The Effects of Habitat Color and Surface Temperature on Habitat Selection of Various *Melanoplus* Grasshopper Color Morphs

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EEB 381 - General Ecology
August 17, 2017
Dr. Israel Del Toro

Abstract

Habit selection plays a large role in the behavior of many organisms. Ectotherms are are unable to produce their own internal heat and rely on environmental sources to maintain an ideal body temperature, so thermoregulation may play an important role in habitat selection. In addition, decreasing the risk of predation may also be an important factor when choosing habitats. Here, we looked at habitat selection in various color morphs of *Melanoplus femurrubrum* and *Melanoplus sanguinipes* grasshoppers to see whether thermoregulation or risk of predation determines where they spend the majority of their time. We found that male grasshoppers are significantly darker than female grasshoppers, but none of our color or predation trials revealed a significant difference in color choice. However, we did see trends that generally supported the thermoregulation hypothesis: lighter grasshoppers tended to spend more time in areas with darker backgrounds while the opposite was potentially shown to be true for darker grasshoppers. These results could have implications for the future of these species, especially in the context of the climate change -- as the earth warms, it is possible that the suitable habitat for the grasshoppers that prefer cooler substrates will decrease, which could cause a change in the composition within these species as a whole.

Key Words:
Grasshopper, morphology, color morphs, predation, temperature, thermoregulation, ectotherms, habitat selection, Melanoplus femurrubrum, Melanoplus sanguinipes

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Introduction

It is well-known that organisms use camouflage to blend into their environments as a way to reduce risk of being adversely affected by predation (Isley, 1938; Edelaar, 2017; Karpestam, 2013; Karpestam, 2016; Manríquez, 2009). While this is without a doubt a key element in habitat selection for organisms, many species must also take thermal factors into consideration (Ahnesjö & Forsman, 2005; Kuyucu & Çalar, 2016). Ectotherms are unable to produce their own internal heat and, as a result, the thermal conditions of potential habitats could play a large role in determining where they are best able to survive.

In a 2005 study by Ahnesjö and Forsman, it was proposed that thermoregulation may play a larger role in the habitat selection of pygmy grasshoppers than habitat coloration, suggesting that dark color morphs which absorb more heat would preferentially stay in areas with lighter coloration and thus lower surface temperatures, relative to areas with darker substrates. It was found that grasshoppers spent significantly more time in areas that would help them maintain their ideal internal body temperatures. Furthermore, it was shown that females select habitats that are, on average, 3°C warmer than the habitats chosen by males, which was proposed to be a result of the body size dimorphism, as females are larger than males. It has also been proposed that darker grasshoppers will prefer slightly higher body temperatures than paler color morphs (Forsman 2000). This is likely because darker colors absorb more light, so dark morphs will naturally be warmer than light morphs.

Here, we partially replicate the 2005 study to see how thermoregulation and predation risk influence habitat selection in two common grasshopper species found at the University of Michigan Biological Station, *Melanoplus femurrubrum* and *Melanoplus sanguinipes*. Observing these species will help us to better understand how ectotherms adjust their body heat in relation to their surroundings, especially in the context of a warming climate.

Melanoplus femurrubrum is the most common grasshopper in North America (Capinera 2004). It is found in many different habitats, but shows a preference for grasslands. At the University of Michigan Biological Station where these grasshoppers are very abundant, we observed a number of different colorations, ranging from a light tan color to a dark brown. This makes *M. femurrubrum* an appropriate organism for semi-replicating the experiments done by Ahnesjö et al. Additionally, *Melanoplus sanguinipes* (Fabricius) is a common migratory grasshopper distributed throughout North America. It can be found in multiple habitats, the most common habitats being meadows and grasslands. The *M. sanguinipes* generally has a body color of tan, gray, and sometimes light green. These grasshoppers are also abundant at the University of Michigan Biological Station and are very similar looking as well as have similar habits to *M. femurrubrum*.

We predicted that habitat selection would be based on minimizing the risk of predation over thermoregulation. To test this, we had three generalized colors from the grasshoppers original habitat to see which one they would stay on. Then we had a plastic bird swing over them while a bird call was played simultaneously to see how they reacted. The objective of these tests were to see is if similar reactions from the male and female grasshoppers were similar to the results of the 2005 study.

