Formica obscuripes use metabolic heat and nest structure to thermoregulate By Lauren Greenleaf

Introduction

Thermoregulation is a critical aspect of an insect's ecology and directly impacts growth, metabolism, and their development (Kadochová and Frouz 2014). Many insects, like honeybees and *Formica obscuripes*, are ectothermic, meaning they regulate their internal temperature through behavioral changes. Social insects, like ants, are much more effective at thermoregulation than solitary insects because they have the capability of building large nests and engaging in more complex behavior (Kadochová and Frouz 2014). By maintaining a high inner temperature of the nest, a colony can achieve greater fitness by increasing reproduction rate (Kadochová and Frouz 2014). Formica obscuripes have also been observed to move the location of their brood to different auxiliary chambers to account for temperature fluctuations, therefore increasing the reproductive success of the colony (Cole 1932). Knowing that both structure and behavior influence the thermoregulation of Formica obscuripes, I was interested in looking at which factor has a greater influence on mound temperature. To do so, I compared dead mounds to live mounds to learn more about the effect of ant presence on a mound's thermoregulation. I also looked at the temperature variation of large versus small mounds. I hypothesize that presence of ants in a mound will have a greater impact than mound size on mound temperature because ant bodies have a high thermal capacity (Kadochová and Frouz 2014).

Previous literature has examined how mound size effects the thermoregulation of *Formica obscuripes*: large mounds are more effective than small mounds at keeping a consistent, high temperature, and large mounds stay warmer than ground and air temperature throughout the day (Conway 1996). *Formica obscuripes* nests also exhibit thermal stratification (Conway 1996) because the top acts as a solar collector (Kadochová and Frouz 2014). These ants can also open

and close entrances to trap or release heat (Henderson and Ackre 1986). We also know that ants have a high concentration of water in their bodies, so when they sun themselves outside the mound and then come back inside, that heat is released from their bodies into the mound, helping to keep it warm overnight (Kadochová and Frouz 2014).

In addition to radiative warming, the ant's metabolic heat helps to keep the nest at a high inner temperature. But it's not just the presence of ants that make the nest warm-building materials also play a critical role in insulating the colony. *Formica obscuripes* are a thatch ant, meaning they use bits of plants to create a thick layer of insulation which has a lower thermal diffusivity than the soil and helps keep the nest at a consistent temperature. It has also been observed that *Formica obscuripes* tunnel into the ground below the thatch to an average depth of 57 inches, independent of how tall the mounds are (Weber 1935).

Methods

<u>Sampling</u>

This study took place within the Columbia National Wildlife Refuge in Eastern Washington at latitude 46, longitude -119, altitude 245 meters (Figure 7). On the evening of Friday, May 12th, 2023, we set up temperature iButtons to record air, ground, and mound temperatures every hour until 8:00 (all times recorded using military time) on Sunday, May 14th 2023. iButtons are small thermometer discs you can program to take a temperature reading at the desired intervals. We chose 4 alive mounds and 2 dead mounds that were equally shaded by golden current. To determine if the mound was dead, we poked it with a stick and looked to see if any ants came out. We then measured the height and width of the mounds with a meter stick. Air temperature measurements were taken by taping iButtons to the top of a meter stick and planting them into the ground 0.5m away from the base of the mound. iButtons duct-taped to flags were inserted 5cm into the ground near the base of mounds to measure ground temperature and iButtons duct-taped to flags were inserted 5cm into the top of the mound to measure mound temperature. Using a Garmin inReach mini, we took the latitude and longitude of each mound and mapped their locations (Figure 7). At time of iButton collection on the 14th, we counted the number of ants present in a 5cm² quadrat three times and then averaged the values. To compare maximum mound width to the difference in temperature between the ground and mound, we used data collected during the month of May in 2003-2023 at the Columbia National Wildlife Refuge by the University of Washington Biology 480 class.

Data Analysis

To get a more detailed understanding of the variation between air, ground, and mound temperatures, we created time categories across each location. Morning was assigned as 4:00 to 10:00, afternoon was assigned to 11:00 to 18:00, and night was assigned to 19:00 to 3:00 based on the general patterns of air temperature rising and falling observed in figure 3. We then averaged the temperatures and preformed an unpaired, two-tailed t-test using excel. We performed a linear regression using excel to test the variance between maximum mound width and the difference between ground and mound temperatures for both live and dead mounds. We also performed an unpaired, two-tailed t-test to test for significance between the number of ants in a quadrat in a small mound in comparison to a large mound.

