

Bumblebee Thermoregulation: Understanding the thermal properties of physical features of bumblebees

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Abstract

Temperature regulation plays an essential role in bumblebees because they need a specific range of temperatures to forage without overheating. Although physiological mechanisms that allow bees to regulate their temperature have been studied, how physical features of bumblebees influence their thermoregulation abilities is still not well understood. This study examined which physical features of bumblebees- color, pile density and length, and size- affect bumblebee temperatures the most when they heat from sunlight exposure. Temperature data was examined from thermal images of 130 bumblebees of 11 different species as they heated under a lamp. The results showed that pile length and color played a significant role in passive heating of bumblebees while the roles of the other factors studied were found to be insignificant. Even small effects of physical features may be favored in particular climates and may have helped drive diversity in bumblebee color and pile properties globally.

Introduction

Thermoregulation is a process that allows the body of an organism to maintain its core body temperature even under extreme conditions such as tropical heat and Arctic cold. This body function has been adapted by several species to ensure their survival under varying environmental conditions. One of the species that uses thermoregulation as a basis to survive are bumblebees. Thermoregulation allows bees to control the heat that spreads throughout their thorax and abdomen as they contract their muscles to fly. Higher thoracic temperatures help bumblebees to forage faster and more efficiently (Heinrich 1996). Maintaining a stable internal temperature is important for bumblebees because it allows them to not overheat when they use energy to forage for food.

Bombus species are notorious for possessing convergent color patterns (Owen 1980). There are over 250 bumblebee species with different color forms (Williams 2007). Bumblebee diversity was studied and found to affect the loss rate of bumblebee workers during foraging (Chittka 2014). Bumblebee colors and color patterns are also thought to have an aposematic or warning function, and this has led to the evolution of mimicry in bumblebees (Steltzer 2010). Studies done by Stiles suggest that bumblebee color diversity may be caused by thermal properties as well as mimicry (Stiles 1979). Thermal properties could play a role in bumblebees using coloration to adapt to various climates (Stiles 1979). Williams found that bumblebees with

all black or mostly black pile are found in tropical environments, bumblebees with red pile are found in the highlands, and bumblebees with more pale yellow and yellow pile are found in mid-temperate environments (Williams 2007). It is argued that in climates like the tropics where the day to day temperature is consistent, bumblebees with black hair are advantageous (Williams 2007). In climates where the temperature varies daily, bumblebees are seen with a lot of pale yellow and yellow pile (Heinrich 1996). Bumblebees adapt and use color diversity to avoid predators but this adaptation could also help bees adapt to extreme conditions if coloration plays a role in bumblebee thermoregulation (Williams 2007). Color traits may affect the ability to cool and heat the body of a bumblebee and therefore may be adaptive for optimizing foraging.

This study examines the relative contribution of physical traits to thermal properties in bumblebees such as pile length, pile density, size, and color. These features vary from species to species when considering the bumblebee population so observing how different species with different traits heat and cool would help give evidence to support that thermoregulation can be enhanced or reduced based on contrasting bumblebee properties. There are currently no studies that show the systematic differences of the effect of either coat color or coat length on temperature excess in bumblebees of different species (Heinrich 1996). The physical effects of color on bumblebee thermoregulation are not yet well understood, particularly when this involves the differences in pile density and size of bumblebee species (Williams 2007). There are predictions based on wind tunnel insulation experiments that pile density is more important in thermoregulation than pile length (Heinrich 1993). Size is one of the major restrictions in regulating high body temperatures (Heinrich 1983). Studies looking at the role bee size plays in thermoregulation have shown that smaller bees have a relatively higher warm-up rate per mass unit than bigger bees (Heinrich 1983). Thoracic temperatures measured during free flight in the field correlated positively with the bees' body mass (Stone 1993). Bees with long hair are better able to keep themselves warm during cold nights but they also struggle with heat stress during hot days (Stiles 1949).

This research project will help to understand which physical properties of bumblebees play the biggest role in thermoregulation and are most advantageous to these bees. It is important to study the effect of thermoregulation on bees because thermoregulation is essential for their ability to efficiently forage and foraging is one of the keys to bumblebees' survival. It is suggested that greater foraging rates of large bees are due to their high thermoregulation abilities in cooler temperatures (Heinrich 1993). Body temperature also affects bees' energy rates and ability to avoid predators (Heinrich 1974).

This thermoregulation research project is building on past research on the effects of coloration on thermoregulatory properties and expanding it to include the pile length, pile density, and size of these bumblebees. Even a small change in color variation, size, pile density, or pile length could have a big impact on thermoregulation which is a possible explanation for why bumblebee species that have differences in these traits thermoregulate at disparate rates. In this study, we used infrared imagery of bees under a full-spectrum lamp to understand which of the factors studied-body size, pile density, pile length, and color- impact bumblebee heating the most and therefore have been most important in their adaptation. This research will be a significant step towards understanding role of external features on thermal properties and adaptation of these bees.

Materials and Methods

Cuticle Experiment

Before examining the thermal properties of whole bumblebees, the thermoregulatory properties of different colored pile on bumblebees were studied using cuticle sample pieces from bees. An analysis was performed of small pieces of dissected cuticle of standard size. Cuticle pieces were obtained from *Bombus huntii* and *Bombus impatiens* by cutting a $0.4 \times 0.3 \mu\text{m}$ piece of abdominal cuticle off of each bumblebee. In total 10 black cuticle pieces, 10 yellow cuticle pieces, and 10 red cuticle pieces were used. The samples were arranged in a circle in a Styrofoam box and a piece of paper separated these samples from the lamp to help diffuse the light. A 1-inch circular gap in the paper was cut out for the infrared camera to capture images. A Philips Duramax 45W indoor flood light bulb was used in this research because it best emulated sunlight and contained infrared wavelengths. The light bulb was placed 20 cm above the cuticle pieces. The FLIR C2 Compact Imaging System was the camera used for these experiments, it took infrared images and could pinpoint the temperature of the samples that the images captured. The FLIR C2 camera was set up to take MSX images from a distance of < 1 m according to the instructions found in the Flir User Documentation Manual, included with FLIR C2 camera (FLIR Manual 6). After the light bulb was turned on an image was taken immediately, then an image was taken of the cuticle every 5 seconds for the first minute, every 10 second for the next four minutes, and every minute for the last 5 minutes (see Fig. 1).

