

ROCKY MOUNTAIN BIOLOGICAL LABORATORY

COLOR PREFERENCE OF *SPEYERIA MORMONIA*.

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2. *Project summary/abstract (<250 words).*

Butterflies detect potential mates by employing tactile and pheromone cues at close range, but wing color detection is the most important initial stage of mate recognition in courtship. Wing color is a long-range signal used by males in mate searching. Mate detection by visual cues can also be affected by the butterfly's spectral sensitivity. Previous work has shown that *Speyeria mormonia* exhibited peak spectral sensitivity at 570nm, near the orange wavelength. The possibility of males *S. mormonia* having a preference for the orange color is supported by their tendency to land on orange-colored flowers with the apparent initial intent to mate. This may suggest that orange act as behavioral cues that stimulate males to pursue a target for mating. The first goal of this study is to determine a) whether males of *S. mormonia* discriminate and have innate preferences between unnatural and naturally occurring variations of "orange" color on the wings of conspecifics within their natural range. I hypothesized that *S. mormonia* males are highly attracted to b) "redder orange" hues, and c) there may be high variation of preference in orange hues in different background settings that can make the some hues cryptic. We performed controlled mate choice experiments in the field over different colored-microhabitats using paint chips with different orange hue degrees. Males did prefer the redder orange hue over all other hues. However, no significant difference of preference was seen between the two middle orange hues, B: 67.37 and C: 63.73.

3. Project narrative/research plan. The project narrative includes the following sections:

i. Introduction:

Butterflies detect potential mates by employing tactile and pheromone cues at close range, but wing color recognition is the most important initial stage of mate detection (Kronforst et al. 2006). Lepidoptera have diverse colorful ornaments, and their hues are produced by the interaction of light with pigments on the wing scales (Stavenga et al. 2014). These wing colors tend to be more vibrant in the choosier sex that is evaluating during courtship (Robertson et al. 2005). Usually, males take the initiative in actively chasing females during courtship, and can distinguish the gender of conspecifics using visual cues and signals alone. Behavioral experiments have shown that males of some species can visually recognize females not by wing size, but by their sexually specific wing color (Li et al. 2017). While the phenomenon of female mate choice has been largely studied male mate choice has only attracted attention in recent years (Tigeros et.al), and this intersexual selection remains poorly understood.

Diurnal butterflies depend essentially on their visual sensory system when they need to detect mates and food resources from long distances (Hidaka 1973; Li et al. 2017). Butterflies have apposition compound eyes, which are composed of thousands of subunit optical receivers called ommatidia. The integration of information from multiple ommatidia produces a “pixelated” image of their surrounding environment, where each ommatidium is equivalent to one pixel. (Land & Nilsson 2012, as cited by Sanchez 2018). Because their image acuity depends on the number of lens or facets aimed at an object, butterflies can be near-sighted (Pyle & Hughes, 1992, p.19). Males however, have an advantage in mate detection by having larger eyes and therefore wider facets called “love” zones, which not only increase sensitivity, but also improve visual resolution by decreasing the blurring effects of diffraction (Rutowski 2000, Land & Nilsson 2012, as cited by Sanchez 2018). Males of *S. mormonia*, do in fact have significantly bigger eyes than females (Sanchez 2018).

Mate detection by visual cues can also be affected by the butterfly’s spectral sensitivity, which is the relative efficiency of the eye to be stimulated and detect light or other signals, as a function of the wavelength of the signal. In other words, the spectral sensitivity of an animal can help detect and discriminate certain colors more than others. Wavelengths that stimulate a maximum response represent a peak spectral sensitivity (Leech & Johnsen 2009). In the study by Sanchez (2018), *S. mormonia* exhibited peak spectral sensitivity at 570nm, which is near the orange wavelength. Although it is not known whether the peak spectral sensitivity is directly correlated to innate color preference in *S. mormonia*, colored-flower-preference field assays showed that this species is more attracted to orange-colored flowers than natural occurring yellow flowers when feeding (Pohl et al. 2011). This predilection for the orange color is reinforced by the tendency of males *S. mormonia* to land on orange-colored *Agoseris aurantiaca* flowers in the field with, in fact, the apparent initial intent to mate (Carol Boggs, personal observation, as cited

by Sanchez 2018). Furthermore, males of a European sister species of *S. mormonia*, *Argynnis paphia*, are known to prefer females with more orange and larger wings (Magnus 1958). This may suggest that orange wings act as visual cues that stimulate males to pursue a target for mating. The species affinity for orange wing colors on females may come about because it is an indicator of mate-quality, which is the case of other butterfly species, the hue of wings may be a byproduct of the larval diet consumed by females, among other environmental factors (Johnson et al. 2014, Talloen 2004, Sanchez 2018).