Results

Live Mounds

We found that the mean width of our 4 live mounds was 51.25cm and the mean height was 23cm (n=4). By looking at the general average temperature trends over 39 hours, we found that mound temperature stayed above ground temperature, except during the peak heat of the day

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because mound temperature rises slower than ground temperature (figure 3). We also found that average mound temperature fluctuates less than average air and ground temperatures (figure 3), and that the mound temperature increased at a less steep rate than air temperature (figure 3). Figure 5 shows the temperature fluctuation of a small mound (width= 25cm, height= 7cm) is much greater than the temperature fluctuation of a large mound in figure 6 (width= 70cm, height= 40cm). From time 6:00 to 14:00, the small mound and large mounds had a rate of temperature change of 2.4 and 4.4, respectively. From time 16:00 to 24:00, the small and large mounds had a rate of change in temperature of -2.1 and -3.8 respectively. These results show that the temperature increases more quickly in the morning through afternoon than it decreases in the evening across both small and large mounds, and the rate at which temperature increases or decreases is faster in large mounds. We found that there is a significant difference between ground and mound temperatures (p=0.001) in the morning, but not between air and ground temperatures (p=0.06) or air and mound temperatures (p=0.96). In the afternoon, there is a significant difference between air and ground temperatures (p=0.00009) and air and mound temperatures (p=0.00007), but not between ground and mound temperatures (p=0.42). At night, there was a significant difference between ground and mound temperatures ($p=1.67^{-15}$) and air and ground temperatures ($p=3.54^{-7}$) but not between air and ground temperatures (p=0.86). These patterns appear because air temperature fluctuates more dramatically than ground temperature.

We also looked at the relationship between the difference in ground vs mound temperature and maximum mound width (n=527) and found a slight positive correlation between the two. Mounds that were warmer than the ground (to the right of the y axis in figure 8) also tended to have a larger maximum width (R^2 = 0.014). In addition, the average number of ants in our smallest mound (width= 25cm, height= 7cm) was 3 ants and the average number of ants in our largest mound (width= 70cm, height= 40cm) was 13.3 ants. Large mounds had more ants than small mounds (p= 0.001, N=?).

Dead Mounds

The mean width of a dead mound is 82.5cm and the mean height is 31cm (n=2). By looking at the general trend lines (figure 4), we can see that average mound temperature stays below air and ground temperature throughout the heat of the day and decreases slower in the evening than it increases in the morning. At night, the ground and mound temperature lines all overlap. We found that, in the morning, ground temperature is lower than mound temperatures (p=0.0001) and that air temp is higher than ground temperatures (p=0.02). Air and mound temperatures were similar (p=0.12). In the afternoon, there is a significant difference (p=0.0005, p=0.001, and p=0.00006) respectively, and at night, there is a significant difference between ground and mound temperatures (p=0.0009), but not between air and mound temperatures (p=0.77).

Figure 9 shows that there is also a slight positive correlation between mound width and the difference in temperature between the ground and dead mounds (n=164) ($R^2=0.014$). Mounds warmer than the ground temperature are to the right of the y axis (figure 9).

Discussion

Thermoregulation is critical to ectotherms like *Formica obscuripes*. As social insects, they can create intricate nests and perform complex behaviors which all help increase the inner heat of their mounds (Kadochová and Frouz 2014). In this study, we found evidence that both mound structure and ant presence influence the internal temperature. When we compare live mounds to dead mounds, we can see that live mounds are more effectively thermoregulating.

Throughout the day, live mound temperature stays very similar to ground temperature, decreases in temperature slowly at night, and stays warmer than both the ground and air throughout the night (figure 3). In previous studies, they studied the ant's behavior and found that ants cluster at the top of the mound in the afternoon, after the peak heat of the day, and then return to their mounds (Kadochová et al., 2019). This behavior could explain why we observed that mound temperature is slow to decrease in the evenings.

In comparison, dead mounds begin warming before the ground, stay cooler than the ground at the warmest part of the day, and have a similar temperature to both ground and air at night. This indicates that the presence of ants is an important part of thermoregulation. Previous studies support the activity of the ants as an important part of their thermoregulation (Conway 1996, Bollazzi and Martin 2010, Kadochová and Frouz 2014) because of the metabolic energy they create and the heat they absorb and release.