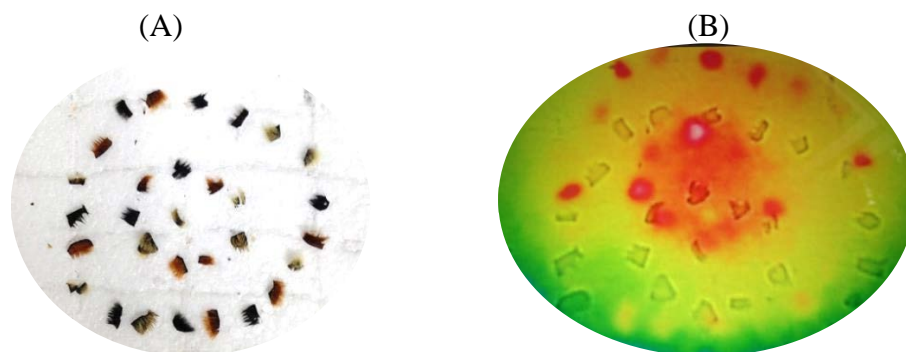


Figure 1: Design of standard cuticle piece experiment. (A) Black, red, and yellow cuticle pieces ($0.4\mu\text{m} \times 0.3\mu\text{m}$) were arranged in random order in a Styrofoam box. (B) An infrared image of the assorted cuticle pieces taken using the FLIR C2 Camera.

Preservation Experiment

Different preservation methods were tested to see if preservation methods affected how whole bumblebees heated. This experiment guided the choice of preservation technique for subsequent experiments. We used three dead queen *Bombus impatiens* for each preservation type and removed their wings (see Fig. 2A-2C). Preservation techniques included a dry bee (sitting out at room temperature for weeks), ethanol preserved bee (dipped in ethanol then dried at room temperature for an hour and a half) and freshly frozen bee (taken out of the freezer twenty minutes before the heating experiment was carried out until all the moisture had evaporated). Each of the three bees had two replicates and each bee was placed in the same Styrofoam box setup used for the cuticle experiment. A Styrofoam box was used because it best

insulated the heat radiated from the light bulb. The bees were pinned to hold them in place. The same heating process used for the cuticle experiment was used for testing preservation methods, an image was taken immediately after the light bulb was turned on then every 5 seconds for the first minute, every 10 second for the next four minutes, and every minute for the last 5 minutes (see Fig. 2D).

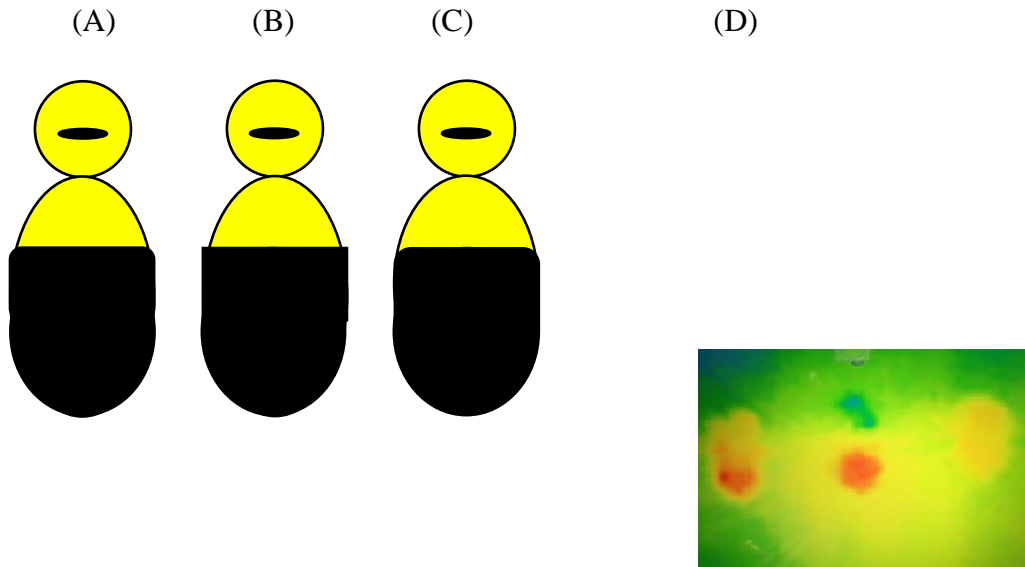


Figure 2: Design of Preservation Experiment: Queen *Bombus impatiens* preserved three different ways. (A) dry bee, (B) ethanol preserved bee, (C) freshly frozen bee. (D) Infrared image taken of the three *B. impatiens* at the 5 minute timepoint. Preservation methods impact bee temperature.

Role of Physical Properties on Whole Bees Heating

The roles that size, coloration, pile density, and pile length of bumblebees played on their thermal properties were examined by taking bees with differences in these factors and putting them in a heating and cooling experiment. Bumblebees were obtained from two local communities in central Pennsylvania, Bellefonte at a cidery and State College at the Pennsylvania State University's Arboretum, and from field sites in two other states, Oregon and California. The species studied in this experiment were *B. impatiens*, *Bombus centralis*, *B. huntii*, *Bombus melanopygus*, *Bombus mixtus workers*, *Bombus ternarius*, *Bombus bimaculatus*, *Bombus perplexus*, *Bombus griseocollus*, *Bombus bifarius*, and *Bombus vosnesenskii* with 130 bumblebees total (see Appendix). To prepare the bumblebees for the main experiment the wings and legs of each bee were removed. The whole bumblebees used were dead so that the temperature of each bee could be located and other outside factors did not affect the heating process.

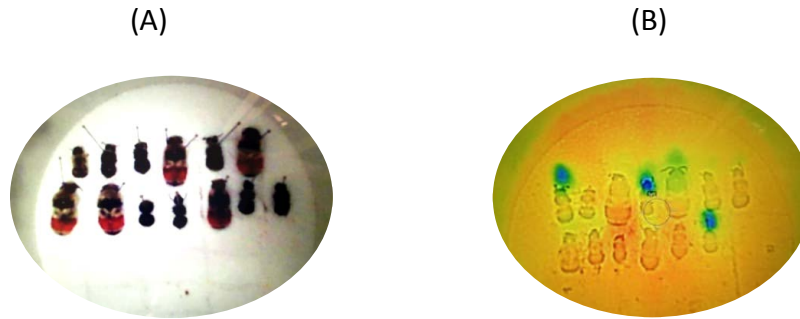


Figure 3: Design for Whole Bee Heating Experiment (A) is one of the trials used for the whole bee heating experiment. There were 13 bees in each trial with 10 trials total. The bees were arranged randomly and pinned down through their thorax in the same container used in the previous experiments. evenly. (B) Infrared image taken of one of the trials used for heating whole bees.

