevational gradient: specimens collected in polar and tundra and subpolar biotopes, specimens collected in boreal and mountain biotopes, specimens collected in temperate biotopes, and specimens collected in Mediterranean biotopes. Arctic and boreal species were characterized by a very low heat stress resistance; median THS was 20 and 40 min, respectively. Temperate and Mediterranean species had the highest heat resistance: THS median of 150 and 400 min, respectively (see Appendices S1 and S3 for the biotope of each specimen).

The phylogenetic signal associated with average THS values was supported only by an approximated Bayes factor of 6.81. According to the scale of interpretation, a Bayes factor >3 but <20 can only be interpreted as a positive but not as a strong statistical support (Appendix S4). Therefore, we concluded that there was at least a positive but poorly supported heat-resistance pattern related to phylogenetic relationships among tested species.

Fresh mass was measured for 1592 specimens before experiments and dry mass for 1276 specimens after experimentation (Appendix S1). Fresh body mass and dry mass varied significantly among species ($p < 0.01$) but not at the interpopulation level ($p = 0.19$). As highlighted by the PCA (Fig. 4), dry and fresh weights appeared to be poorly correlated with THS, contrary to the different temperature variables that appeared logically and notably correlated with each other. This trend was consistent with the results of the CA performed on the following linear regression (LR): THS \sim mTWQ +

dry weight + fresh weight (global $R^2 = 0.47$, $p < 0.01$). Because the PCA confirmed that the 3 temperature variables (mean T° , mTWQ, and mTCQ) were highly correlated to each other, we only considered 1 of these measures in the LR-CA. We selected mTWQ because it corresponds to the average temperature occurring during the flying season of bumblebee males. For the different explanatory variables, we obtained the following unique (U) and combined (C) contributions to the global R^2 : mTWQ, $U = 0.251$, $C = 0.161$; dry weight, $U = 0.001$, $C = 0.035$; and fresh weight, $U = 0.033$, $C = 0.158$. Therefore, among the tested predictive variables, only the mean temperature of the warmest quarter appeared to explain a significant part of the variability in THS.

Relationship Between Population Trends and THS

Analyses of aggregate data related to the Red List status (following IUCN criteria) showed significant differences between species displaying a positive population trend and species with a negative, stable, or unknown trend ($p < 0.01$) (Fig. 5). However, the category of species with positive population trends could have been heavily driven by *B. terrestris*. No significant difference was detected between declining, stable, and unknown categories ($p > 0.20$). Species associated with high THS values tended to increase their relative abundance and their geographical range, whereas species in regression were associated with low THS. There was no specific pattern for stable species, which showed a large range of THS (Fig. 5).

Discussion

At high temperatures, organisms lose neuromuscular function (i.e., heat stupor), making them sensitive to environmental challenge or unable to fill their biological functions (Huey & Kingsolver 1989; Goller & Esch 1990; Overgaard & MacMillan 2017). Although bumblebees may regularly experience extreme temperatures during heat waves when foraging outside (for workers) or during their nuptial behavior (for males), few researchers have evaluated resistance to heat stress to date (Martinet et al. 2015; Oyen et al. 2016). We found at large geographical scale a very high interspecific variability but a very limited interpopulational variability in heat stress resistance (Fig. 1). At the community level, heat waves could therefore be mostly detrimental for thermally sensitive bumblebee species by inducing strong disturbances due to hyperthermic stress.

Inter- and Intraspecific Variability of THS and Biotope Effect

Variability in heat stress resistance was high, ranging from 20 min for arctic species (e.g., *B. polaris*) to

