Grasshopper coloration and its effect on camouflage behavior of *Melanoplus spp.* communities at the National Bison Range

Daniel J. De Jesús

Advisor: José C. Wharton

Summer 2017

Abstract

Grasshoppers play very important roles in grassland ecosystems worldwide and have an immense potential economic importance in western US due to their periodic outbreaks in rangelands. In grasshoppers, color polymorphism has been associated with the need for crypsis and camouflage. Previous studies have noted that because distinct types of substrates differ greatly in various significant factors like temperature, humidity, structure and color, it is not yet fully understood why grasshoppers prefer certain substrates rather than others. Because these animals are such important drivers for many environmental processes, a better understanding of how their anatomical adaptations, like coloration, influence their behavior is essential. In this experiment, I studied how grasshopper coloration affects their preference towards specific substrate colorations. The particular hypothesis tested was that different *Melanoplus spp.* grasshopper communities that live in the National Bison Range select substrate colors based on their body coloration. We found that, although the hypothesis was not fully supported, this study suggests that these communities of grasshoppers generally prefer substrate colors that represent the color of vegetations in their natural habitat. Furthermore, it was observed that this preference may be guided by how uniform is the vegetation cover of the habitat in which the communities live.

Introduction

Over time, organisms have been developing a diversity of evolutionary strategies in order to survive. Those who manage to do so at the right time, reproduce, while those who do not, go extinct. This constant evolutionary pressure has driven life into producing a variety of strategies that aid their survival. Among these strategies, coloration could be one of the most evident adaptations that are displayed by living things. The biological use of colors has been commonplace throughout most of the world’s habitats: organisms are able to use coloration as a defense mechanism against predators, or as a tool that facilitates catching prey, or even as a garment to attract potential mates. For example, some orthopteran populations have managed to exhibit a variety of colorations that help them survive and reproduce, one of these adaptations being the density-dependent warning coloration in locust populations (Sword 1999). Although regular grasshoppers do not use density-dependent morphological changes, they are able to manifest color polymorphism to a certain degree.

Color polymorphism in grasshoppers has been associated with the need for crypsis and camouflage (Valverde and Schielzeth 2015). For instance, the results of a study conducted by Forsman (2002) affirm that color polymorphism in pygmy grasshoppers (*Tetrix undulata*) is influenced more strongly by selection imposed by visually-guided predators, than by fitness effects of better thermoregulation. This would suggest that grasshopper coloration has a stronger correlation with their survival than other external ecological factors, hence the need for an improving camouflage. This arises the question of whether or not this is a widespread phenomenon throughout grasshopper communities of different species or if it is a result of the specific ecological contexts of pygmy grasshoppers.

Grasshoppers play very important roles in grassland ecosystems worldwide. They comprise significant components of biodiversity, contribute greatly to grassland function, and exhibit an immense potential economic importance due to their periodic outbreaks in western US rangelands, often eliciting urgent human intervention (Branson 2006). Because these animals are such important drivers for many environmental processes, a better understanding of how their anatomical adaptations influence their behavior is essential. Moreover, because distinct types of substrates differ greatly in various important factors like temperature, humidity, structure and color, it is not yet fully understood why grasshoppers prefer certain substrates rather than others (Ahnesjö and Forsman 2006).

In this experiment, I sought to address this knowledge gap by studying how the coloration of grasshopper populations of the *Melanoplus* genus (Orthoptera: Acrididae) affects their preference for specific environmental colorations. I focused on determining if there is a relationship between grasshopper coloration and their camouflage behavior. The particular hypothesis tested was that different *Melanoplus spp.* grasshopper communities that live in the National Bison Range select substrate colors based on their body coloration.

Materials and Methods

Field site

Established over one hundred years ago, the National Bison Range (NBR) is one of the first and premier wildlife refuges in the United States of America. It is located in the center of the Flathead Indian Reservation in western Montana and its scenery includes mountain ranges in every direction. On the NBR lives a variety of wildlife across the plant and animal kingdom, including an array of grasshopper species and the wild bison that had been saved by members of the Confederated Salish and Kootenai Tribes at a time when the bison were on the verge of extinction (Upton 2014).

