

Does an achromatic border alter color discrimination
of male *Speyeria mormonia* butterflies
during mate searching?

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Abstract:

Larval food stress for the butterfly *Speyeria mormonia* causes less pigmentation in female butterflies. For this reason, males may approach females with higher pigmentation more often because it is a sign that they are healthier. Past studies have shown that this could be true, but they have not taken into account the markings on the butterflies' wings. The achromatic black markings in contrast with the orange color stimulus of the wing could cause the male butterflies to perceive the color stimulus differently than they would without the markings. I added a black border to butterfly decoys made from three different colored Pantone papers, representing a super stimulus, high pigmentation, and low pigmentation, and observed approaches by males to see whether the achromatic border makes a difference in the way males perceive the color stimulus. I hypothesized that I would see a 1:1 ratio of approaches to butterfly decoy pairs with the border, and that the ratio for decoy pairs with the border would not differ from those without the border. Only the super stimulus and high pigment pair with borders did not deviate from a 1:1 ratio of approaches by males. Comparing results for decoy pairs with the border and without the border, only the high pigment and low pigment pairs differed, with more approaches to the low pigment decoy with the border than without the border. This suggests that while pigmentation does matter in the likelihood of approach by a male, so does the black border and markings. Females with low pigmentation will still be approached because of their achromatic markings causing the male butterflies to perceive the color stimulus differently.

Introduction:

Larval nutrition in butterflies can have major impacts on their life history. The butterfly *Speyeria mormonia* (Lepidoptera: Nymphalidae), is found in montane environments and eats native *Viola* species as larvae (Fipps, 2020). Some of the effects of larval food stress in the lab, which is equivalent to drought stress in the field, include lower body mass, lower potential fecundity, and shorter wing length, which may impact their flight (Boggs and Freeman, 2005). Male *S. mormonia* butterflies have larger eyes than females which are important for patrolling, flying in search of females. Caterpillars that are starved in the lab grow to adults with smaller eyes, negatively affecting their ability to patrol for females (Fipps, 2020). Nutrition can also impact the pigmentation of the adult butterfly wings. When female caterpillars are partially starved in the lab, they have less wing pigmentation, which manifests as less red wings to the human eye. This is because the production of pigmentation is very costly in nitrogen which comes from their larval diet. It has been hypothesized that female butterflies under food stress as larvae may trade the production of pigmentation for the production of eggs. Thus healthier adult female butterflies may have redder wings, and males may prefer females with redder wings (Fipps, 2020).

Past research at RMBL has shown that *S. mormonia* males may favor redder females (Alejandro, 2020). This was found by observing approaches made by males to butterfly decoys made out of four different colored paint chips. Two of the colors were matched by the human eye to butterfly wings. The other two colors were chosen to be higher and lower pigmentation than what is naturally occurring in *S. mormonia* wings to the human eye (Alejandro, 2020). However this experiment is being repeated again this summer with Pantone color samples that have been matched to the closest spectral reflectance curve to that of the butterflies wings.

Both the past study and the current study did not take into account the black markings on *S. mormonia* wings. Females in particular have a thick black band along the outside of their wings. Color signals can change depending on the physiology of the receiver, illumination, and the background color in contrast with the signal color (Davis et. al 2022). Most studies focusing on how background color affects visual perception have been done with color backgrounds. Many fewer have been done with achromatic backgrounds. In humans, brightness discrimination with achromatic backgrounds was easiest when the background and color stimuli are similar in brightness (Whittle, 1992). In other words, if the achromatic background is darker compared to the color stimuli it is harder to tell the difference in brightness between different color stimuli. A study done on zebra finches (*Taeniopygia guttata*) showed that they experienced changes in color perception and discrimination similar to that found in humans when viewing a color stimulus against an achromatic black background (Davis et.al, 2022). For the purposes of my project, the orange scales of the butterflies wings are the color stimulus, while the achromatic black border is the background.

I asked whether adding an achromatic border to butterfly decoys will alter the color discrimination by male *Speyeria mormonia* butterflies during mate searching. I predicted that the approach ratio for all pairs of decoys with black borders will be 1:1, and the approach ratios for decoy pairs with black borders will not differ from the approach ratios for decoy pairs without a border.