Methods:

The study was conducted using *M. femurrubrum* and *M. sanguinipes* grasshoppers from the UV field at the University of Michigan Biological Station in Pellston, Michigan. The UV field is a grass plain that is occasionally disturbed by human activity. A trail runs through the center. The field is surrounded by a temperate forest nearby a cabin community. Forty specimens were collected along different habitat types such as the sand trail, native vegetation, and dirt patches. While collecting, we aimed to create an equal proportion of males to females.

M. femurrubrum and *M. sanguinipes* grasshoppers were initially kept and cared for within a tank for up to a week. Vegetation from their natural habitat and sand were provided as well as water-soaked paper towel for drinking. Paper towel was replenished every day. During the experiment, each specimen was given its own centrifuge tube supplied with vegetation and water-soaked paper towel. Specimens were separated and their centrifuge tubes were numbered in order to accurately record each individual's experimental results. After experimentation, the grasshoppers were released back into the UV field.

This study and its methodology follows a study conducted by Jonas Ahnesjo and Anders Forsmanon on pygmy grasshoppers but has been modified from the original study. Our study consists of four experiments testing the color preference: color preference without any further manipulation, color preference with respect to heat, color preference with respect to predation, and color preference with respect to predation and temperature changes in environmental substrates. For the control experiment, the bottom of a ten gallon glass tank was divided into two rows with six columns creating a checkered pattern of alternating between tan, green, and black colored paper. The colors from the bottom extended up the side of the glass tank;

therefore, none of the outside environment was visible from the inside of the tank. These colors were obtained by photographing the sand, vegetation, and shadows of the natural environment and isolating them using Photoshop version 13.0.1x64. Additionally, the papers were attached to the inside of the tank and covered with plastic to eliminate potential contamination between trails, and each color was equally represented within the tank. The temperature of each colored paper was measured five times in different locations by using a digital thermometer, creating an average temperature for each color.

Color Trials

To begin the trial, a single *M. femurrubrum* or *M. sanguinipes* was selected and placed in the center of the tank under a plastic cup for three minutes in order to acclimate to its environment. Then, the cup was removed and the trial was recorded for twenty minutes. During that time, we looked in the tank every minute to see what color the grasshopper was on. Plastic wrapping containing holes for breathing covered the top of the tank to ensure the grasshoppers would not escape. After the trial, the tank was cleaned with alcohol wipes and the *M. femurrubrum* and *M. sanguinipes* individual continued onto the next experiment.

Predation Trials

For the experiment testing color preference with respect to indoor and outdoor predation, the same setup was followed with exception to the introduction of a predator and the length of the trials. A plastic bird model was hung from fishing line and attached to a meter stick. After the three minute acclimation period, the trial began and the position of the grasshopper was recorded every 20 seconds. After 1 minute, the predation simulation began and the call of the house sparrow, - a bird native to the region - was played while the bird model was swung above the tank. The trial time for this experiment was shortened to five minutes in order to decrease the chance that the grasshoppers acclimated to the stimuli.

Heat Trials

Again, for the experiment testing color preference with respect to temperature changes in environmental substrates, the same setup was followed as the first experiment with exception to the location of the trial. The trials were conducted in tanks outside in order for the different colored backgrounds to absorb sunlight and change temperature in accordance with the wavelengths that they absorb. Tanks were left in the sun for thirty minutes before the onset of trials.

Data Analysis

After experimentation, the sex and color of each specimen was evaluated and recorded. We photographed each grasshopper and utilized Photoshop to isolate their colors. The color of the wings above the thorax was used as our measure for grasshopper color. Then, we recorded the RGB and Lightness values of the color swatches; these values were later used to assess a gradient of grasshopper colors from light to dark.

All data analysis was done using R version 3.3.2. The program ggplot2 in R was implemented to create all the graphical representations. An ANOVA test allowed us to determine whether or not there was a significant difference in the average temperature of the different color backgrounds for each experiment. Lastly, a scatter plot containing error bars was created to evaluate if there is a significant difference in the average percent of time spent on each colored background for each color of grasshopper.

Results

A Welch two sample t-test revealed a color difference between males and females - males were found to be significantly darker than females (t-test, $p<2.2e-16$). In the color trials, no significant difference was found between the proportion of time that males and females spent on each color (Wilcoxon test; Proportion on Tan: p=0.5156; Proportion on Green: p=0.9818; Proportion on Black: p=0.6923). Similarly, no significant difference was found between the proportion of time that males and females spent on each color in the predation trials (Wilcoxon test; Proportion on Tan: p=0.4555; Proportion on Green: p=6926; Proportion on Black: p=0.3717), the color & heat trials (Wilcoxon test; Proportion on Tan: p=0.9823; Proportion on Green: p=0.5883; Proportion on Black: p=0.3494), or the predation & heat trials (Wilcoxon test; Proportion on Tan: p=0.5114; Proportion on Green: p=0.6263; Proportion on Black: p=0.8803).

