Heat waves: Stupor in Arctic Bumblebees
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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-endothermic 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 rondouli from Pyrenees [n=45], B.(Pstyrus) flavius [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].

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 III, Fig.1) at 40°C where temperature and humidity were controlled. Each Petri dish was tipped 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. flavius) 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.flavius) 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).

Discussion
According to the Bergmann’s rule [13], the arctic species B.alpinus and B.balteatus are heavy weight bumblebees. They are also characterised by a low heat stress resistance (Fig.5). 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 ecoclimatic constraints. B.flavius, 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 allopatic 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].