

Introduction

The current worldwide biodiversity undergoes one of the greatest mass species extinction in earth's history [1]. The **biodiversity decline** results from numerous interacting factors. Among these factors, **climate change** has been pointed out as one of the major causes of extinction in several groups of organisms [2]. Climate change is related to an increase of frequency of extreme event such as **heat waves** [3].

Bumblebees are robust and hairy bees with **hetero-endotherm metabolism** [4] that enable them to live in some of the highest-elevation and most northern ecosystems. Their hotspot species diversity areas (mountains, Arctic, Subarctic and Boreal regions) are also the hardest regions hit by climate change [5].

The goal of this study was to develop a new experimental device to determine the **heat stress resistance** under **hyperthermic stress** of small insects in field lab. We tested our device and approach on different bumblebee species in order to predict consequences of heat waves.

Material and Methods

We sampled 144 males belonging to five different species from Eastern Pyrenees and North of Scandinavia : two taxa with an **arctic distribution**: (*B.alpinus* [n=16], *B.balteatus* [n=22] (Fig.4), both belonging to *Alpinobombus* subgenus, [6]); three **boreo-alpine** (mountainous) taxa (*B.(Pyrobombus) monticola scandinavicus* from Sweden, *B.monticola rondoui* from Pyrenees [n=45], *B.(Psithyrus) flavidus* [n=31]) and one **widespread and ubiquitous** species (*B. (Bombus s.s.) lucorum* [n=30]). We used **only males** as they display simple and constant behaviour and they normally do not take shelter in thermoregulated underground nests as the females could do [7]



Figure1: Picture of the experimental device (HerpNursery II)

After sampling, specimens are placed in a fridge at 8°C (standby temperature according to Heinrich (1972) [7]) during one day. After 24 hours, insects were placed individually in breakthrough Petri dishes. The Petri dishes with specimens were placed in the **incubator** (Herp Nursery II®, Fig.1) at 40°C where temperature and humidity were controlled . Each Petri dish was flipped over at regular intervals (1-2 minutes) to check if the specimen was able to flip from up-down to normal position. When specimens became no more able to return in normal position, they have been assumed to be in "**Heat Stupor**".

An insect is said to be entering into "heat stupor" [8,9] when it falls on its back, is unable to turn, and loses its normal reflexes [10].The extremities are then shaken by muscle spasms [11] that appear just before death [9,12] (Fig.3). The **Time before Heat Stupor** (THS) is measured for each specimen tested.

Results

B. lucorum, which is the more **ubiquitous** species, has the **longest THS** (median = 242 minutes) while other species stretch from Boreo-Alpine taxa (intermediate THS: *B. monticola* and *B. flavidus*) to species with a centred arctic distribution (low THS: *B. alpinus* and *B. balteatus*) (Fig.2).

Statistical tests confirm these results: arcto-alpine species (*Alpinobombus*) are characterised by a very low heat stress resistance (t-test Welch, *p-value* >0.01, Tab.1) while boreo-alpine species (*B.monticola* and *B.flavidus*) have a higher heat resistance (t-test Welch, *p-value* <0.01, Tab.1) than arctic species but a weaker heat resistance than a widespread and ubiquitous species as *B.lucorum* (t-test Welch, *p-value*<0.01, Tab.1).

Table 1: Values of the Welch two sample t-test to compare the heat stress resistance (THS) of different bumblebee species. Only the *p-values* < 0.01 were considered significant (Bold). For the two distinct allopatric populations (*Bombus monticola rondoui* and *Bombus monticola scandinavicus*), only the comparison between these two populations was performed.

Species	<i>B. balteatus</i>	<i>B. lucorum</i>	<i>B. flavidus</i>	<i>B. monticola ssp.</i>	<i>B. monticola rondoui</i>
<i>B.alpinus</i>	t = -0.11 p-value= 0.92	t = -13.07 p-value<0.001***	t = -9.43 p-value<0.001***	t = -9.58 p-value<0.001***	NA
<i>B. balteatus</i>		t = -11.07 p-value<0.001***	t = -7.06 p-value<0.001***	t = -7.50 p-value<0.001***	NA
<i>B. lucorum</i>			t = 7.92 p-value<0.001***	t = 6.22 p-value<0.001***	NA
<i>B. flavidus</i>				t = 1.71, p-value= 0.092	NA
<i>B. monticola scandinavicus</i>	NA	NA	NA	NA	t = -0.80, p-value= 0.43