An ANOVA test and linear model revealed no significant difference between the time that grasshoppers with varying lightness spent on each background color for the full data (p=0.8381, Adjusted R^2= -0.008572), the color trial data (p=0.4494, Adjusted R^2= -0.002524), the color x heat data (p=1, Adjusted R^2= -0.06333), the predation trial data (p=0.9941, Adjusted R^2 = -0.05608), and the predation x heat data (p=0.9984, Adjusted R^2=-0.05846).

Discussion

Sex

Males were significantly darker in color than females (Figures 1 & 2). This may be attributed to sexual selection. Sexual selection is a theory that explains exaggerated characteristics in males or other sexual dimorphisms that are unlikely to contribute to their survival (Clutton-Brock 2009). These traits may have been selected for due to the advantage they give males in intraspecific competition or female preference for mates with these traits (Clutton-Brock 2009). Darker color in males may provide some survival advantage in terms of thermoregulation which would therefore not deem it as a trait associated with sexual selection. Our analysis suggests that trends regarding thermoregulation exist but are not statistically significant. Furthermore, this difference in coloration may simply be attributed to sexual dimorphism. Sexual dimorphism is the morphological difference between males and females, not including primary sexual characters (organs that produce gametes in males and females) (Oxford Reference). However, there was some overlap in terms of the distribution between female and male colors which argues against this proposal.

Color Trials

Though none of the data was shown to be statistically significant, it can be seen that the lighter the grasshoppers were, the less amount of time they spent on the tan proportions of the tank and the more amount of time they spent on the black proportions (Figure 3). Similarly, the darker the grasshoppers were, the more time they spent on the tan proportions of the tank and the less amount of time they spent on the black proportions. In females, it was seen that lighter specimens tended to spend more time on the green backgrounds while the opposite was found in males, though the correlation is not strong. These findings provide support for the thermoregulation hypothesis, even though the grasshoppers were not subjected to increased temperature during these trials. However, an alternative hypothesis may contribute to the trends observed in males. Perhaps darker males spend more time on lighter backgrounds in order to become more apparent to females. Becoming more visible to females may increase their reproductive success and therefore present an evolutionary advantage. Our experiment did not focus on mating behaviors, but further investigation may present interesting findings regarding this hypothesis.

Color x Heat Trials

In the Color x Heat trials, it can be seen that light female grasshoppers tended to spend a larger proportion of time on the tan portions of the tank while light male grasshoppers tended to spend a larger proportion of time on the black part of the tank (Figure 4). However, our trends were not statistically significant. Exposing our tanks to direct sunlight for 30 minutes created a sort of greenhouse in terms of temperature. The temperatures in the tank were higher than those typically experienced by grasshoppers in the wild. Additionally, due to the rectangular shape of our tanks, some of the sides casted shadows. Many grasshoppers were observed to spend time in the shaded areas over being in areas of direct sunlight. This may have been an attempt to minimize the stress caused by the intense heat. Therefore, every color in a shaded area would have been preferred as opposed to direct sunlight, confounding our test of color preference in the presence of heat.

Predation Trials

Once again, despite the fact that none of the findings for the predation trials were shown to be statistically significant, it can be seen that lighter grasshoppers tended to spend less time on the tan portions of the tank and more time on the green and black portions (Figure 5). This was shown to be true for both sexes. Light female grasshoppers tended to spend more time on the black portions of the tank, while light male grasshoppers spent nearly equal amounts of time on the black and the green portions.

Additionally, it can be seen that most of the grasshoppers spent either 0% or 100% of the time during the predation trials on just one color. Though there is not strong evidence that one color is preferred over another, this behavior is still interesting because it suggests that the grasshoppers remained motionless throughout the majority of the trials. This could be an adaptive behavior -- remaining still could prevent predators from seeing the grasshoppers and thus decrease their risk of being caught.

Predation x Heat Trials

From our predation and heat trials, although the results are not statistically significant, we see that lighter females tended to spend more time on green colored backgrounds (Figure 6). Darker colored males spent more time on green colored backgrounds and lighter colored males spent more time on both tan and black backgrounds. Here we are presented with the trade-off between coping with predation and thermoregulation. The results are not statistically significant. It is difficult to say which played a bigger role, especially with the confounding variables of unnatural heat intensity and shading within the tanks and the reduction of movement due to predation.