Field sampling and locations

Sampling was conducted during the month of July 2017 at the NBR. Three locations were sampled for *Melanoplus spp.* grasshoppers using an insect net (30.5 cm diameter, 68 cm height). The nets were swung horizontally over the ground where grasshoppers were spotted, and were checked periodically to confirm collection. Then, the captured individuals were stored in plastic bags until brought to the laboratory space for further examination. The date, elevation and coordinates were recorded for each general location where grasshoppers were sampled (Table 1).

The first location, named Pauline, is part of a small, flat valley that consists mainly of a diversity of grass and forb species, as well as dirt. The second location, Triskey, is located at a hillside, is located at a higher elevation than Pauline and more humid than the other locations because a small stream runs through the area. The last location is High Point; it is the least flat and most rocky of all sampled locations and it sits at one of the highest peaks in the NBR.

Insect identification

The target species for this experiment were: *Melanoplus sanguinipes* and *Melanoplus bivittatus.* According to survey data gathered by the Belovsky Lab for the database of the NBR Long Term Research in Environmental Biology (LTREB), these species are in the most abundant genus of grasshoppers at the NBR. This genus of insect herbivores was identified by determining the presence of a spur-like appendage located on the throat of all grasshoppers of the Melanoplinae subfamily. The collected animals were then identified to species, sex, degree of development (nymph or adult) and size (length from head to end of abdomen), and a coloration category was assigned to each individual. The assigned coloration categories were: brown, light brown and beige. These categories were assigned according to the coloration patterns of each species, and were a representation of how clear or dark was the tone intensity of the individual complexions.

The animals were identified to species using the book by Ralph D. Scott, "Montana Grasshoppers, Katydids & Crickets: A Pictorial Field Guide to the Orthoptera" (2010). *M. sanguinipes* nymphs generally have a discontinuous line that runs through their hind femur, while the adults have long forewings and bear a row of dark spots centrally. Comparatively, *M. bivittatus* adults have two pale yellow stripes that run from the tip of the head to the forewings, are usually bigger and have an overall darker complexion than sanguinipes individuals. Only adult grasshoppers and larger nymphs (>15mm overall length) were used. All *M. bivittatus* individuals that were used were in the adult stage.

There is a possibility that some adult, female *M. sanguinipes* were, in fact, *Melanoplus femurrubrum*. Although all *M. sanguinipes* nymphs and adult males were identified correctly, I was not able to identify the adult females with 100 % confidence. However, I can be certain that no *M. femurrubrum* nymphs were present during collections done before July 19.

Laboratory setup

Similar to the strategy that was used by Ahnesjö and Forsman (2006), in this experiment, the inside space of a glass tank (50 cm long, 25 cm wide, 32 cm tall) was divided into 4 equal sections. The floor and walls of each section were then covered with sheets of construction paper of a specific color. The selected colors were: beige (HEX: #fbdd9d), gray (HEX: #bfbcb6), green (HEX: #37775b) and brown (HEX: #5c4033). These section colors were chosen as a depiction of real environmental tones to which the grasshoppers are exposed in their natural habitats (i.e., beige resembles dry vegetation, gray resembles rocky terrain, green resembles vegetation, and brown resembles bare soil).

The glass tank was set outdoors, at ground level, in the shade. In order to avoid having large fluctuations of light availability and temperature across the different sections, a heat lamp (Halogen, FL25° beam, 100 W, 120 V) was set approximately 1 m above the ground, over the center of the tank, and was left on to heat the tank for at least 30 minutes before starting a set of trials. Temperatures varied only 1-2 °C across floor sections.

Running the trials

Grasshoppers were only used once within 24 hours of their collection. Before starting each trial, the temperature inside of the tank was carefully measured at different points in each floor section using an infrared laser thermometer (Tekpower DT-8380). Each of the 4 sections was measured 5 times (in its four corners and its center) for a total of 20 temperature values taken before every trial.

