

The influence of pile length and body size on rates of heat loss in the bumble bee, *Bombus vosnesenskii*

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Introduction

Individual bumble bee species can exist at a wide range of latitudes and altitudes, exposing themselves to extreme temperature ranges. As heterotherms, bees are required to deal with a variety of thermal demands. They must be able to deal with extreme ambient temperatures, but their production of metabolic heat required for flight adds more complex demands. They need to be able to warm themselves quickly to ensure mobility and retain that heat to improve efficiency, but sometimes need to facilitate heat loss to avoid overheating. Thermal biology becomes especially important during the intermittent flights required for foraging. As soon as a bee lands on a flower it begins cooling to a point that will make it unable to fly. To continue flying between flowers bees will have to maintain an elevated body temperature while feeding or rewarm themselves after feeding. Both of these strategies have been shown to be significant energetic costs for heterothermic insects, and these costs have measurable consequences on the foraging decisions bees make (Nieh et al. 2006, Waddington 1990, Heinrich 1972). When facing thermal challenges bees will have to respond either by shifts in morphology or behavior.

Foraging bees have been shown to adjust behavior in response to different conditions. When feeding from flowers with more concentrated nectar, bees are more likely to maintain high body temperatures while foraging to increase foraging speed (Nieh et al. 2006, Waddington 1990). Bees also may choose not to maintain the body temperature necessary for flight and instead to choose to walk between flowers. Heinrich (1983) showed that smaller bees and bees foraging at cooler temperatures are less likely to thermoregulate while foraging and more frequently choose to walk between flowers. While these behavioral strategies could facilitate foraging in the diverse temperatures bumblebees encounter, morphologies that decrease cooling rates could decrease energetic costs and increase foraging rates in cool temperatures, increasing colony growth.

One of the most fundamental responses to decreasing temperatures is increased body size as originally put forward by Bergmann (1847). Increased body size decreases the surface area to volume ratio which in turn decreases heat loss. This pattern has been shown to improve the

thermoregulatory ability of large bees (Bishop and Armbruster 1999). The predicted size variation across environmental gradients has been investigated in bumblebees with mixed results. Classen et al. (2017) found that on gradients going up Kilimanjaro there were fewer large bee species, but the species that persisted across broad gradients were larger at higher elevation. Peat et al. (2005) studied body size of bees from the tropics to Northern Europe. When grouped by habitat temperature (cold, hot, or temperate) they found that bees from hot climates were the largest and bees from temperate climates were the smallest. It's possible that body size is under too many other selective factors (resource availability, foraging efficiency, aerodynamic demands) to show strong responses to temperature.

Beyond body size, bumblebee's thermal performance could also depend on their abundant pile. Unlike body size, pile length is probably not under as intense and varied selection as body size. Pelage is a relatively rare character among insects that has not been well studied. The variations and benefits of fur in *Colias* butterflies has been well documented across environmental gradients; however, a similar body of work for *Bombus* is lacking (Kingsolver 1983). Several studies have found the expected pattern in pile length across environmental gradients; in cooler climates bees have longer hair (Peat et al. 2005, Peters et al. 2016). However, these studies do not quantify the benefits of pile but assume it's benefits based off a small body of previous work (Morgan and Heinrich 1987, Church 1960). The thermal effects of bumblebee pile have rarely been measured and all previous measurements have only compared cooling rates of bees with and without hair. These measurements show that pelage has a significant effect on a bee's cooling. However, complete lack of hair is not the most ecologically relevant metric. Given the variation that has previously been measured across bee populations, it seems that the next logical step would be to measure the consequences of variation at that scale.

Furthermore, while variation in pile length has been documented in field caught bees, it's unclear whether these patterns are genetically or developmentally determined. The developmental conditions an insect experiences can have dramatic effects on its adult morphology (Atkinson, 1994). It's possible that plasticity in pile length serves as a way for bees to respond to environmental changes within a generation. This would allow colonies to tune the thermal biology of developing workers to current temperatures, improving foraging efficiency. Tuning workers to forage in current climatic conditions would be an incredible display of developmental plasticity and could buffer bumblebees from the effects of climate change.