Conclusion

Though our results regarding the effects of heat and predation were not statistically significant, most trends did support the claim that thermoregulation plays a larger role in habitat selection, which contradicts our predictions. This information could be used in future management efforts for conserving biodiversity in the face of climate change. Grasshoppers and perhaps other ectotherms may choose their habitats based on maintaining optimal internal temperatures. Perhaps increasing temperatures may cause darker color morphs to spend more time in the tan sand which would make them more susceptible to predation. This may cause lighter color morphs to persist in the population while the darker morphs decline. Overtime, the lighter colors may reach fixation.

Acknowledgements

We would like to thank our Professor Israel Del Toro for the helpful experiment design suggestions as well as help us figure out graphs to use from R. We also want to thank the insects professor, Dr. Brian Scholtens, for showing us how to identify the grasshopper genus and species. Thanks to Nicholas Elton for his help in tank construction and to Sherry Webster for her assistance with acquiring the materials that were necessary to make this experiment possible.

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Figures

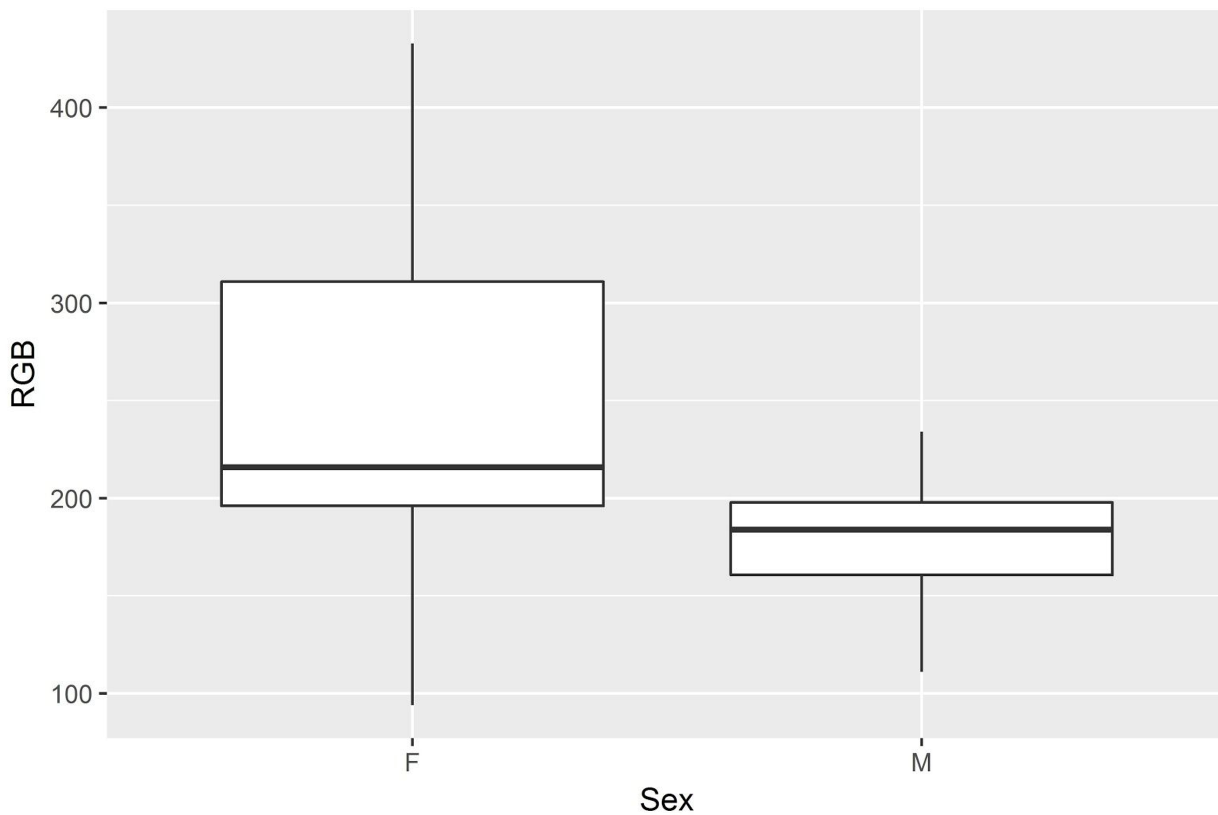

Figure 1. The difference between the sum of RBG values separated by sex. A significant difference was found between male and female coloration -- males were found to be darker than females (t-test, $p < 2.2 \times 10^{-16}$).