The thermoregulation experiments were carried out under a surface light bulb using the FLIR C2 infrared camera to locate and process the temperature of each individual bee while the surface light bulb simulated sunlight to heat the bumblebees. These experiments were carried out 10 times with 13 bees placed at random in each trial. The bumblebees were placed into two rows and pinned on both sides of the abdomen and underneath the thorax in the same Styrofoam box used before so that the infrared camera could properly capture the temperature of each bee without light dispersion being a complication (see Fig. 3A). A plastic cylinder was placed over the bumblebees in the box so the FLIR C2 camera could fit into the hole that the cylinder made and capture thermal images. The FLIR C2 camera was set to take images from a distance of < 1 m and the display temperature setting was used (FLIR Manual 6). An image was taken immediately after the light bulb was turned on. After the first image, an image was taken of the thirteen bumblebees every 20 seconds for the first minute then every minute for the next nine minutes (see Fig. 3B). This allowed the camera to have more time to calibrate between images, avoiding too many environmental temperature fluctuations in the data. All of the images were uploaded and analyzed in FLIR Tools on a Surface Pro 3.

Each bumblebee was phenotyped after the heating experiment was finished. Size was accounted for by measuring the intertegular distance of each bee (Cane 1987). Pile length was measured by taking 4-6 individual hairs off of the top of the thorax and the first segment of the abdomen and finding their length under a microscope then averaging the lengths of all the hairs obtained. It was found that the length of hairs on the thorax was the same on the abdomen for each species. Pile density was found a similar way by shaving hair from a 0.5 μm cuticle area off of the top of the thorax and the second segment of the abdomen then counting how many hairs were in each area. The percentage of each color was found by making a color map of each species used in the whole bee heating experiment (see Appendix).

Results

Results were obtained from the heating experiments on bumblebee cuticle pieces, bumblebees preserved different ways, and the total of 130 bumblebees used for studying different physical features of whole bumblebees. The average heating curve of the different colored cuticle pieces studied was calculated (see Fig. 4) and this curve displayed that the black cuticle pieces heated more overall than both the yellow and red pieces. There was a 1°F difference between the black cuticle pieces and the red and yellow ones. The curve also showed that red and yellow cuticle pieces heated similarly throughout the experiment, there was only a

0.1°F difference between these two samples, with red cuticle heating more than yellow cuticle. As seen in Fig. 5, preserving bumblebees different ways did have an impact on the amount of heat they displaced. The dry bee heated with a larger degree of difference than both the freshly frozen and ethanol preserved bees (see Fig. 5). For the main experiment, the physical features of whole bumblebees were compared to the temperature of the bees at 240 seconds which was in the middle of the experiment. Bee size (see Fig. 7), Black coloration in the thorax and the abdomen (see Fig. 8), Pile length (see Fig. 9), Pile density in the thorax and the abdomen (see Fig. 10), and starting temperature for each trial (see Fig 11) all were compared to temperature and analyzed using a multiple regression.

The multiple regression was done in Statistical Package for the Social Sciences Software. The multiple regression compared the temperature at 240 seconds which was in the middle of the experiment, to each of the physical features studied individually. The temperature at 240 seconds was also compared to the starting temperature for each trial and each trial itself to see if these factors impacted temperature as well. The multiple regression found the p-value of each factor to see if it was significant. When the multiple regression analysis was studied (see Fig. 6), black coloration in the thorax and pile length were the only physical features that had significant p-values. Pile length had the biggest impact on temperature out of all of the physical properties with a p-value of 0.001 followed by black coloration in the thorax which had a p-value of 0.038. The trials and starting temperatures of each trial were shown to have significant p-values as well, both had p-values of < 0.001.

Average Heating Curve for Different Colored Cuticle Pieces

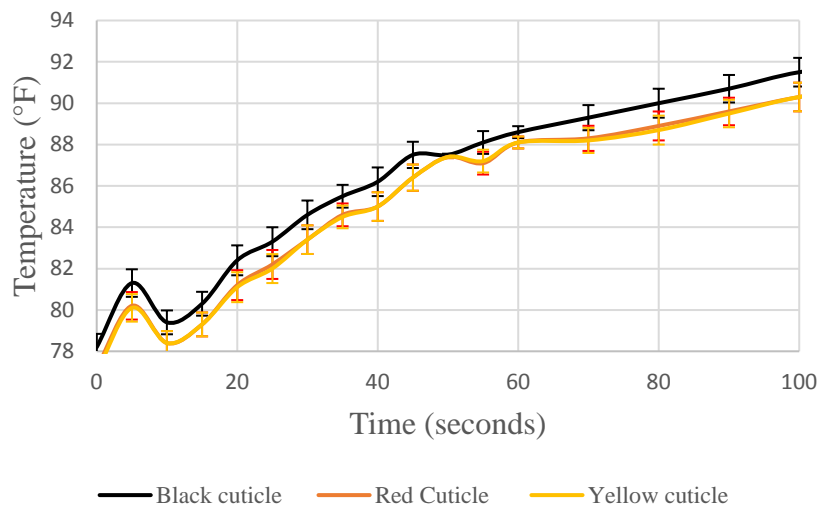


Figure 4: Average heating curve for the black, red, and yellow cuticle pieces (3µm x 4µm). The temperature of each cuticle piece in thermal images using FLIR Tools Software. Black cuticle pieces had a large degree of difference compared to the red and yellow cuticle pieces. Red and yellow cuticle pieces had a small degree of difference between each other.