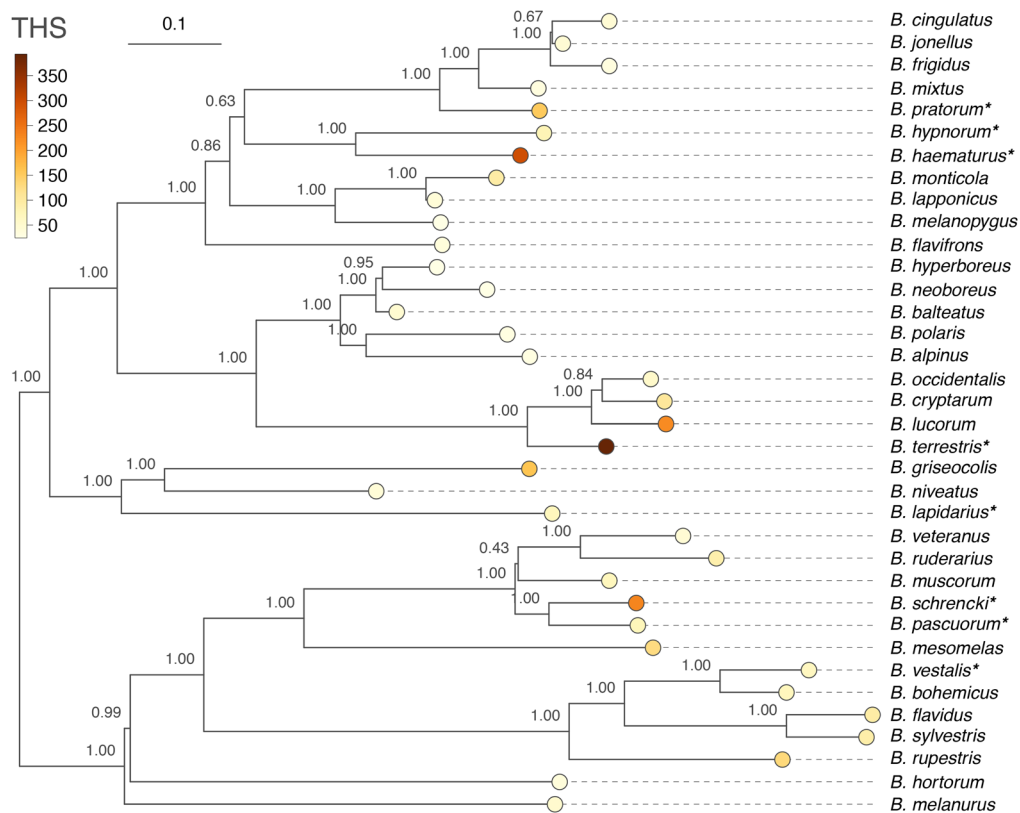


Figure 3. Bayesian phylogeny of target *Bombus* species. Maximum clade credibility (MCC) tree obtained from posterior trees inferred by Cameron et al. (2007) (see text for details). Tip nodes are colored according to the minimum thermal resistance (THS) averaged for each species. Posterior probabilities are reported for each internal node in the tree. An asterisk indicates species is associated with a positive population trend (as defined by the International Union for Conservation of Nature [Nieto et al. 2014]).

more than 10 h for Mediterranean ones (e.g., *B. terrestris*). Such a large interspecific variability in heat resistance has also been observed in other morphologically homogeneous groups of animals (e.g., Tomanek 2010; García-Robledo et al. 2016). Heat stress resistance variations could reflect the diversity of habitat and therefore the ecoclimatic constraints encountered in specific geographic distribution (Rasmont & Iserbyt 2012). Our results suggest that the more the geographic distribution of a species is restricted to the north or in high altitudes, the less resistance they have to hyperthermic stress. This could be explained based on simple selection mechanisms. Because high temperatures in the Arctic region constitute a recent climatic disruption, no specific selection for heat shock gene could have occurred there, in contrast with genes involved in resistance to the cold (Liu et al. 2020). Although genetic structure is known to occur among conspecific populations (Lecocq et al. 2017; Ghisbain et al. 2020), species with a wide distribution are exposed to more variable climatic parameters and may be less sensitive to modified ecoclimatic conditions (Kingsolver & Buckley 2017). Our results confirm this hypothesis. The widespread *B. terrestris* and *B. lu-*

corum (Rasmont et al. 2015) exhibited a lower heat sensitivity than spatially restricted species belonging to the phylogenetically close *B. magnus* or *B. cryptarum*. For *B. xanthopus*, although its distribution is insular (Corsica), the wide biotope diversity encountered in its geographical range could explain its especially high heat resistance (Rasmont & Adamski 1995). Overall, our results suggest that heat tolerance varies a lot among bumblebee species, a trend that can be associated with the extreme diversity in their habitats. Therefore, tolerance to extreme temperatures is likely a limiting factor in their distributions.