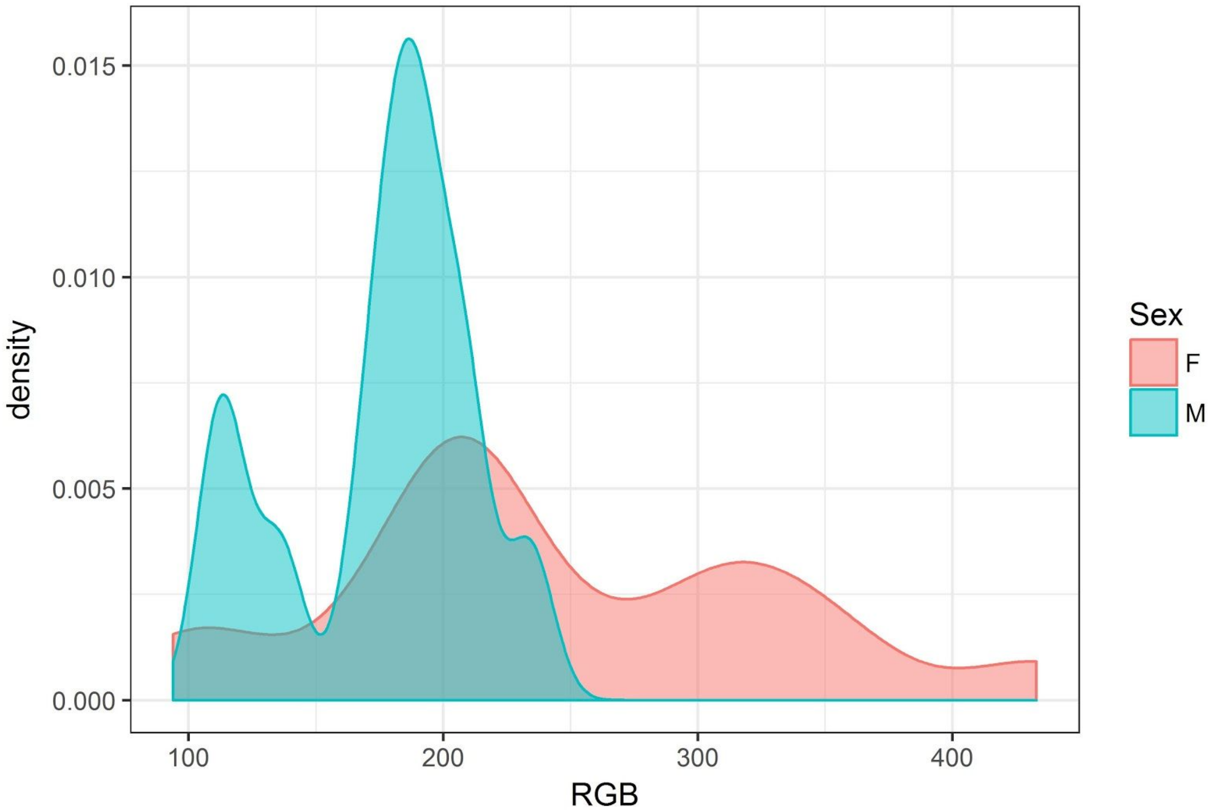

Figure 2. A density plot representing the color differences between male and female grasshoppers. Once again, we see that males are significantly darker than females (t-test, $p < 2.2 \times 10^{-16}$).