Average Heating Curve of Bumblebees
Preserved using Different Methods (Dry bee v.
Ethanol preserved bee v. Freshly Frozen bee)

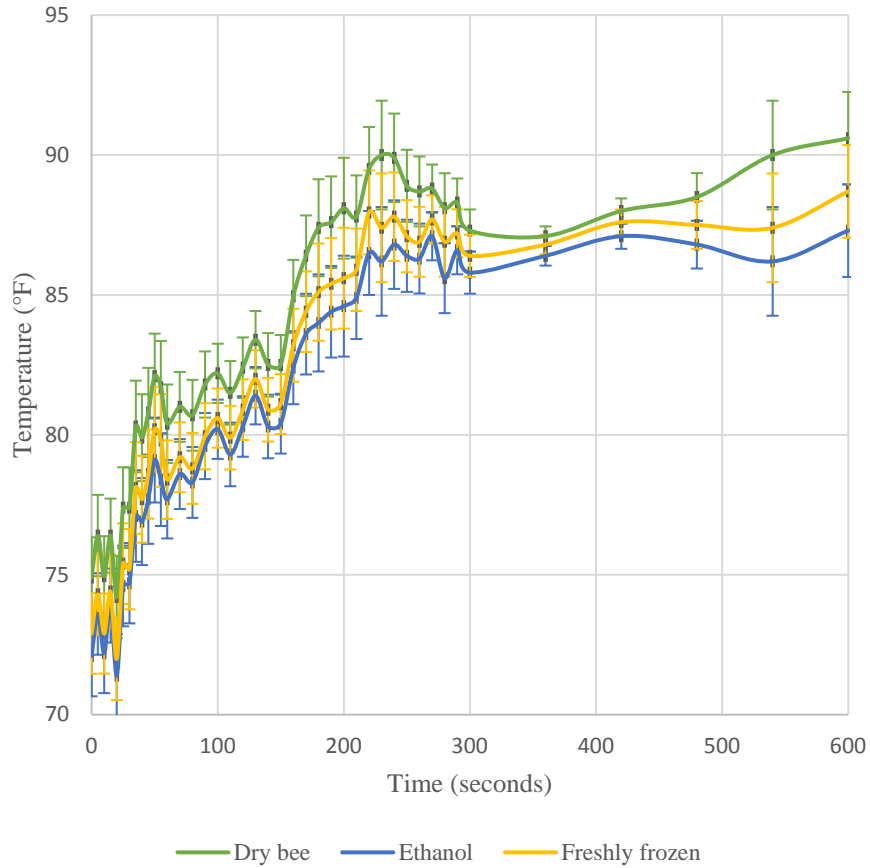


Figure 5: Average heating curve for dry, ethanol, and freshly frozen preserved whole bumblebees. Temperatures were taken from infrared images over time while they were heating under a lamp. Preservation method did impact heating since each bee heated differently. The dry bee had a large degree of temperature difference compared to the other preservation methods studied.

Multiple Regression Analysis for All Physical Features Studied including Trial and Starting Temperature for each Trial

Model	Significance (p-value)
Starting Temperature	0
Size	0.941
Black in Thorax	0.038
Black in Abdomen	0.111
Yellow in Abdomen	0.245
Pile length	0.001
Pile density (Thorax)	0.215
Trial	0

Figure 6: Multiple Regression Analysis. This model was made using a multiple regression statistical analysis to find out if the physical features studied (size, pile length & density, color) had significant p-values and therefore impacted temperature. Starting room temperature and trial were also studied to see if they impacted temperature. From the results shown black coloration in the thorax, pile length, trial, and starting room temperature had significant p-values and therefore influenced heating in this experiment.

Temperature at 240 seconds v. Size

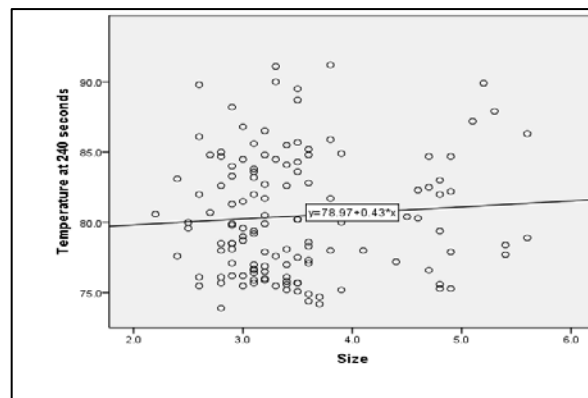


Figure 7: Temperature at 240 seconds v. Size. This figure compares the size of the bees in each trial to their temperature at 240 seconds to see if size impacted the temperature at this time. The size was found to be insignificant.

Temperature at 240 seconds v. Black Coloration in the Thorax and Abdomen

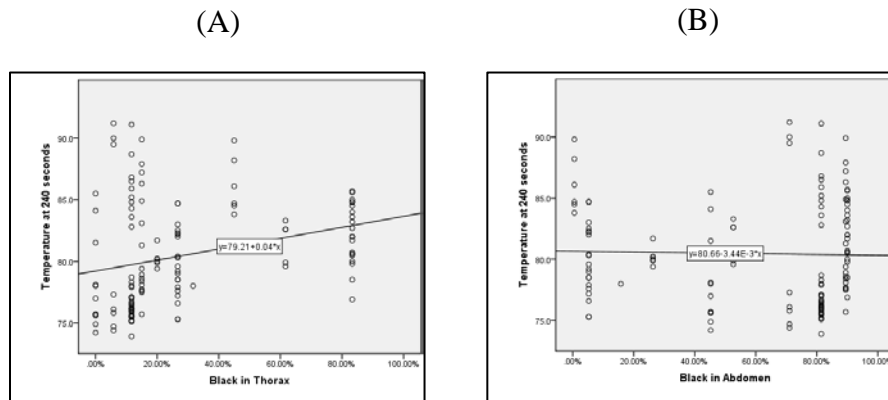


Figure 8: Temperature at 240 seconds v. Black Coloration in the Thorax and Abdomen. This figure compares the black coloration in the thorax (A) and abdomen (B) of the bees in each trial to their temperature at 240 seconds to see if black coloration impacted the temperature at this time. The black coloration in the abdomen (B) was found to be insignificant, therefore it did not influence the temperature of each bee. Black coloration in the thorax (A) was significant ($p = 0.038$).