Here we describe a common garden study quantifying the effects of variation in pile length and body size across altitudinal and latitudinal gradients on a wide spread bumble bee's (*Bombus vosnesenskii*) cooling rates. We also compare findings for common garden reared bees with data for field collected bees from across the same gradient to determine the effects developmental conditions have on hair length and body size.

Methods

Field captured workers

To measure the variation in worker morphology under the environmental conditions experienced across the bee's range, *B. vosnesenskii* workers were captured from the field in the summer of 2015 through opportunistic hand netting. After capture, bees were assigned a unique ID number, massed, then placed on ice until transfer to a -20 °C freezer. Accessible capture sites with abundant workers were selected that thoroughly covered altitudinal and latitudinal gradients.

Common Garden Colonies

To determine if morphological variation was determined by genetics or developmental conditions we reared bees from queens captured across altitudinal and latitudinal gradients in common garden conditions. *B. vosnesenskii* queens were caught in the spring of 2016 prior to them establishing nests. Queens were netted while foraging and placed in a cooler until they were transported to the USDA-ARS Pollinating Insect Research Unit's (PIRU) (Logan, Utah USA). Capture locations ranged in latitude from 36.122° N to 45.826° N and in altitude from 60m to 2,187m. **(fig1)**.

At PIRU, queens were placed in 2.25L plastic queen initiation box (Biobest, Leamington, ON) and encouraged to begin colonies following a modified version of the methods described by Evans et al. (2007). After being assigned unique identification numbers queens had access to 500mg of pollen and *ad libitum* nectar provided in a 60ml reservoir. The initiation boxes were then moved to a dark room held at 27 ± 1 °C and 55-60% relative humidity. Queens were monitored for signs of nest establishment and once five workers were enclosed the colony was moved to a 7.75 L plastic hive box (Biobest, Leamington, ON). Once a colony had at least 20 workers it was transferred to facilities at University of Wyoming for use in experiments. Throughout the colonies' lifespans they were monitored and managed to ensure colony health and growth.

Measurement of cooling rates

To determine cooling rates, we massed euthanized bees and then inserted 36-gauge T-Type thermocouples 2mm into their thoraces. Using a heat gun (3 stage heat gun, Milwaukee Tool, Brookfield, WI USA), we heated bees to ~45 °C and placed them in glass chambers (7.8cm long, 1.7 cm diameter) attached to an air pump with a flow rate of 100 ± 6 ml/min. This flow rate exposed the bees to average wind speeds of 0.0073m/s. We left bees to cool until they were near

ambient temperature, logging their temperature using a thermocouple data logger (Pico Technology USB TC-08, St. Neots, United Kingdom) and then removed them from the chambers and massed them again.

We constructed cooling curves (temperature vs. time) for each bee. Erroneous noise can occur in thermocouple data due to bumping the bee, shifts of the bee's body, or electrical noise. To ensure such noise would not affect our data we did not analyze curves with erroneous noise that would lead to incorrect calculations of cooling rates. For each cooling trial we extracted and analyzed the minute of cooling starting 30 seconds after peak temperature. This 30 second delay ensured that we only analyzed data from bees that were fully settled in the test chambers. From the resultant cooling curves, we estimated standardized cooling rates by taking the slope of a trendline fit to the relationship between $\ln((T_{\text{core}} - T_{\text{ambient}})/(T_{\text{initial}} - T_{\text{ambient}}))$ and time (**fig2**). This slope provides a standardized cooling rate that is independent of body temperature or ambient conditions.

Morphology

Intertegular width (ITW) was used to estimate the size of bees as it is a robust indicator of body size that does not vary with feeding status or drying (Vogt and Dillon 2013). ITW was measured on images of bees' thoraces from under a dissecting scope with the tegula level with a 5mm microruler. In ImageJ or LabOptix we determined the proper scaling for the image by measuring the microruler in pixels. Then, we measured the distance between the bee's tegula and converted it to millimeters using the known scaling factor.