After this, a grasshopper was placed in the center of the tank, where all sections met, under a plastic cup (9.5 cm diameter, 12 cm height). The animals were then allowed to habituate to the temperature of the tank for 3 minutes before being released inside the tank by removing the plastic cup. In order to document in what section each animal was spending more time, grasshopper positions were recorded every 30 seconds for 5 minutes, yielding a minimum of 10 observations per individual. If a grasshopper was observed standing on the line where two sections met, with their bodies positioned so that half of the animal would be in one section and the other half in another section, then, only in these instances were the observations counted twice, yielding 2 observations, 1 for each of the sections.

Statistical analysis

All statistical analyses were done using R (R 3.4.1, GUI 1.70, El Capitan build), RStudio (version 1.0.153) and R Commander (version 2.3-2, XQuartz 2.7.11, xorg-server 1.18.4). A two-way contingency table was done to verify if the sampled population had a different amount of grasshoppers of a specific coloration category, according to species. In order to determine if differently colored grasshoppers chose different section colors, I ran a two-way contingency table with the assigned coloration categories as the independent variable and the selected sections as the dependent. This test was done individually for each species.

Additionally, to identify if grasshoppers of different elevations chose different section colors, I ran a two-way contingency table with the locations as the independent variable and the selected sections as the dependent. Finally, to establish if temperature had a relationship with the selection process of the grasshoppers, I regressed the individual temperatures of each section against the total amount of observations in the same section.

Results

In terms of the sampled populations, 75 individuals were used in total for this experiment, of which 50 were *M. sanguinipes* and 25 were *M. bivittatus*. Of the 75 individuals, 38 were females and 37 were male. Within the sampled population, there were 20 individuals of the beige category, 28 of the light brown category and 27 of the brown category. Finally, regarding the sampled locations, there were 22 individuals from Pauline, 22 from Triskey and 31 from High Point (Table 2).

M. sanguinipes had a significantly higher amount of light brown individuals, while *M. bivittatus* had a significantly higher amount of brown individuals (Table 2, $X^2 = 14.713$, df = 2, p-value = 0.0006384). Selection of section colors varied significantly among all coloration categories in *M. sanguinipes* (Table 3a, $X^2 = 84.607$, df = 6, p-value = 3.981e-16) and *M. bivittatus* (Table 3b, $X^2 = 61.948$, df = 6, p-value = 1.808e-11).

Moreover, the selected sections were significantly different among individuals from High Point (Table 4, $X^2 = 129.58$, df = 3, p-value < 2.2e-16) and Triskey (Table 4, $X^2 = 26.21$, df = 3, p-value = 8.618e-06), but not for individuals from Pauline (Table 4, $X^2 = 7.3929$, df = 3, p-value = 0.06038). Lastly, according to the regressions, the section temperatures did not have a significant relationship with the selection process of the respective section color (p-values = > 0.05).

Discussion

In this study, I sought to explore how the behavior of some *Melanoplus spp.* Communities in the National Bison Range is affected by their different body colors. Several locations at the National Bison Range were sampled for grasshoppers and the collected animals were then studied for their preference in specific substrate colors, thereby advancing scientific understanding of how grasshopper coloration correlates with their actions.

Selection of section colors was not equal among the coloration categories of both species of grasshoppers. For *M. sanguinipes*, section beige was the most selected color of all. Beige individuals selected section beige a significantly higher amount of times, and the light brown individuals also selected section beige the most, followed closely by section brown. In contrast, brown individuals selected section green the most, followed by section beige (Table 3a). For *M. bivittatus*, section beige was also the most selected color. Again, beige individuals of this species selected section beige significantly more than other colors. At the same time, brown individuals also selected section beige the most, while the light brown individuals selected section brown the most, followed by section green (Table 3b).