Temperature at 240 seconds v. Pile length

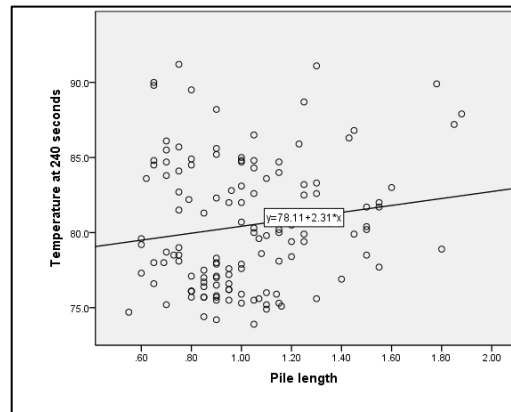


Figure 9: Temperature at 240 seconds v. Pile length. This figure compares the pile length of the bees in each trial to their temperature at 240 seconds to see if pile length impacted the temperature at this time. The pile length was found to be significant ($p = .001$).

Temperature at 240 seconds v. Pile density of the Thorax and Abdomen

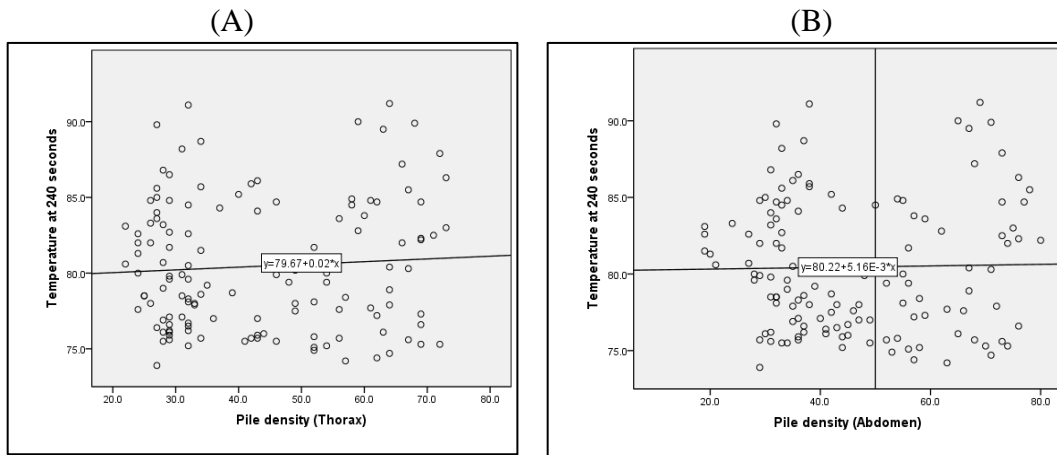


Figure 10: Temperature at 240 seconds v. Pile density of the Thorax and Abdomen. This figure compares the pile density of the thorax (A) and the pile density of the abdomen (B) of the bees in each trial to their temperature at 240 seconds to see if pile density impacted the temperature at this time. Pile density was found to be insignificant.

Temperature at 240 seconds v. Starting Temperature for each Trial

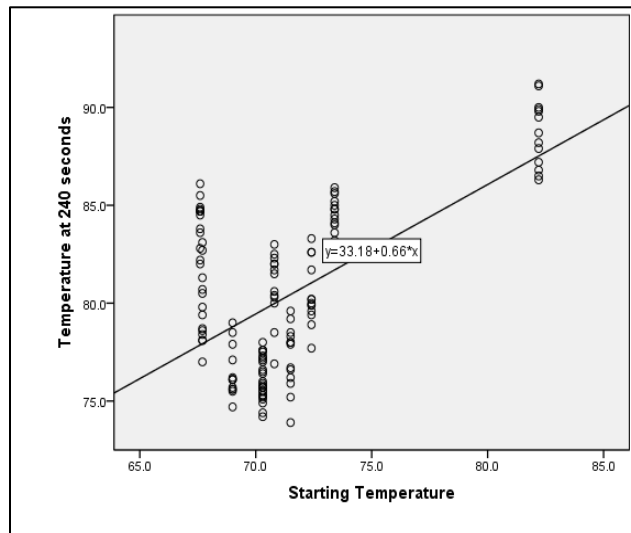


Figure 11: Temperature at 240 seconds v. Starting Temperature for each Trial. The starting temperatures for each trial were looked at to see if they had an impact on the temperature at 240 seconds. The starting temperatures were found to be significant ($p = 0.00$) so they did influence the overall temperature of each bee. This shows that trials must be done on the same day consecutively to negate the trial effect.

Discussion

The results from the cuticle experiment suggest that coloration plays a role in thermoregulation but whole bees were also studied to see if color impacted temperature when the bees had their internal tissue intact. The preservation methods experiment was carried out to see if preserving bees different ways impacted their heating process. This experiment suggested that preservation methods did impact heating because each bee did not heat the same way when they were preserved differently. The dry bee heated with to a larger degree than the freshly frozen and ethanol preserved bees and the ethanol preserved bee heated to a smaller degree than the bees preserved using other methods. Since preservation methods impacted heating, freshly frozen bees were chosen to be used in the main experiment studying the thermal properties of whole bees because they best emulated live bees from the wild.

This study investigated which physical features of bumblebees impacted temperature regulation. Although the process of thermoregulation is understood, the physical factors that affect it have not been studied so this project worked to fill in those research gaps. From the multiple regression statistical analysis done comparing the impact that each physical feature- size, pile density, pile length, and color- had on the temperature of each bee. Pile length was shown to be the most significant and therefore had the biggest impact on the heating process. Color was also significant but had a smaller contribution.

This provides further evidence to the suggestion that color diversity could possibly be caused by a combination of mimicry and thermoregulation (Stiles 1979). Different colored bumblebee pile could be advantageous in different climates, which would explain why completely black and mostly black bumblebees are typically found near the equator, whereas lighter colored bees tend to be found in mid-latitudes (Heinrich 1996).

Conclusion

Previously the effect of physical features of bumblebees on thermoregulation had been studied using correlation to mimicry but not studied individually. This research is provides empirical evidence that different physical properties of bumblebees effect thermoregulation in their bodies. The p-values found for both pile length and color were significant and also much lower than the p-value for pile density suggesting that pile length and color impact temperature more than pile density. Size was also found to not be significant compared to the temperature, revealing that it is less important than color and pile properties. Since pile length was shown to have a large influence on temperature, there is a possibility that insulation effects can help control temperature with shorter pile enabling more heating than longer pile, which may retain internal temperature better.