In contrast with the interspecific analysis (Fig. 1a), our results depicted a low variability in heat stress resistance among conspecific populations (Fig. 1b). Recently, Pimslar et al. (2020) described the same pattern in *Bombus vosnesenski*: no significant regional differentiation in gene expression at intraspecific level under high temperatures exposure (CTmax). However, for the most widespread species, *B. terrestris*, which lives in habitats ranging from warm to cool temperate climates, our results showed a statistical marginal significance, in contrast to the other studied taxa with a more restricted

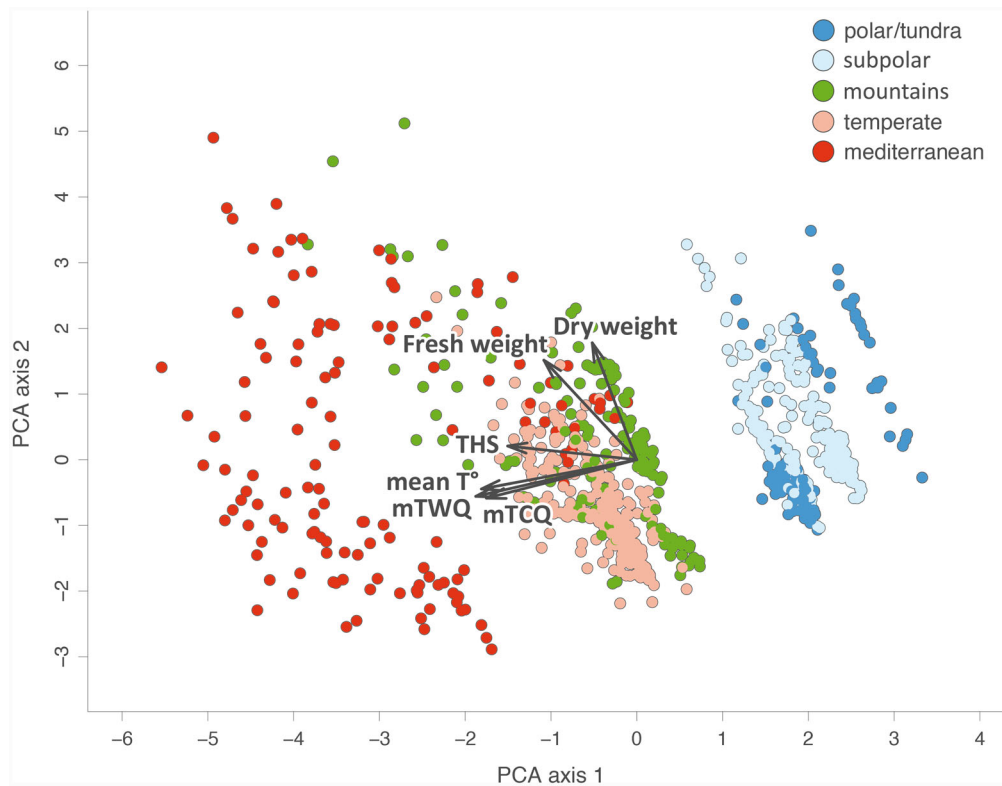


Figure 4. Principal component analysis (PCA) of the time before heat stupor (THS) and several covariables (mean T , mean annual temperature of collecting site; $mTCQ$, mean temperature of the coolest quarter; $mTWQ$, mean temperature of the warmest quarter; weight, tested specimen weight; colors, biotope where the specimen was collected; arrows, different variables in the PCA and their contribution to principal axes 1 and 2).

geographic range. Overall, these results underline that bumblebees are cold-adapted organisms with local adaptation probably more tuned on cold tolerance across a species range, whereas their ability to adapt to high temperatures seems to be more limited, which explains their high sensitivity to climate change.

At the interindividual level, differences in THS between biological units may reflect innate individual variation in heat resistance (Oyen & Dillon 2018). Thermal limits may also differ between individuals based on their own evolutionary or developmental histories (Bertheaux et al. 2004). For some populations, previous selection (through recent extreme climatic events) of resistant individuals could explain the low interindividual variability. On the contrary, high interindividual variability in THS (e.g., observed in *B. terrestris* or *B. lucorum*) could be explained by a larger intrapopulation genetic diversity or a mechanism behind the ability of these species to occupy more diverse climatic zones.