The selection patterns of the beige grasshoppers may be presenting camouflage behavior, where they tend to visit places that look more like themselves in order to blend in and stay away from predators that rely on vision. Nevertheless, the fact that the brown grasshoppers of both species did not spend more time selecting section brown suggests that other phenomena could also be occurring. In these cases, although they are not selecting habitats that are similar in tone to their bodies, they may still be trying to hide or forage for food. For example, *M. sanguinipes* individuals chose section beige the most, followed by section green. Both of these sections represent dry vegetation and green vegetation, respectively. Hence, these grasshoppers may be displaying attraction to colors that look like good hiding and foraging places in their natural habitat. Comparatively, most *M. bivittatus* grasshoppers chose section beige, followed by section brown. These grasshoppers, having a naturally more intense tone of brown, may then be more attracted to brown than is *M. sanguinipes*. However, *M. bivittatus* exhibited the same behavior of preference towards beige, a color that is very present in their grasslands ecosystem.

When analyzing the location data, grasshoppers at higher elevations showed a stronger preference towards section beige. For example, individuals from Pauline, the location with the lowest elevation, selected section beige more than other sections, but at a marginally significant level (Table 4, p-value = 0.06038), meanwhile individuals from High Point, the highest location, had the strongest preference towards beige (p-value < 2.2e-16). Correspondingly, individuals from Triskey, the location with the second highest elevation, showed the second highest preference to beige (p-value = 8.618e-06). The observed pattern aligns with the hiding and foraging hypothesis. For instance, Pauline, being in a valley, has the most consistent presence of vegetation. High point, a rocky, mountainous location, possesses a more irregular presence of vegetation, with rocks that turn the landscape into patches of grasses. Communities living in an environment with a continuous presence of grasses, like Pauline, are perhaps behaviourally less motivated to actively search for cover in vegetation. On the other hand, grasshoppers that live in places with patchy terrains, where cover is less available, may be more adapted to constantly seek for a good hiding and foraging site.

In conclusion, the pattern observed in this experiment establishes an important reasoning concerning why grasshoppers prefer certain environmental colors. Although the hypothesis that different grasshoppers choose colors based on the color of their complexion is not fully supported, the data compiled in this study suggest that these communities of grasshoppers generally prefer substrate colors that represent the color of vegetation in their natural habitat. Furthermore, it was observed that this preference may be guided by how uniform and consistent is the vegetation cover of the habitat in which the communities live. This behavior seems to be a depiction of camouflage and foraging, but not of crypsis. The implications of these phenomena furthers our understanding on how grasshopper coloration correlates with their behavior and gives an insight into how it can be further studied. In order to better understand if grasshoppers prefer substrates according to their ecological context, I highly encourage doing the study with grasshopper populations that live in habitats with an abundance of green vegetation. In order to improve this experiment’s methodology, I recommend doing the study indoors where temperature and light availability could be further controlled. Also, using a consistent amount of nymphs throughout species is advised.

Acknowledgements

I would like to thank the whole UNDERC West team, specifically my advisor, José C. Wharton, for helping me throughout my research project. I would also like to thank Katherine L. Barrett and Dr. David G. Flagel for helping with all of the research materials and the statistical analyses. Many thanks to Dr. Gary Belovsky for letting me have the great experience of participating at UNDERC West for the summer of 2017.

Literature cited

Ahnesjö, J. and A. Forsman. 2006. Differential habitat selection by pygmy grasshopper color morphs; interactive effects of temperature and predator avoidance. *Evolutionary Ecology*. 20:235-257.

Belovsky, G. E., J. B. Slade, B. H. Stockhoff. 1990. Susceptibility to predation for different grasshoppers: an experimental study. *Ecology*. 71(2):624-634.

Branson, D. H., A. Joern, G. A. Sword. 2006. Sustainable Management of Insect Herbivores in Grassland Ecosystems: New Perspectives in Grasshopper Control. *BioScience*. 56(9):743-755.