A limitation of this study was the highly significant impact of trial on temperature. This shows that each trial for the experiment must be considered separately and cannot be considered together since each trial started and ended at different temperatures. One source of this error is that the trials were not done on the same day and therefore had different starting temperatures and humidity. This is further supported by the significant role of starting temperature on heating (see Figure 11). Another source of error could be that not all the moisture evaporated out of each bee at the same rate for each trial, which would have a major impact on their heating.

For future directions, to obtain more cogent results this experiment could be repeated measuring the heat of multiple whole bees in one trial to get rid of trial effect. After that experiments to understand how color effects thermoregulation could be done by studying live bees. One way to do this would be by putting bumblebees of different species with different

color forms into a heat chamber and measuring the rate and at what temperature they knockout to see how different color forms affect how much heat they can withstand in a certain amount of time (Martinet 2015). Although there is more research to be done, the results of this project suggest a correlation between thermoregulation and coloration in bumblebees.

Acknowledgements

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Appendix Physical Features and Temperatures of Different Bumblebee Species

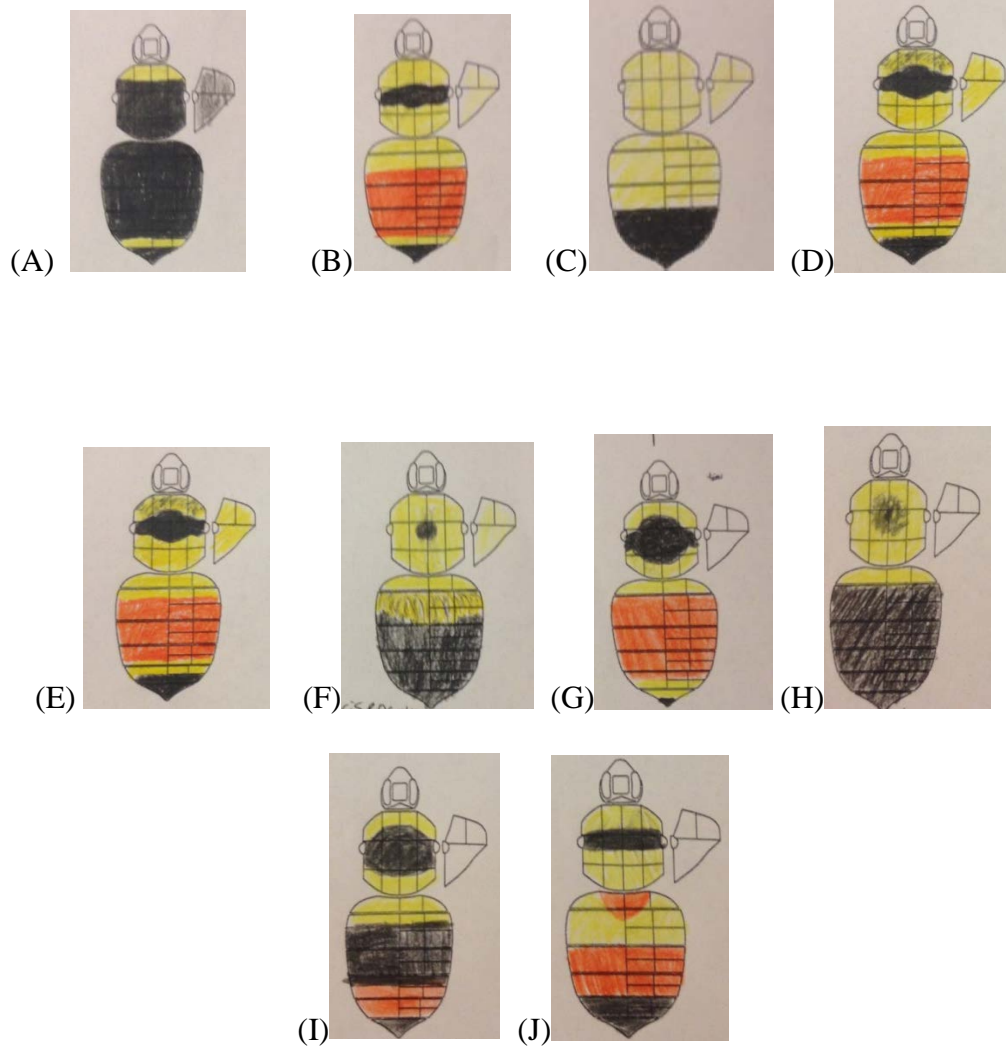
Bee	Temperature at 240 seconds	Starting Temperature	Size	Black in Thorax	Black in Abdomen	Pile length	Pile density (Thorax)	Pile density (Abdomen)	Trial
Huntii Queen	77.2	70.3	4.4	26.67%	5.26%	0.95	62	57	1
impatiens 1	77.5	70.3	3.5	15%	89.47%	0.85	49	42	1
impatiens 2	77.6	70.3	3.3	15%	89.47%	1	56	66	1
Perplexus 1	77	70.3	3.4	0%	45.21%	0.9	36	49	1
Perplexus 2	75.7	70.3	2.8	0%	45.21%	0.8	43	52	1
Griseocollis	77.3	70.3	3.6	5.83%	71.05%	0.6	69.00	59.00	1
Huntii	77.6	70.3	2.4	26.67%	5.26%	0.95	24.00	46.00	1
Melanopygus	78	70.3	3.8	31.67%	15.79%	0.65	26.00	43.00	1
Bimac 1	77.1	70.3	3.6	11.67%	81.47%	0.8	29.00	40.00	1
Bimac 2	76.4	70.3	3.1	11.67%	81.47%	0.85	27.00	41.00	1
Bimac 3	76.5	70.3	3.2	11.67%	81.47%	0.9	32.00	43.00	1
Bimac 4	76.6	70.3	3.1	11.67%	81.47%	0.95	29.00	37.00	1
Bimac 5	75.7	70.3	3.5	11.67%	81.47%	0.9	56.00	68.00	1
Huntii Queen 1	77.9	71.5	4.9	26.67%	5.26%	0.9	64.00	72.00	2
Huntii Queen 2	76.6	71.5	4.7	26.67%	5.26%	0.65	69.00	76.00	2
Huntii 1	79.2	71.5	3.1	26.67%	5.26%	0.6	35.00	39.00	2
Huntii 2	79.6	71.5	2.5	26.67%	5.26%	0.6	32.00	34.00	2
Huntii 3	78.5	71.5	2.8	26.67%	5.26%	0.75	25.00	32.00	2
Perplexus	78	71.5	4.1	0%	45.21%	0.9	49.00	47.00	2
Bimac 1	76.2	71.5	2.9	11.67%	81.47%	0.95	29.00	31.00	2
Bimac 2	78.3	71.5	3.6	11.67%	81.47%	0.9	32.00	36.00	2
Bimac 3	73.9	71.5	2.8	11.67%	81.47%	1.05	27.00	29.00	2
Bimac 4	78	71.5	2.8	11.67%	81.47%	0.69	33.00	38.00	2
Bimac 5	76.7	71.5	3.1	11.67%	81.47%	0.85	32.00	45.00	2
Bimac 6	75.2	71.5	3.4	11.67%	81.47%	1.1	32.00	44.00	2