To get pile for length measurements we used forceps to pull hairs from directly in front of the bee's right tegula and then taped those hairs to microscope slides. Pictures of the hair bunches were taken using the dissecting scope. Proper scale was determine by taking a picture of a 1mm microruler and then not adjusting the camera's magnification or focus between that picture and the hair images. For each bee, 5 hairs were randomly selected and measured in ImageJ or LabOptix.

Data analysis

All data were analyzed in R version 3.5.2 (The R foundation, Vienna, Austria) via Rstudio version 1.1.463 (Rstudio, Boston, MA). The relationship between pile length and cooling rate was determined by simple linear regression. The relationship between body size and cooling rate was found with regression including both a linear and a squared term to account for the non-linear effect of body size on cooling rate. When analyzing the relationship between pile

length and capture site temperatures we normalized pile length by intertegular width to eliminate the combined effects of body size variation by site and body size's impact on pile length.

Climate data for capture sites was extracted from WorldClim version 2 (Fick and Hijmans, 2017). Trends were analyzed using the weighted average minimum July temperature from 1960-1990 in a 5km radius zone around the capture site.

Results

Increases in body size and pile length both significantly decreased cooling rates. Pile length varied over three fold across common garden reared workers and decreased cooling rates in a linear fashion ($p < 0.001$, $r^2 = 0.09$, $n = 483$). Body size had a stronger and nonlinear effect on cooling rates. The best fit line included negative linear and positive squared terms (both $P < 0.001$, $R^2 = 0.31$, $n = 483$). Pile length increased slightly with body size ($P < 0.001$, $r^2 = 0.02$), so to ensure pile length reduced cooling more than was explained by body size alone we also regressed the residuals of the body size cooling rate relationship with pile length and still found significant results ($P < 0.001$, $r^2 = 0.05$). **(Fig. 3)**

The morphometrics' relationship with minimum July temperature at queen's capture site was the opposite of our prediction. Average body size and normalized pile length both increased significantly with increasing July minimums. This trend held true for cooling rate's relationship with temperature as well. Bees reared from warm climate queens had lower average cooling rates than bees from cold climate queens. **(Fig4)**

For field caught workers, normalized pile length increased with increasing July minimum temperatures as it did in common garden reared bees. Body size, however, did not. The relationship between body size and July minimum for field captured bees proved to be relatively flat **(Fig 4)**.

Discussion

Body size and pile length increases both had the expected effect on cooling rate. Body size's effect has been recorded before for a multitude of species including bumblebees (Church 1960, Bishop and Armbruster 1999). This is, however, the first measurement of piles effectiveness at levels of variation that are actually encountered in field settings. Despite numerous studies measuring variation in pile length across environmental gradients, no studies have quantified the effect of variation at that level (Peat et al. 2005, Classen et al. 2017). The importance of pile for reducing convective heat loss has largely been based off a small body of work comparing heating rates before and after hair removal (Church 1960, Morgan and Heinrich 1987). The effectiveness

of varying pile at the scale that we measured was notably small. A greater than three fold increase in pile length only decreased cooling rate 24%. While increasing pile length does decrease convective cooling it does not seem like an especially efficient or effective means of doing so for bumblebees.

The patterns in body size we saw across our study are do not match our predictions or the findings of previous studies. Excluding tropical bees, most work shows that larger bees are more common in cold environments and that, within species, individuals tend to be larger in cooler places, likely due to their increased thermoregulatory ability (Peat et al. 2005, Classen et al. 2017, Peters et al. 2016). The flat relationship we show between environmental temperature and body size of field captured workers suggests that *B. vosnesenskii* uses strategies other than body size to deal with cold or that body size is constrained by other things such as flight efficiency demands or resource limitation. The discrepancy between the body size trend seen in our field caught versus common garden reared workers is likely a result of insects' developmental responses to rearing temperature. Typically, insects reared in warm temperatures achieve smaller adult body sizes (Atkinson, 1994). It seems that common garden bees from cold climate queens are responding to higher than expected rearing temperatures by decreasing body size, while those from warm climates are experiencing close to normal rearing temperatures in our lab, so are not putting forth a body size response.