Methods:

Choice of Color for the Decoys:

The colors were determined by the collaboration of Chloe Keck, Dan Chappel, and Daniel Spieser at the University of South Carolina. Spectral reflectance measurements were taken of butterfly wings with varying pigmentation, and spectral reflectance measurements were taken of over 100 different Pantone colors. Using Dan Chappel's R pathway, butterfly wing measurements were matched to Pantone colors in the database by calculating the spectral reflectance curve most similar to the butterfly wing curve. The colors chosen were 716 U for the super stimulus meaning that it has higher pigment than what is naturally occurring in *S. mormonia* wings, 2014 U for high pigment, and 7573 U for low pigment.

Decoy Construction:

To make my butterfly decoys, I cut out Pantone color samples 60 mm in length, the size of a large female butterfly, in the colors 716 U (super stimulus), 7573 U (low pigment), and 2014 U (high pigment). I drew a 1.5 mm thick black border with a fine tip Sharpie all the way around the outside of the rectangle. The width of the border was determined by the wingspan and border width of 16 *S. mormonia* female images. I used ImageJ to find the average border width for a large female butterfly with a 60 mm wingspan. I then hot glued the paper to the tip of a metal flag after taking off the flag.

Field Observation:

All of my observations were made in the Kettle Ponds in Gothic, Colorado (Figure 1). In the field I observed two decoys at a time, setting them out a meter apart and level with the ground in places with a high density of *S. mormonia*. Decoys were placed so that the height of the decoy

corresponded to the average height of vegetation. I rotated through the pairs so that I observed different pairs each day. My observations were made between 900 h and 1500 h. I observed and recorded approaches to my decoys by male *S. mormonia* butterflies. I counted approaches as times when male butterflies fluttered around several inches above my decoys and it was clear that they were looking at it.

Statistical Analysis:

To analyze my data, I performed a Chi-square test for each pair of my decoys and compared each pair of my decoys with data collected by Chloe Keck using decoys in corresponding colors without the border.

Results:

Was the Approach Ratio 1:1?:

The only decoy pair where a 1:1 ratio was observed was the "super stimulus vs high pigment". In the other two pairs, the more pigmented decoy received significantly more approaches (Table 1, Figure 2).

Was the Approach Ratio the Same for Decoys with and Without Borders?:

Approaches to decoy pairs with and without borders did not differ significantly, with the exception of the pair "high vs low pigment". In that case, the pair with borders had significantly more approaches to the low pigment decoy than did the pair without borders (Table 2, Figures 3, 4, 5).

Discussion:

Males were able to distinguish among all pairs with borders except for the super stimulus and high pigment pair. This is likely because increased pigmentation does not matter after a certain point to patrolling males. Wing pigmentation production requires nitrogen that the butterfly consumes as a larva, once pigmentation reaches its upper limit it becomes a waste of nitrogen to produce more pigment (Fipps 2020). These results differ from Saimara Alejandro's (2020), in that she did see a difference with more approaches to her super stimulus than high pigmentation. This is likely because without having done spectral reflectance measurements, her super stimulus could have still been within the threshold of natural wing color where higher pigmentation does get more approaches. Alternatively, the butterflies could have responded to wavelengths in the paint chips that are not found in pigments on butterfly wings.

Males approached the low pigment decoy with the border more than the low pigment decoy without the border. My second hypothesis was that the approach ratios for the decoys with the black borders would be different than those without. This was not true for the pair 716 U and 2014 U because they are essentially the same color to the butterflies. This was also not true for the pair 716 U and 7573 U, however it was true for the pair 2014 U and 7573 U. In the high pigment and low pigment pair, the pair with borders saw more approaches to 7573 U than the pair without borders. This shows that the border does make a difference in color discrimination, and that lower pigmented butterflies are still approached because of their border. While lower pigmented butterflies will still be approached less than higher pigmented butterflies it is not as

much less as the decoys without borders would lead us to believe. Future studies could look at how borders of different thickness affect the number of approaches made by male butterflies.

Larval food stress affects male and female *S. mormonia* butterflies in different ways. In males, food stress leads to smaller eyes which can be detrimental to mate searching. In females, larval food stress leads to less wing pigmentation. While males prefer females with higher pigmentation, this project showed that females with lower pigmentation may still be approached due to their achromatic markings changing the visual perception of their wings to males. It is important to understand how food stress effects species like *S. mormonia*, because with climate change there will be more droughts and other events causing food loss.