Phylogenetic Signal in Heat Resistance

We did not find a strong phylogenetic signal associated with THS (Fig. 3; Appendix S4), suggesting evolutionary patterns that were independently acquired

among species. The presence of a weakly supported phylogenetic signal could be influenced by groups of closely related species distributed within the same habitat (e.g., all *Alpinobombus* species limited to arctic areas and all displaying low THS) (Figs. 2 & 3), in contrast to other more widespread groups (e.g., *Pyrobombus*) in which closely related species are associated with very different THS values and different habitats. *Pyrobombus* and several other bumblebee lineages can drastically vary in their climate occupancy, with sister lineages separated by vicariance adaptations that could relate to climate adaptation (Hines 2008).

Heat Resistance and Body Mass

Given the biophysics of heat transfer and the relation of the insect's mass and heat resistance (Bergmann 1847; Heinrich & Heinrich 1983), larger organisms are generally expected to be less able to thermoregulate at high temperatures and should therefore be more sensitive to hyperthermic stress. Previous work on 3 bumblebee species suggests that only 1 species (*B. buntii*) shows a relationship between thermal limits and fresh body mass (Oyen et al. 2016). According to this study, CT_{max} increases with body mass but this relation is the opposite

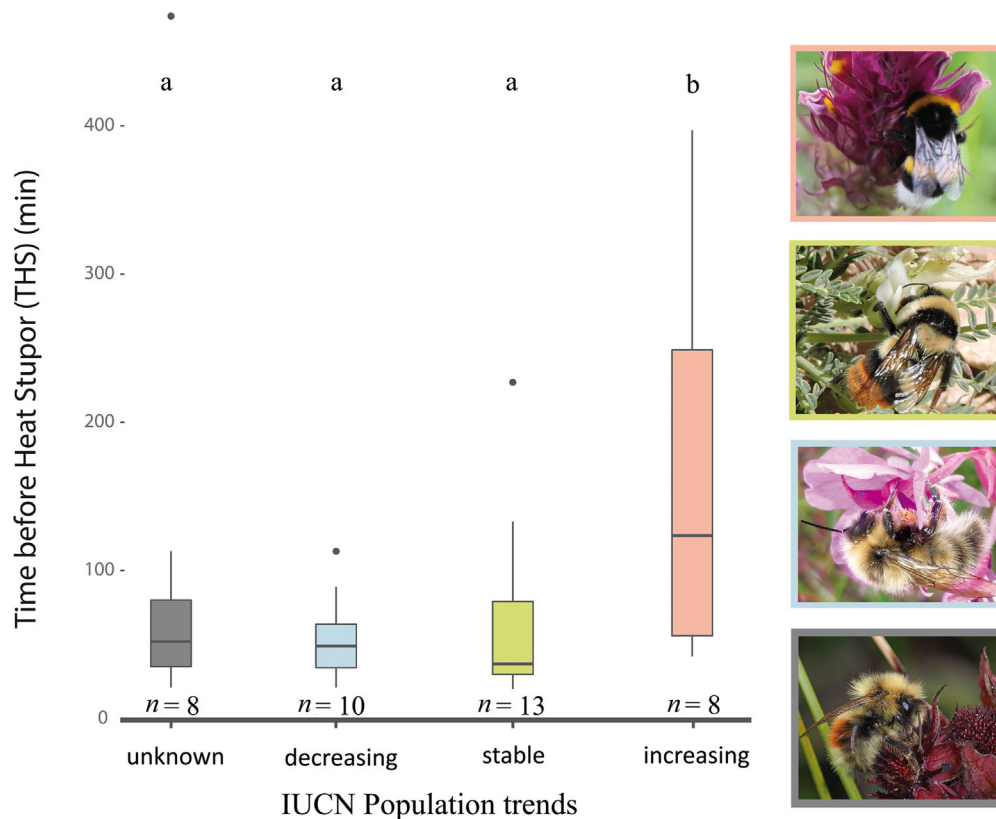


Figure 5. Relation between the heat resistance (THS) of bumblebee species and population trend status as defined by the International Union for Conservation of Nature (IUCN [Nieto et al. 2014]): decreasing, stable, increasing, or unknown evolution of population size (numbers above x-axis line, number of species per category; gray, *Bombus lapponicus* [P. Rasmont, Chokurdakh, Russia]; blue, *Bombus polaris* [P. Rasmont, Toolik, Alaska, U.S.A.]; green, *Bombus niveatus* [M. Boustani, Lebanon]; red, *Bombus terrestris* [M. Folschweiller, Belgium]; letters above boxes, groups supported by a post hoc multiple comparison test [Kruskal–Wallis, $p < 0.05$]; horizontal lines in boxes, median; whiskers, minimum, first quartile, third quartile, and maximum values; dots, extreme values). Each sampled species is sorted by its International Union for Conservation of Nature status.