Pile length also did not vary as expected with environmental temperature which again does not match findings of previous work on the subject (Peat et al. 2005, Peters et al. 2016). Bees from warm places had longer pile and, in turn, lower convective cooling rates. These unexpected results coupled with the relatively weak effect pile had on convective cooling suggest that pile may serve a function other than protection from convective heat loss for *B. vosnesenskii*.

Fur is almost always viewed as a mean of insulation since that is the purpose we most often find it serving in animals we regularly encounter. However, it is not the only function it can have. Hair or feathers have been shown before to reduce radiative heat gain in certain situations (Walsberg et al. 1978). Specifically, a thick coat of dark hair or feathers can reduce heat gain in moderate wind speeds such as those bees experience while flying when overheating can be a concern. This occurs because a dark coat captures radiative heat at its surface and holds it away from the animal's body. With a moderate wind, the coat will lose the captured heat to convection before the heat is able to conduct down the hairs to the body. *B. vosnesenskii* is notably a very dark bee with the vast majority of its thorax and abdomen being covered in black pile. Given the patterns in hair length we see and previous work showing the potential for fur as a heat shield it seems reasonable to think that these bees might be using pile as a defense from overheating due to radiative heat gain.

It's also possible that pile length isn't driven by thermal demands at all. Variation in pile could aid in pollen capture, pollen detection, water retention, or numerous other things. To fully understand what drives pile variation more work must be done to better understand how pile effects and interacts with things such as pollen, water, and various forms of heat transfer.

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Figures

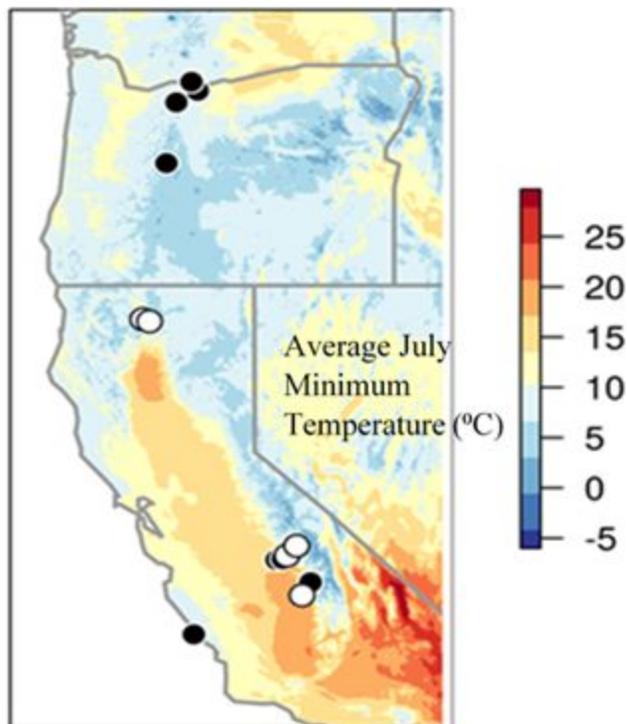


Figure 1 Capture locations for *B. vosnesenskii* individuals. Black dots are capture sites for queens that initiated the common garden colonies. White dots are capture sites for workers. Colors represent average July minimum temperatures for 1960-1990 from WorldClim (Fick and Hijmans, 2017).