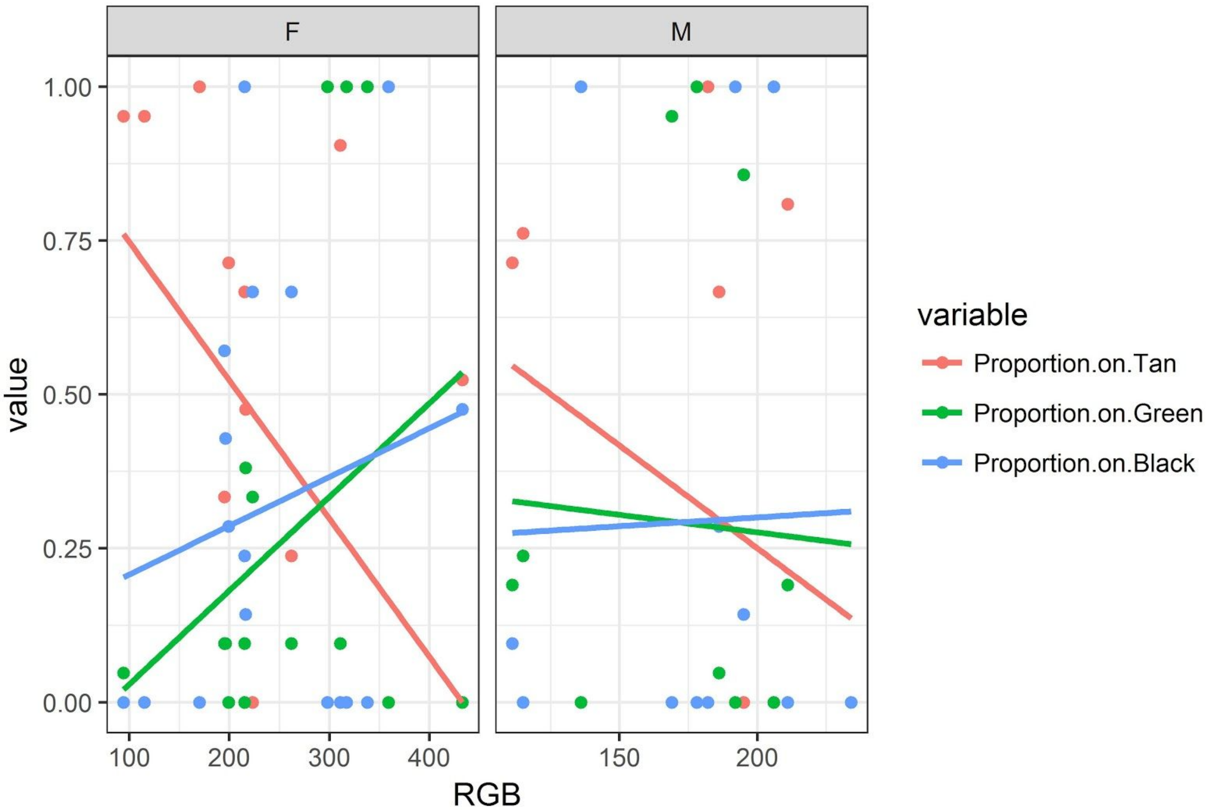

Figure 3. Color Trials; the proportion of time spent on each color separated by sex. No significant relationships were found (p=0.4494, Adjusted R^2= -0.002524), but general trends show that lighter grasshoppers tend to spend less time on lighter background colors and more time on darker colors. The opposite is true for dark grasshoppers.