to that of the heat transfer rule (Bergmann 1847; Heinrich & Heinrich 1983). In the same study, the low elevation species tolerate hotter temperatures than the high elevation species despite their body mass (Oyen et al. 2016). Our study at the continental scale did not confirm these preliminary results because our heat resistance estimator was consistent across bees varying in body mass. The THS variation was poorly related to dry mass considering all data gathered (Fig. 4). Interestingly, our linear model showed that fresh mass explained a larger part of the variance of THS than dry mass. Fresh mass reflects the hydration of organisms, and this parameter could have a significant effect on heat stress resistance, as observed in other groups (Leiva et al. 2019). In the field, bumblebees may regularly experience differences in the nutritional quality of their resources (Vanderplanck et al. 2014). Poor nutritional quality alters foraging activity (Pankiw et al. 2004) and influences physiological conditions (Stabler et al. 2015), whereas high-quality diets could have a buffering effect of heat stress on

colonies (Vanderplanck, Martinet et al. 2019). In other insects such as fruit flies or beetles, feeding raises CT_{max} by increasing the body mass of the organism or by improving nutritional or hydration status (Nyamukondiwa & Terblanche 2009; Chidawanyika et al. 2017).

Heat Resistance and Population Trend

Our results highlight that expanding bumblebee species (Nieto et al. 2014; Rasmont et al. 2015), such as *B. baematurus* and *B. schrencki*, present a particularly high resistance to heat stress (Fig. 5). These species live in areas with a continental climate and are therefore naturally exposed to a large range of temperatures during their life cycle (Rasmont et al. 2015). Their recent expansion could be partially explained by an evolutionary selected ability to tolerate extreme temperature variations. Although our results emphasize only a significant relationship between heat stress resistance and positive population trend, the rarity or decline of

species such as *B. polaris*, *B. jonellus*, and *B. hyperboreus* could at least be partially explained by their low THS. Following species-specific physiological characteristics, some species could naturally present a higher plasticity (e.g., activation of transcriptional cascade of heat shock response) and phenological or behavioral adaptations, which would reduce the deadly effect of heat stress (Valladares et al. 2014). This could explain the different responses to our thermal resistance test between species with a similarly widespread geographic distribution, including species living in diversified habitats. For some of the studied species (8 of 39), the available data to formally assess population trends are insufficient (“unknown” [Nieto et al. 2014]). Although the unknown category may not be included in formal analyses (Arbetman et al. 2017), we still applied this classification because our results showed that the majority of these taxa had a low THS, indicating the urgency of obtaining data for a monitoring and a safeguarding management of these key pollinators.

In the context of current and future climate change, a reaction through a physiological or biochemical response (i.e., resilience, acclimation capacity) to stressful environmental temperatures may facilitate species conservation in transformed ecoclimatic environments (Stillman 2003; Somero 2010; Gunderson et al. 2017). Interspecific differences in thermal tolerance could be a key factor in explaining and predicting climate-driven range shifts of pollinators. With our measurements of hyperthermic stress resistance in bumblebees across many populations and species, we have provided valuable insights into recent population declines and range shifts (Kerr et al. 2015). We suggest that shifts in the distribution of bumblebee species could be partly driven by their heat stress resistance during heat wave events. Our empirical results should be integrated in models facilitating mechanistic predictions of the climate change effects on future distributions of these vital pollinators. Limited local adaptations observed in bumblebees represent a critical factor in the physiological threshold that could make certain species very sensitive to environmental changes.

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Author Contributions

B.M., S.D., and P.R. conceived and designed the experiments. B.M., E.Z., M.B., and K.P. conducted the experiments. B.M. and S.D. analyzed the data. B.M., S.D., G.G., E.Z., K.P., T.L., M.B., R.B., D.M. and P.R. wrote the paper.

Supporting Information

Additional information is available online in the Supporting Information section at the end of the online article. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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