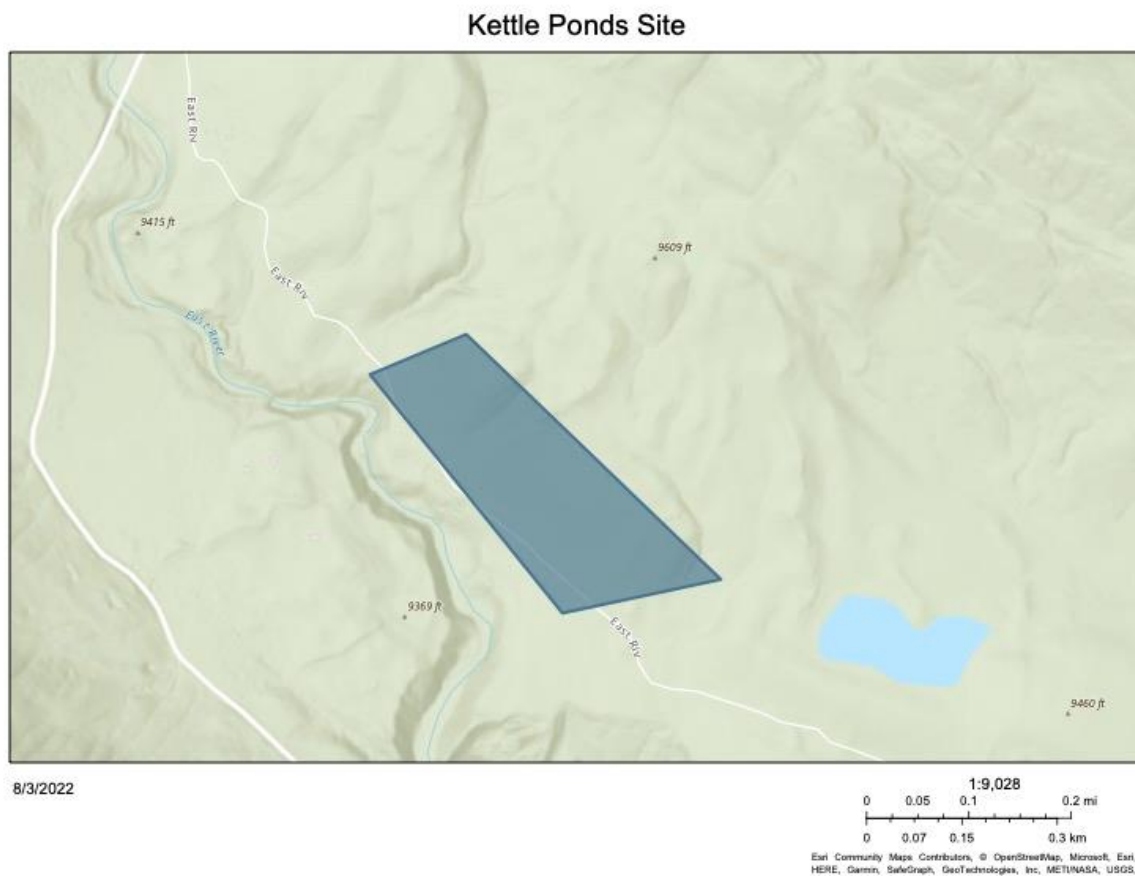


Figure 1: The kettle ponds site where I made all of my observations.