Forsman, A., K. Ringblom, E. Civantos, J. Ahnesjö. 2002. Coevolution of color pattern and thermoregulatory behavior in polymorphic pygmy grasshoppers *Tetrix undulata*. *Evolution*. 56(2):349-360.

Scott, R. D. 2010. Montana Grasshoppers, Katydids & Crickets: A Pictorial Field Guide to the Orthoptera. *MagpieMtGraphics*.

Sword, G. A. 1999. Density-dependent warning coloration. *Nature*. 397(6716):217.

Upton, B. 2014. Returning to a Tribal Self-Governance Partnership at the National Bison Range Complex: Historical, Legal, and Global Perspectives. *Public Land & Resources Law Review*. 35:51-145.

Valverde, J. P. and H. Schielzeth. 2015. What triggers colour change? Effects of background colour and temperature on the development of an alpine grasshopper. *BMC Evolutionary Biology*. 15:168.

Other references

Colvin, J., R. J. Cooter. 1995. Diapause induction and coloration in the Senegalese grasshopper, Oedaleus senegalensis. *Physiological entomology*. 20(1):13-17.

Forsman, A. 2011. Rethinking thermal melanism hypothesis: rearing temperature and coloration in pygmy grasshoppers. *Evolutionary Ecology*. 25(6):1247-1257.

Yoon, C. K. 1999. Inside Story of Nature's Foul-Tasting Beauties. *New York Times*, 2 Feb. 1999. *Biography in Context*.

Figures

Table 1. Sampled locations information. The date, elevation and coordinates were recorded at each general location where grasshoppers were sampled.

Location	Dates sampled (July 2017)	Coordinates	Elevation (m)
Pauline	13, 19, 27	47.342170, -114.277073	795
Triskey	19, 27	47.314281, -114.189297	1220
High Point	13, 16	47.315770, -114.209040	1426

Table 2. Sampled populations information. Species at the left, coloration categories on the top. *M. sanguinipes* had a significantly higher amount of light brown individuals, while *M. bivittatus* had a significantly higher amount of brown individuals (X^2 = 14.713, df = 2, p-value = 0.0006384).

	Beige	**Light brown**	**Brown**	Total
M. sanguinipes	14	25	11	50
M. bivittatus	6	3	16	25
Total	20	28	27	

Table 3a. ***M. sanguinipes*** **selection of section colors.** Coloration categories on the left and section colors on the top. Selection of section colors varied significantly among all coloration categories (X^2 = 84.607, df = 6, p-value = 3.981e-16). Note: the numbers represent the amount of times grasshoppers of a particular coloration category were observed on a section color.

	Beige	**Gray**	**Green**	**Brown**	Total
Beige	73	32	30	7	142
Brown	32	17	61	13	123
Light brown	99	46	40	71	256
Total	204	95	131	91	

Table 3b. ***M. bivittatus*** **selection of section colors.** Coloration categories on left and section colors on top. Selection of section colors was not equal among all coloration categories (X^2 = 61.948, df = 6, p-value = 1.808e-11). Note: the numbers represent the amount of times grasshoppers of a particular coloration category were observed on a section color.

	Beige	**Gray**	**Green**	**Brown**	Total
Beige	42	3	4	11	60
Brown	76	32	43	32	183
Light brown	1	0	11	18	30
Total	119	35	58	61	

Table 4. Elevations and selection of section colors. Elevations on left and section colors on top. Note: The selected sections varied significantly among individuals from High Point (elevation: 1426, X^2 = 129.58, df = 3, p-value < 2.2e-16) and Triskey (elevation: 1220, X^2 = 26.21, df = 3, p-value = 8.618e-06), but not for individuals from Pauline (elevation: 795, X^2 = 7.3929, df = 3, p-value = 0.06038). Note: the numbers represent the amount of times grasshoppers of a specific location were observed on a section color.

Elevation in m	**Beige**	**Gray**	**Green**	**Brown**	Total
795	68	63	42	51	224
1220	87	32	59	55	233
1426	168	35	88	46	337
Total	323	130	189	152	