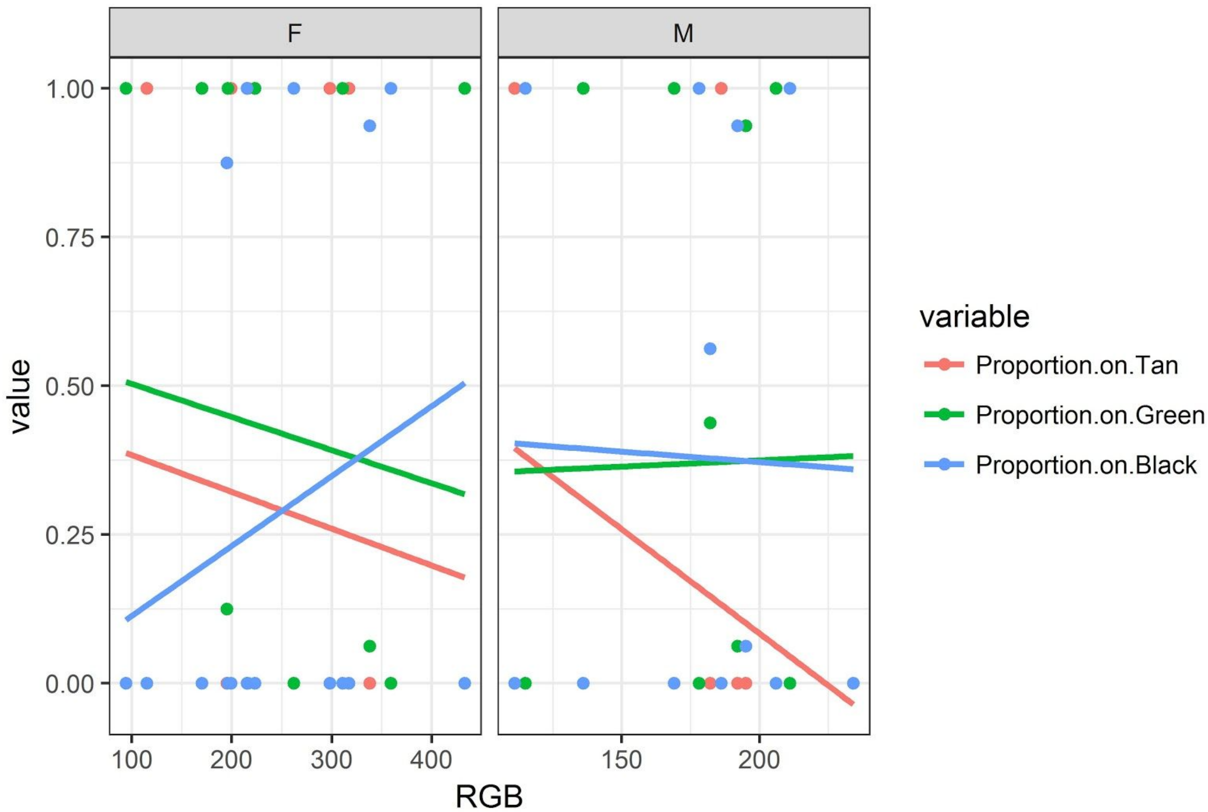

Figure 4. Predation Trials; the proportion of time spent on each color separated by sex. No significant relationships were found (p=0.9941, Adjusted R^2 = -0.05608). However, we still see the same general trend that was observed in the Color Trials: lighter grasshoppers tend to spend less time on light colors and more time on darker colors. The opposite is true for dark grasshoppers. Additionally, it's important to note that the majority of grasshoppers remained on one color during the Predation Trials (value=0.0 or 1.0).