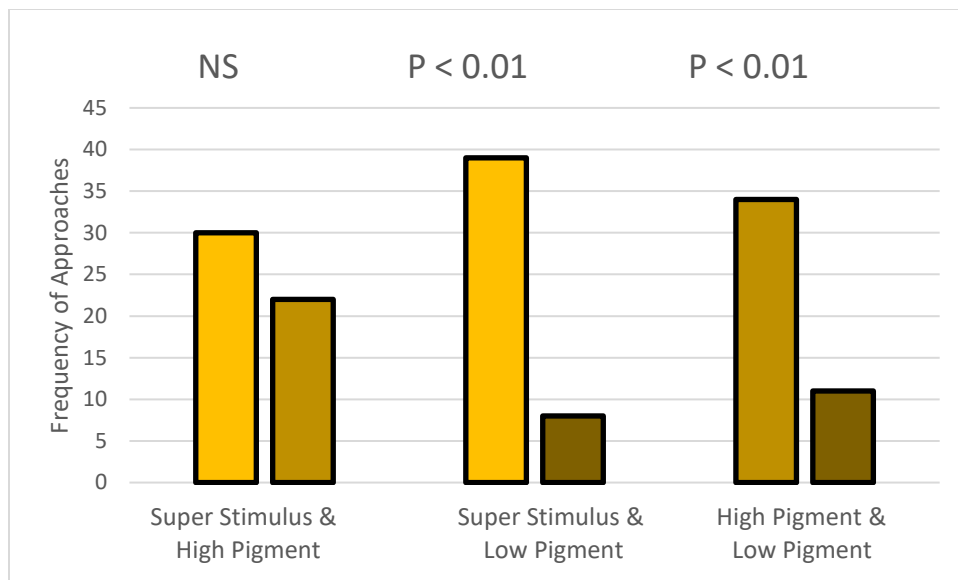


Figure 2: The approach ratio for decoy pairs with borders.

Decoy Pair	Observed #	Expected #	Chi - square	df	p - value
Super Stimulus	30	26	1.2	1	ns
High Pigment	22	26			
Super Stimulus	39	23.5	20.4	1	p < 0.01
Low Pigment	8	23.5			
High Pigment	34	22.5	11.7	1	p < 0.01
Low Pigment	11	22.5			

Table 1: Chi-square results comparing pairs of decoys with borders.

Decoy Pair	Observed # Border	Observed # no Border	Expected # Border	Chi-square	df	p - value
Super Stimulus	30	26	28	0.3	1	ns
High Pigment	22	23	24			
Super Stimulus	39	30	40	0.167	1	ns
Low Pigment	8	5	7			
High Pigment	34	23	41	24.5	1	p < 0.01
Low Pigment	11	2	4			

Table 2: Chi-square results comparing decoy pairs with borders to those without borders.

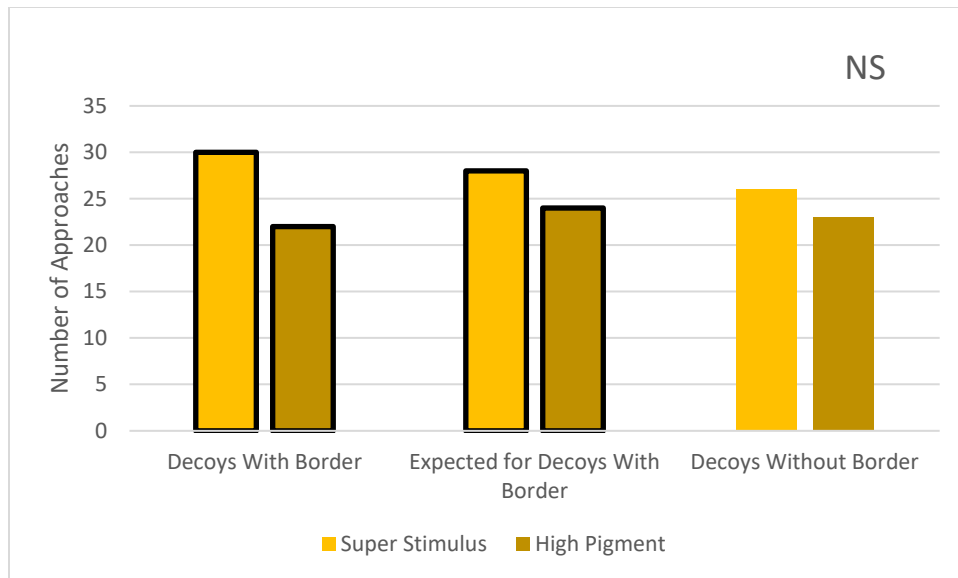


Figure 3: I recorded a total of 52 approaches by males to 716 U (super stimulus) and 2014 U (high pigment) pairs with borders. The proportion of approaches to each decoy did not differ significantly from 1:1. The results for decoys with the border did not differ significantly from those without the border.