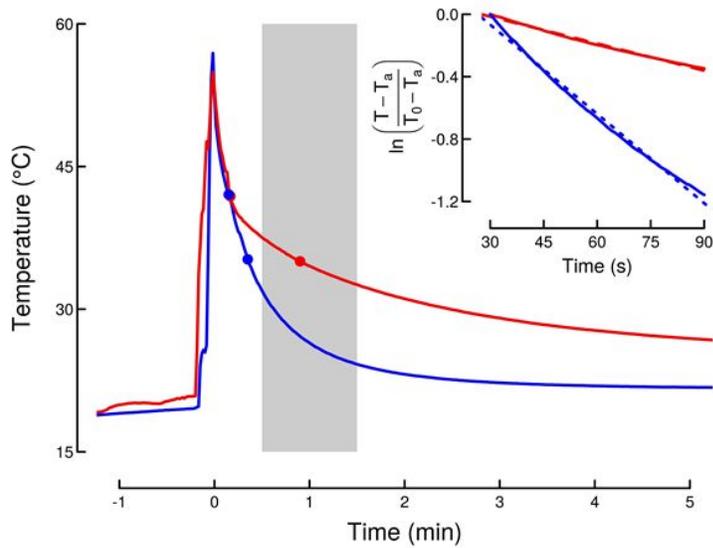


Figure 2 Cooling curves from a well insulated (red) and poorly insulated (blue) bee. The dots on the curves show the points where each bee is at 42C and 35C. The inset plot shows the standardized curves from the shaded region of the graph with the dashed lines showing the linear fit whose slope was used to calculate cooling rates. The more negative slope of the blue line indicates that higher cooling rate of the poorly insulated bee.

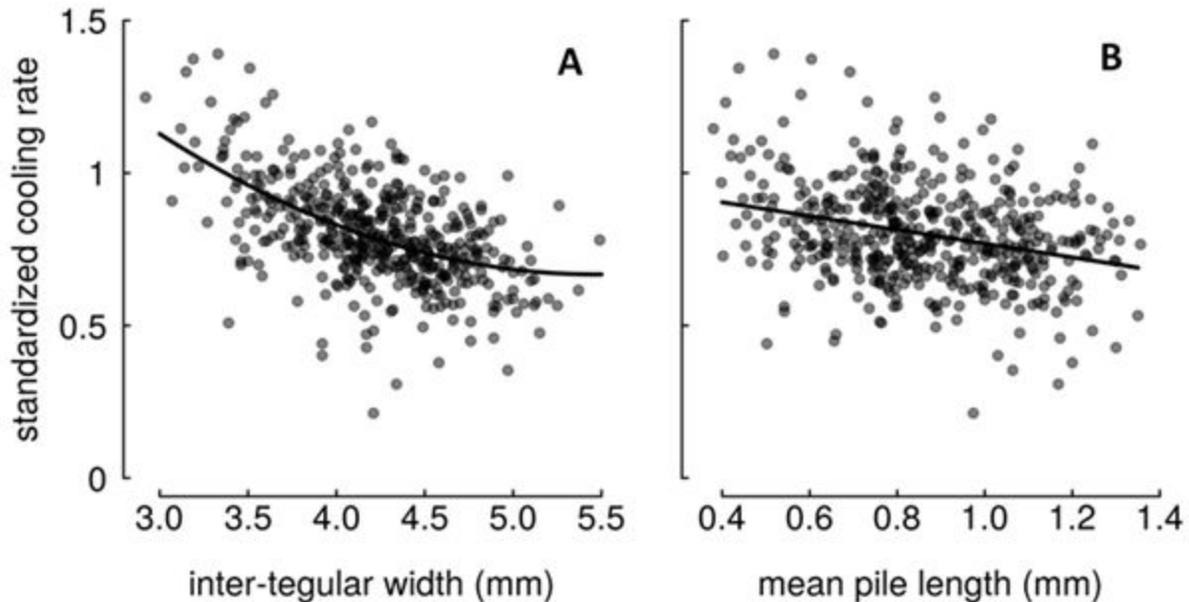


Figure 3 Body size (A) and pile length (B) plotted against standardized cooling rates. Increases in both metrics decreased cooling rates (ITW $p < 0.001$, $R^2 = 0.31$, Pile length $p < 0.001$, $r^2 = 0.09$). Body size had the predicted strong, non-linear effect on cooling rate. Pile length's effect on cooling rate was relatively weak.