However, the presence of ants is not the only factor contributing to the overall inner temperature of the mounds- mound size also has an influence. Larger mounds have been shown to be better at thermoregulation than small mounds (Conway 1996), which is supported by our results. Figure 5 shows the temperature fluctuation of a small mound- the mound temperature stays below ground temperature and is not warmer than air or ground temperatures throughout the night, whereas figure 6 shows a large mound gets even warmer than ground temperature. Conway 1996 also found that large mound temperature stays above ground temperature at almost all times of the day, which is reinforced by our results.

Large mounds may also support a greater colony size (Conway 1996), which in turn would affect the inner temperature because more ants leads to more activity which results in more metabolic heat released. Our findings also support this explanation because, in a large

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mound, we can see that the temperature increases much faster than in a small mound (figure 6 vs figure 5) and there is a significant difference in the number of ants per quadrat in the small mound (n=3) vs the large mound (n=13.3)(p=0.001). However, when we compared maximum width to the difference between ground and mound temperatures, there was no significant relationship between the two across both live and dead mounds (figure 8, figure 9). In this study, we looked at maximum mound width (cm) as a measurement of size because width more directly impacts the surface area of the mound that is exposed to the sun as opposed to height (Scherba, 1962).

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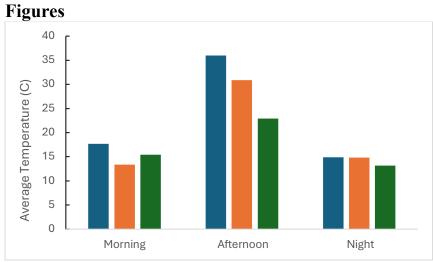


Figure 1: The variation between ground temperature (orange) and air temperature (blue) and mound temperature (grey) is greatest is the afternoon in dead mounds. In the morning, there is a significant difference between ground and mound temperatures (p=0.0001) and between air and ground temperatures (0.02), but not between air and mound temperatures (p=0.12). In the afternoon, there is a significant difference between all three categories (p=0.0005, p=0.001, and p=0.00006) respectively. At night, there is a significant difference ground and mound temperatures (p=0.0009), but not between air and mound temperatures (p=0.07) or air and ground temperatures (p=0.77).

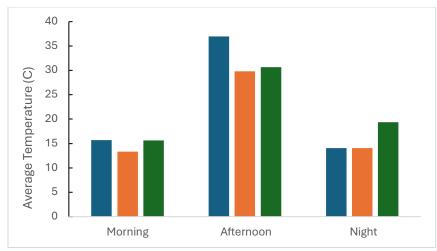


Figure 2: The difference between ground temperature (orange) and air temperature (blue) and mound temperature (grey) is greatest is the afternoon in live mounds (n=4). In the morning, there is a significant difference between ground and mound temperatures (p=0.001) but not between air and ground temperatures (p=0.06) or air and mound temperatures (p=0.96). In the afternoon, there is a significant difference between air and ground temperatures (p=0.00009) and air and mound temperatures (p=0.00007), but not between ground and mound temperatures (p=0.42). At night, there is a significant difference between ground and mound temperatures (p=0.0009) but not ground and air (p=0.76) or air and mound (p=0.07).

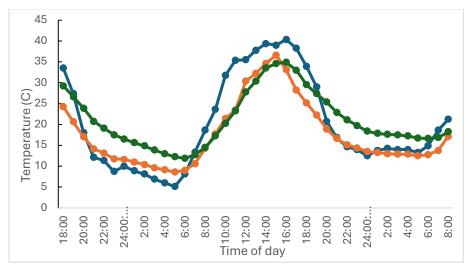


Figure 3: This graph depicts the relationships between air temperature (blue), ground temperature (orange), and mound temperature (grey) averaged across the four live mounds studied. Visual trends show that, as the ground temperature increases, mound temperature follows very closely until the air and ground temperature begin to decrease, at which point the mound temperature stays above both ground and air temperature throughout the night. The mound temperature also decreases in the evening slower than it increases in the morning.