Heat stress resistance of different bumblebee species

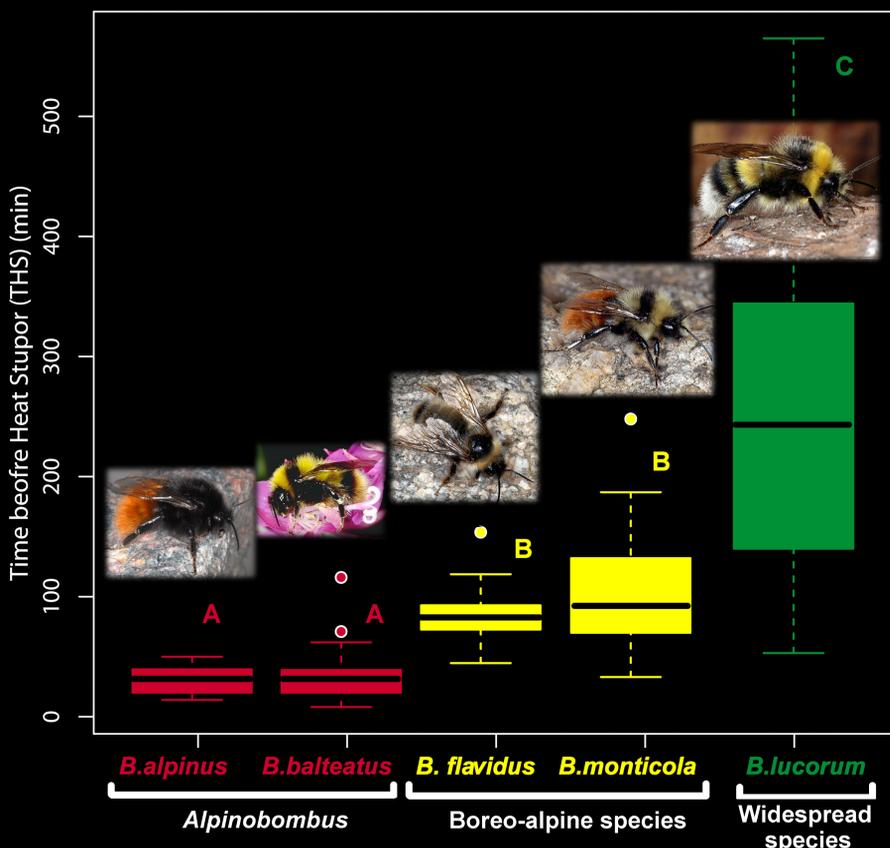


Figure 2: Boxplots of the time before heat stupor (THS) for five bumblebee species: Arctic centred species (A): *Bombus alpinus* and *Bombus balteatus*; Boreo-Alpine species (B): *Bombus flavidus* and *Bombus monticola*; Widespread species (C): *Bombus lucorum*. Circles = extreme values

Discussion

According to the Bergmann's rule [13], the **arctic species** *B.alpinus* and *B.balteatus* are heavyweight bumblebees. They are also characterised by a **low heat stress resistance** (Fig.2). *B. monticola*, which shows a large **boreo-alpine** distribution, is a lightweight species with a **higher heat stress resistance**, as expected according to **Bergmann's rule**.

The similar low heat stress resistance between *B.alpinus* and *B.balteatus* could result from their closely phylogenetic relationship [6] or from their identical eco-climatic constraints. *B.flavidus*, which is likely the cuckoo species of *B.monticola* [14], does not have a significant different heat stress resistance compared to its host (*B.monticola*).

Our results show that there is **no difference** in heat stress resistance **between the two allopatric populations** of *B.monticola* (Tab.1).

Conclusions

This experimental device allows an estimation of the **heat stress resistance** of insects *in natura*. This provides a **practical protocol**, especially in the context of the current climate changes.

These results suggest that **heatwaves** could quickly lead to **fatal consequences** for bumblebee species (e.g.*Alpinobombus*), as it has been suggested by Rasmont & Iserbyt (2012) [15].



Fig.3: Scan this QR-code to access the video of Heat Stupor in bumblebees



Fig.4: *Bombus balteatus* (Dahlbom, 1832)