Bimac 7	75.9	71.5	3.1	11.67%	81.47%	1	29.00	36.00	2
Bimac Queen	75.6	69	4.8	11.67%	81.47%	1.3	67.00	73.00	3
Bimac 1	77.9	69	3.2	11.67%	81.47%	1	33.00	35.00	3
Bimac 2	76.2	69	3	11.67%	81.47%	0.95	32.00	37.00	3
Bimac 3	77.1	69	2.9	11.67%	81.47%	0.9	31.00	36.00	3
Bimac 4	76.1	69	2.6	11.67%	81.47%	0.8	28.00	30.00	3
Bimac 5	75.5	69	2.6	11.67%	81.47%	0.95	28.00	34.00	3
Bimac 6	76.1	69	2.8	11.67%	81.47%	0.8	29.00	41.00	3
Griseocollis 1	74.7	69	3.7	5.83%	71.05%	0.55	64.00	71.00	3
Griseocollis 2	76.1	69	3.4	5.83%	71.05%	0.8	63.00	65.00	3
Huntii 1	79	69	3	26.67%	5.26%	0.75	28.00	34.00	3
Huntii 2	78.5	69	2.9	26.67%	5.26%	0.73	31.00	32.00	3
Impatiens	75.7	69	3.5	15%	89.47%	0.85	34.00	29.00	3
Perplexus	75.6	69	3.4	0%	45.21%	1.07	29.00	31.00	3
Huntii Queen 1	84.7	67.6	4.9	26.67%	5.26%	1.15	69.00	77.00	4
Huntii Queen 2	84.7	67.6	4.7	26.67%	5.26%	1	62.00	73.00	4
Huntii Queen 3	82	67.6	4.8	26.67%	5.26%	0.95	66.00	74.00	4
Huntii Queen 4	82.2	67.6	4.9	26.67%	5.26%	0.79	69.00	80.00	4
Bimac 1	84.8	67.6	3.6	11.67%	81.47%	0.65	61.00	55.00	4
Bimac 2	83.6	67.6	3.5	11.67%	81.47%	0.62	56.00	59.00	4
Bimac 3	82.8	67.6	3.6	11.67%	81.47%	0.96	59.00	62.00	4
Ternarius 1	86.1	67.6	2.6	45%	0.53%	0.7	43.00	35.00	4
Ternarius 2	83.8	67.6	3.1	45%	0.53%	0.7	60.00	57.00	4
Ternarius 3	84.5	67.6	3	45%	0.53%	0.65	58.00	50.00	4
Ternarius 4	84.7	67.6	2.8	45%	0.53%	0.7	46.00	32.00	4
Perplexus	85.5	67.6	3.4	0%	45.21%	0.7	67.00	78.00	4
Impatiens	84.9	67.6	3.9	15%	89.47%	0.8	58.00	54.00	4
Bimac 1	75.9	70.3	3.2	11.67%	81.47%	1.14	43.00	44.00	5
Bimac 2	75.5	70.3	3.3	11.67%	81.47%	0.9	46.00	49.00	5
Bimac 3	75.5	70.3	3	11.67%	81.47%	1.05	41.00	33.00	5
Bimac 4	76	70.3	3.2	11.67%	81.47%	1.1	44.00	45.00	5
Bimac 5	75.1	70.3	3.5	11.67%	81.47%	1.16	52.00	56.00	5
Bimac 6	75.2	70.3	3.9	11.67%	81.47%	0.7	54.00	58.00	5
Perplexus 1	75.7	70.3	3.1	0%	45.21%	0.85	42.00	36.00	5
Perplexus 2	74.9	70.3	3.6	0%	45.21%	1.1	52.00	53.00	5
Perplexus 3	74.2	70.3	3.7	0%	45.21%	0.9	57.00	63.00	5
Huntii Queen 1	75.3	70.3	4.9	26.67%	5.26%	1	72.00	74.00	5
Huntii Queen 2	75.3	70.3	4.8	26.67%	5.26%	1.15	69.00	70.00	5
Griseocollis 1	75.8	70.3	3.4	5.83%	71.05%	0.9	52.00	54.00	5
Griseocollis 2	74.4	70.3	3.6	5.83%	71.05%	0.85	62.00	57.00	5