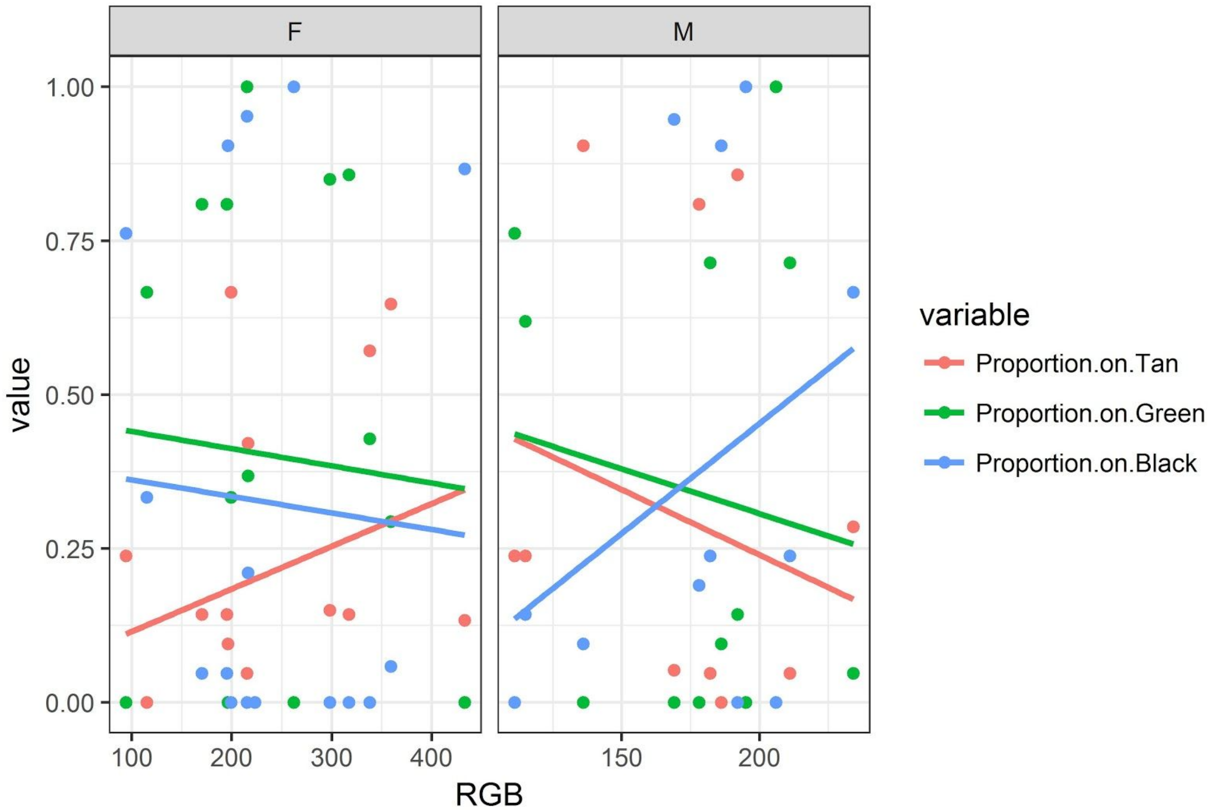

Figure 5. Color x heat trials; the proportion of time spent on each color separated by sex. No significant relationships were found (p=1, Adjusted R^2= -0.06333). The general trends show no preference in light females for any color over another, while light males tend to prefer dark colors over light colors. The opposite is true for light males.

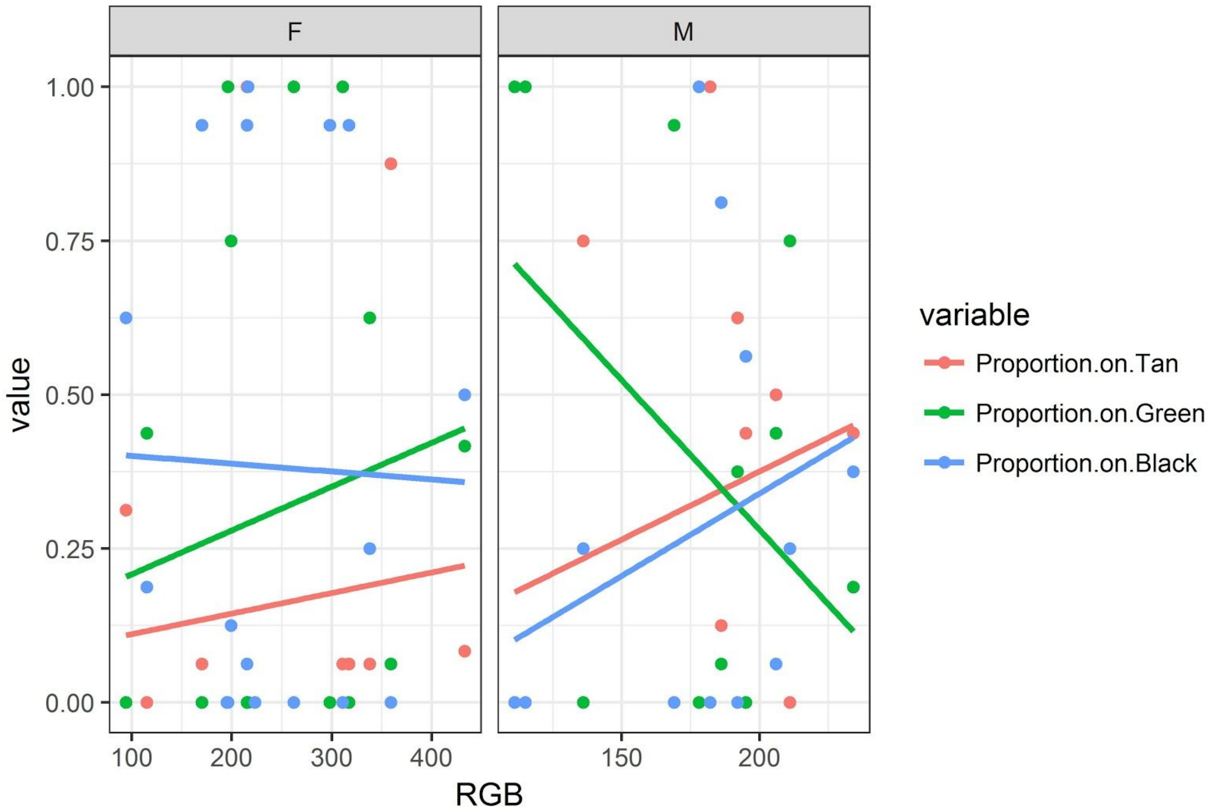

Figure 6. Predation x heat trials; the proportion of time spent on each color separated by sex. No significant relationships were found (p=0.9984, Adjusted R^2=-0.05846). Dark females showed a slight preference for dark substrates, but light females showed no real preference for any color. Dark males, on the other hand, showed a strong preference for green and light males showed a slight preference for lighter colors.