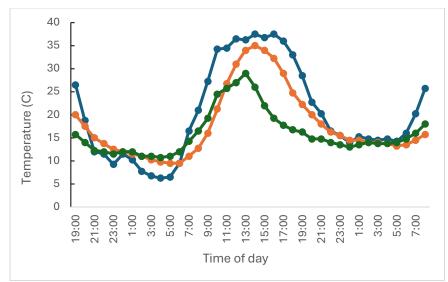


Figure 4: This graph depicts the trends between air temperature (blue), ground temperature (orange), and mound temperature (grey) averaged across the two dead mounds studied. Visual trends show mound temperature generally stays below air and ground temperature throughout the day and decreases slower in the evening than it increases in the morning.

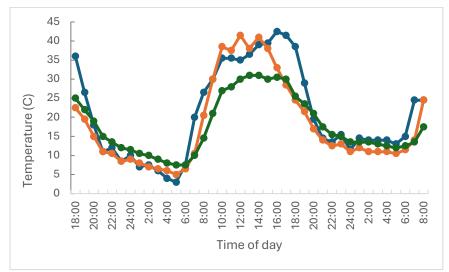


Figure 5: The temperature of a small mound rises at a rate of 0.41 from 6:00 to 14:00 and decreases at a rate of -0.47 from 16:00 to 24:00. The mound temperature stays close to air and ground temperature overnight and does not increase to ground temperature in the afternoon. This mound has a width of 25cm and a height of 7cm. We used iButtons in the air, ground, and mound to record temperatures from 18:00 on Friday, May 12th 2023 to 8:00 on Sunday, May 14th 2023.

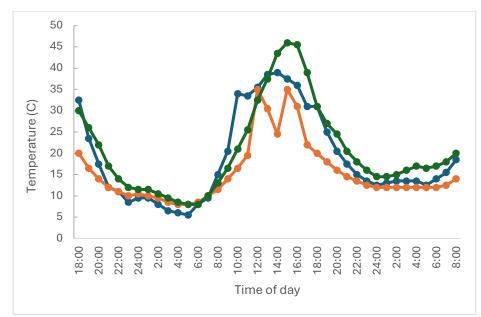


Figure 6: The temperature fluctuation of a large, live mound shows that a large mound will reach higher temperatures than both the ground and air. It has a rate of increase in temperature of 0.23 from 6:00 to 14:00 and decreases at a rate of -0.26. This mound has a width of 70cm and a height of 50cm. We used iButtons in the air, ground, and mound to record temperatures from 18:00 on Friday, May 12th 2023 to 8:00 on Sunday, May 14th 2023.



Figure 7: Map shows locations of 4 live mounds in green and 2 dead mounds in red. This study occurred within the Columbia National Wildlife Refuge in Eastern Washington from 18:00 on May 12th, 2023 to 8:00 on May 14th, 2023 and used a Garmin inReach mini to map the mounds. Latitude is 46, longitude is -119, and altitude is 245 meters.

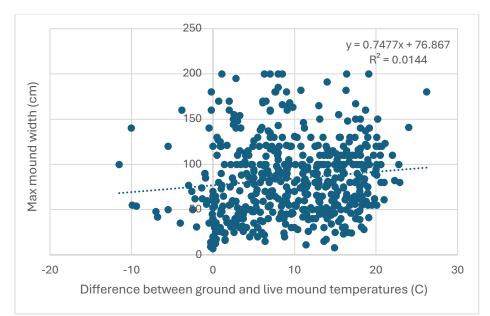


Figure 8: There is no significant relationship between the maximum width of a live mound and the difference in temperature between the ground and mounds ($R^2=0.014$), although there is a slight positive relationship between mound size and temperature difference as seen by our positive trend line. Warmer, larger mounds are in the upper right quadrant of the graph. Data were collected from 2003-2023 by students in Biology 480, and each point represents a mound (n=527).

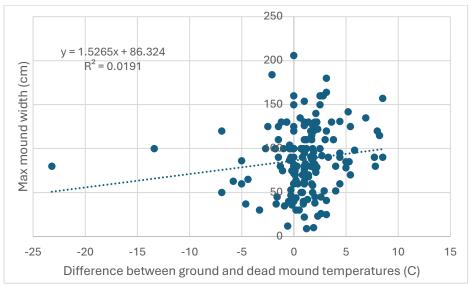


Figure 9: There is no significant relationship between the maximum width of a dead mound and the difference in temperature between the ground and mounds ($R^2=0.019$), although there is a slight positive relationship between mound size and temperature difference as seen by our positive trend line. Warmer, larger mounds are in the upper right quadrant of the graph. Data were collected from 2003-2023 by students in Biology 480, and each point represents a mound (n= 164).