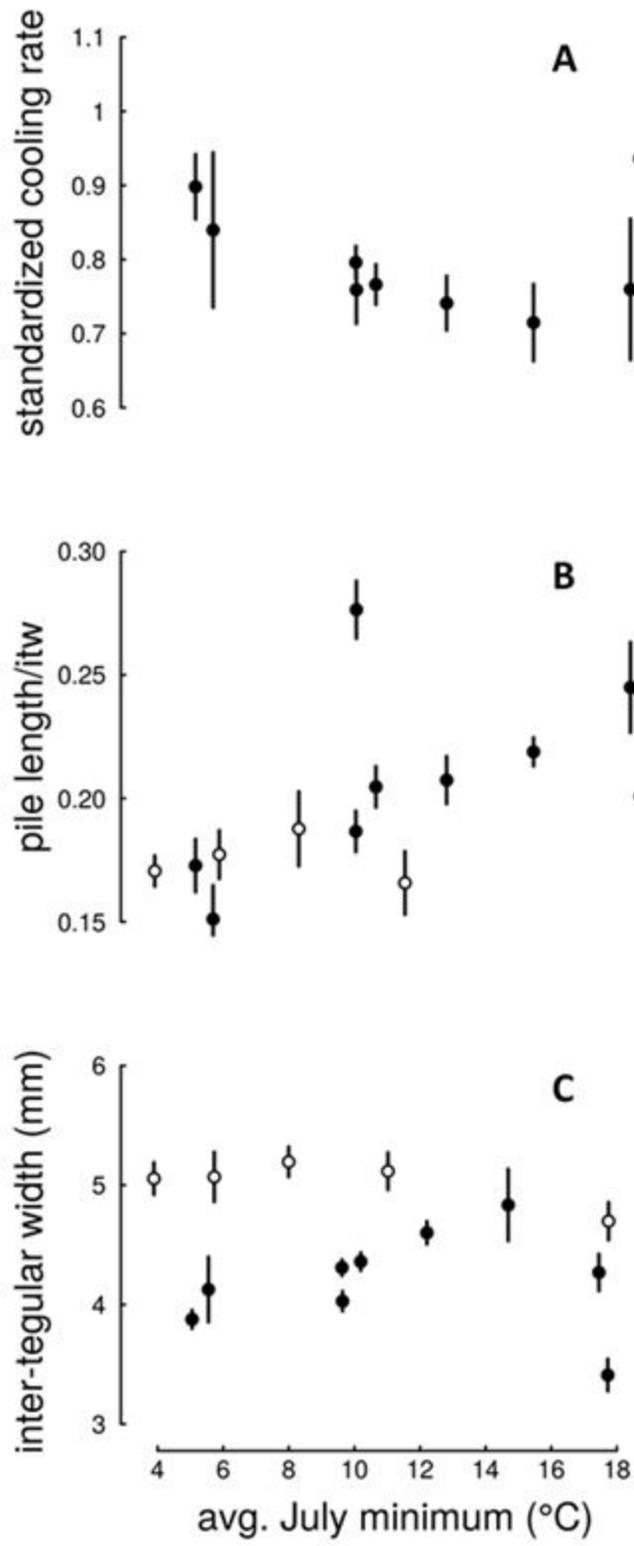


Figure 4 The variation in average cooling rate (A), normalized pile length (B), and body size (C) plotted against average average July minimum temperatures from 1960-1990 (Fick and Hijmans, 2017). The results ran counter to our predictions with bees from warm climates being larger, longer haired, and cooling slower. In B and C black points represent common garden reared bees while white points represent field captured workers. Points are means for bees from each site. Error bars are standard errors.

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