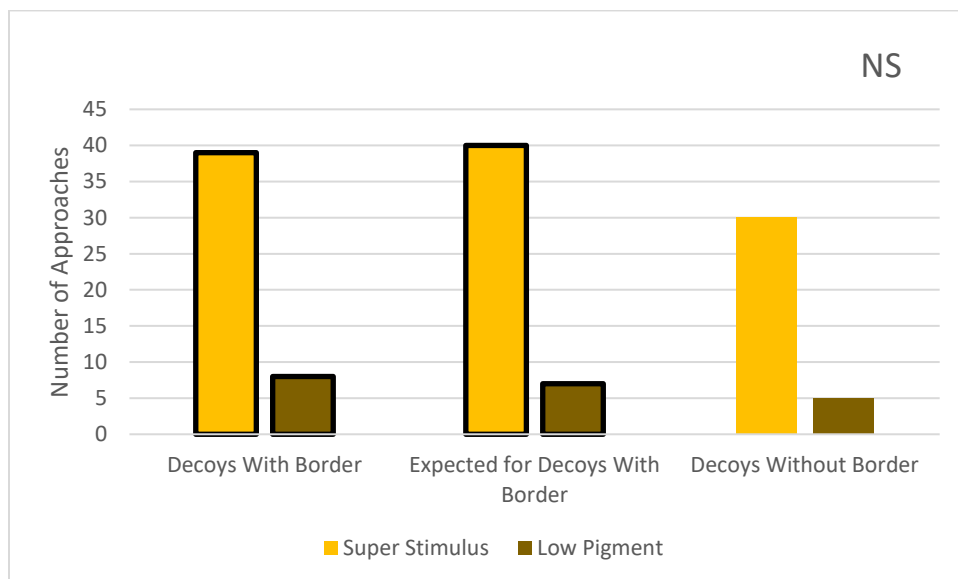


Figure 4: I recorded a total of 47 approaches by males to 716 U (super stimulus) and 7573 U (low pigment) pairs with borders. The proportion of approaches to each decoy differed significantly from 1:1. The results for decoys with the border did not differ significantly from those without the border.

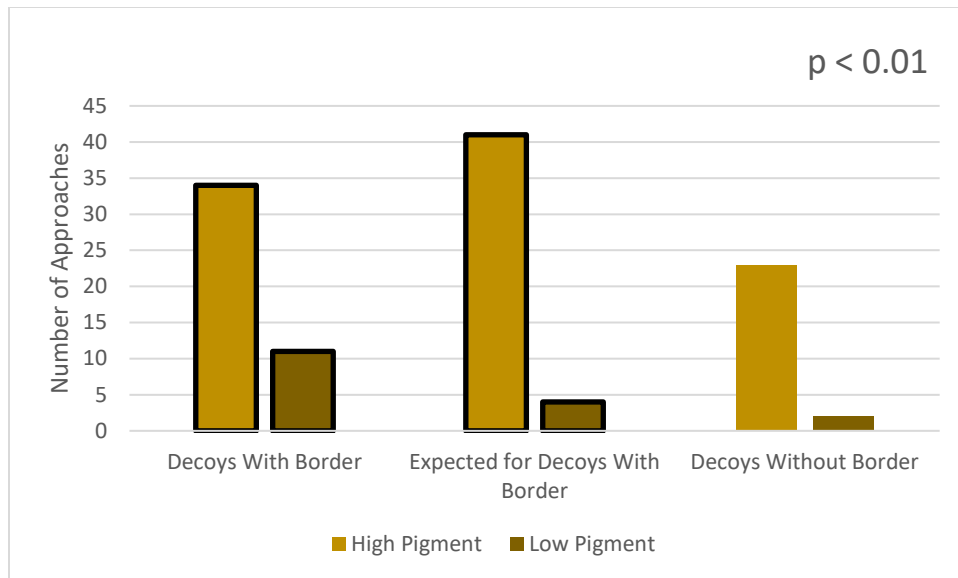


Figure 5: I recorded a total of 45 approaches by males to 2014 U (high pigment) and 7573 U (low pigment) pairs with borders. The proportion of approaches to each decoy differed significantly from 1:1. The results for decoys with the border differed significantly from those without the border.

References:

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