Centralis 1	81.7	72.4	3.8	20%	26.32%	1.55	52.00	56.00	6
Centralis 2	79.9	72.4	2.9	20%	26.32%	1.25	46.00	48.00	6
Centralis 3	80.2	72.4	3.5	20%	26.32%	1.15	49.00	51.00	6
Centralis 4	79.4	72.4	3.1	20%	26.32%	1.25	48.00	52.00	6
Centralis 5	80.2	72.4	3.5	20%	26.32%	1.5	49.00	52.00	6
Centralis 6	80	72.4	3.9	20%	26.32%	1.05	54.00	55.00	6
Mixtus 1	83.3	72.4	2.9	61.67%	52.63%	1.3	26.00	24.00	6
Mixtus 2	82.6	72.4	3.4	61.67%	52.63%	1.05	32.00	27.00	6
Mixtus 3	79.6	72.4	3	61.67%	52.63%	1.07	29.00	28.00	6
Mixtus 4	82.6	72.4	2.8	61.67%	52.63%	1.3	24.00	19.00	6
Mixtus 5	79.9	72.4	3.2	61.67%	52.63%	1.45	31.00	29.00	6
Impatiens Queen 1	78.9	72.4	5.6	15%	89.47%	1.8	64.00	67.00	6
Impatiens Queen 2	77.7	72.4	5.4	15%	89.47%	1.55	61.00	63.00	6
Bimac 1	77	67.7	3.1	11.67%	81.47%	0.85	43.00	47.00	7
Bimac 2	78.7	67.7	3	11.67%	81.47%	0.7	39.00	42.00	7
Impatiens Queen 1	79.4	67.7	4.8	15%	89.47%	1.2	54.00	56.00	7
Impatiens Queen 2	78.4	67.7	5.4	15%	89.47%	1.2	57.00	58.00	7
Perplexus	78.1	67.7	2.9	0%	45.21%	0.75	52.00	55.00	7
Vosnesenskii 1	82.7	67.7	3.2	83.33%	90%	0.75	29.00	33.00	7
Vosnesenskii 2	80.7	67.7	2.7	83.33%	90%	1	28.00	27.00	7
Vosnesenskii 3	80.5	67.7	3.2	83.33%	90%	1.2	32.00	35.00	7
Vosnesenskii 4	79.8	67.7	2.9	83.33%	90%	1.1	29.00	31.00	7
impatiens 1	83.1	67.7	2.4	15%	89.47%	1	22.00	19.00	7
Impatiens 2	81.3	67.7	2.9	15%	89.47%	0.85	24.00	20.00	7
impatiens 3	78.1	67.7	3.4	15%	89.47%	1.15	32.00	32.00	7
impatiens 4	78.6	67.7	3.6	15%	89.47%	1.08	34.00	37.00	7
Bimac 1	85.2	73.4	3.6	11.67%	81.47%	0.9	40.00	42.00	8
Bimac 2	84.3	73.4	3.5	11.67%	81.47%	1.05	37.00	44.00	8
Bimac 3	85.9	73.4	3.8	11.67%	81.47%	1.23	42.00	38.00	8
Vosnesenskii 1	84.5	73.4	3.3	83.33%	90%	0.8	32.00	33.00	8
Vosnesenskii 2	85.7	73.4	3.5	83.33%	90%	0.75	34.00	38.00	8
Vosnesenskii 3	84.8	73.4	3.2	83.33%	90%	1	29.00	34.00	8
Vosnesenskii 4	85.6	73.4	3.1	83.33%	90%	0.9	27	33	8
Vosnesenskii 5	84.8	73.4	2.7	83.33%	90%	1.05	26	29	8
Vosnesenskii 6	85	73.4	2.8	83.33%	90%	1	27	30	8
Vosnesenskii 7	83.2	73.4	3.1	83.33%	90%	1.25	28	31	8
Vosnesenskii 8	84	73.4	2.9	83.33%	90%	1.15	27	31	8
Vosnesenskii 9	83.6	73.4	3.1	83.33%	90%	1.1	27	32	8
Perplexus	84.1	73.4	3.4	0%	45.21%	0.75	43	36	8
Griseocollis 1	91.2	82.2	3.8	5.83%	71.05%	0.75	64.00	69.00	9

Griseocollis 2	90	82.2	3.3	5.83%	71.05%	0.65	59.00	65.00	9
Griseocollis 3	89.5	82.2	3.5	5.83%	71.05%	0.8	63.00	67.00	9
Bimac 1	91.1	82.2	3.3	11.67%	81.47%	1.3	32.00	38.00	9
Bimac 2	88.7	82.2	3.5	11.67%	81.47%	1.25	34.00	37.00	9
Bimac 3	86.8	82.2	3	11.67%	81.47%	1.45	28.00	31.00	9
Bimac 4	86.5	82.2	3.2	11.67%	81.47%	1.05	29.00	36.00	9
Ternarius 1	89.8	82.2	2.6	45%	0.53%	0.65	27	32	9
Ternarius 2	88.2	82.2	2.9	45%	0.53%	0.9	31	33	9
Impatiens Queen 1	89.9	82.2	5.2	15%	89.47%	1.78	68	71	9
Impatiens Queen 2	87.9	82.2	5.3	15%	89.47%	1.88	72	73	9
Impatiens Queen 3	87.2	82.2	5.1	15%	89.47%	1.85	66	68	9
Impatiens Queen 4	86.3	82.2	5.6	15%	89.47%	1.43	73	76	9
Perplexus 1	81.5	70.8	3	0%	45.21%	0.75	34	19	10
Huntii Queen 1	82.3	70.8	4.6	26.67%	5.26%	0.9	69.00	76.00	10
Huntii Queen 2	80.3	70.8	4.6	26.67%	5.26%	1.05	67.00	71.00	10
Huntii Queen 3	80.4	70.8	4.5	26.67%	5.26%	1.5	64.00	67.00	10
Huntii Queen 4	82.5	70.8	4.7	26.67%	5.26%	1.25	71.00	73.00	10
Huntii Queen 5	83	70.8	4.8	26.67%	5.26%	1.6	73.00	75.00	10
Vosnesenskii 1	78.5	70.8	2.9	83.33%	90%	1.5	25.00	31.00	10
Vosnesenskii 2	82	70.8	3.1	83.33%	90%	1	26.00	32.00	10
Vosnesenskii 3	80.6	70.8	2.2	83.33%	90%	1.35	22.00	21.00	10
Vosnesenskii 4	76.9	70.8	3.2	83.33%	90%	1.4	28.00	35.00	10
Vosnesenskii 5	80	70.8	2.5	83.33%	90%	1.15	24	28	10
Vosnesenskii 6	82	70.8	2.6	83.33%	90%	1.55	24	29	10
Vosnesenskii 7	81.7	70.8	3.2	83.33%	90%	1.5	29.00	33.00	10

Color Forms of Each Bee Species



(A) *B. vosnesenskii*, (B) *B. huntii*, (C) *B. perplexus*, (D) *B. melanopygus*, (E) *B. bimaculatus*,
 (F) *B. griseocolis*, (G) *B. ternarius*, (H) *B. impatiens*, (I) *B. mixtus*, (J) *B. centralis*

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