The first goal of this study is to determine a) whether males of *S. mormonia* have innate preferences between unnatural and naturally occurring variations of “orange” color on the wings of conspecifics within their natural range. Furthermore, I hypothesize that b) *S. mormonia* males are highly attracted to redder hue used in the experiment, which is outside the range of orange hues that females naturally exhibit in wings. I also hypothesize c) that there may be high variation in discrimination of orange hues in different microhabitats where the background colors can make the hues appear cryptic. The null hypotheses would be a) no significant preference of orange hues, b) male preference or constancy of yellow-orange hues, and, c) no variation of preference/discrimination in the different backgrounds.

ii. Methods:

A. Study Sites and Populations

The butterfly *Speyeria mormonia* (Lepidoptera: Nymphalidae) is found over a large elevational range in the Colorado Rocky Mountains (1500–3000m). Located in the Rocky Mountain Biological Laboratory Gothic, Gunnison County, CO, *S. mormonia* are typically found along the mountainous ranges in the open grasslands where various species of Compositae, which are their adult food source, and *Viola* species host plants, which are their larval food source, are present. Our study site was the southern area of South Gothic, where *S. mormonia* seemed to be abundant. We also sampled females in meadows near the Deer Creek Trail and the Longterm *Speyeria* Site for hue matching of females.

B. Hue Degree Identification of Females

In order to determine female wing hues I captured 79 females of *S. mormonia* from the Deer Creek Trail and the Longterm *Speyeria* Site. After I matched their wings hue to 21 paint chips (TABLE). Due to the species lack of wing UV reflectance, the paint chips I used are non-glossy paint chips, since these are the least that reflect light. When matching the paint chips with the female’s wings there was special attention on the dorsal hindwing and forewing because these regions are assumed to be important for mate recognition (Sanchez 2018). However, since newly emerged females have their wings closed the ventral hindwing and forewing was also evaluated (Boggs, personal communication).

Speyeria mormonia is a protandrous species, which means that species are characterized by males that emerge from the pupal stage earlier than their female conspecifics. Because average dorsal forewing chromaticity decreases as wing wear is increased (Sanchez 2018) it was imperative to collect these females and match their hues to paint chips at the beginning of their emergence from pupal stage. The peak of female emergence was around end of July (Boggs, personal communication). For the experiment, a restricted and representative of two matched paint chips were chosen by the extremity of naturally occurring hues, and two unnaturally occurring hues. The paint chips were cut into the average size of a female.

C. Hue Degree Identification of Flowers

In order to decrease the possibility of misinterpreting the interaction between the butterflies and the paint chips as nectar detecting behavior I identified the hues of the flowers present in and around the study site, using the 21 paint chips as previously done with the females (TABLE). The range of hues of the flowers in the majority falls off the range of female hues except for the hue of *Agoseris aurantiaca* which was also present in one female. However, it has been previously noted that the *S. mormonia* approaches this flower with courtship behavior.

D. Male Mate Preference Experiment

Within the South Gothic site there are four plots in total, two in road and two in vegetation. These plots present different background colors, such as road (light brown) and dry meadow (olive green). The difference in background color gave us additional information of behavior stimuli variation where the paint chips hue may not be as easy to discriminate.

The behavior of patrolling male butterflies interacting with the different predetermined orange paint chips simulating 'perched' females to examine the males' response to color was recorded. Because these butterflies were not lab-raised their prior experience was not controlled, and voluntary color preference could reflect a combination of visual ability and innate preference.

These paint chips were set in the wild near natural populations of *S. mormonia*. Two orange hue paint chips, of the four already determined, were placed one meter apart from each other. Those in vegetation plots were staked with dowels and insect pins just above the top of the vegetation, and those in road plots were be placed in the ground (Ellers & Boggs 2003). Because two paint chips were be set at a time, six possible combinations can be set in each four plots. In total 24 trials were conducted.

After placing them I watched for males of the species and their "courtship" interactions with the paint chips. While watching for males we tried to identify their sex by their veins as they have thinner veins than their conspecifics. The courtship interactions included changing their direction of their flight path towards the paint chip, approach within 20cm to 25cm of the paint chip, and unfrequently, landing on the paint chip. I recorded all observations during 10:00am to 4:00pm, when weather is permitting and *S. mormonia* seemed to be active. Each combination trial required 40 to 50 observations.

E. Analysis

Because the paint chips represent a binary variable we performed Chi-square tests to determine whether *S. mormonia* males preferred one hue over the other. The expected ratio was 1:1. We performed six Multi-way/ Chi-square Contingency Table Analyses to see the number of occurrences of each hue. The preference of each hue was assessed by treating each “interaction” period by an individual butterfly as a replicate, and comparing the proportion of approaches butterflies made to each of the hue paint chips with the null expectation of one half.

It is very unlikely the same males will be observed multiple times due to their low recapture rate (Boggs, personal communication), but to minimize the potential for pseudo replication all trials are to be performed at several replicated plots and times.

iii. **Results:**

Male *S. mormonia* chose the Carmelized orange hue over Cornucopia orange hue significantly more often than expected (126:42 observed vs. 84:84 expected, Chi-square test: $\chi^2=103.51$, $cv= 3.84$, $P<0.05$; Fig 1). Male *S. mormonia* chose the Carmelized orange hue over the Osage orange hue significantly more often than expected (63:21 observed vs. 42:42 expected, Chi-square test: $\chi^2= 20.02$, $cv= 3.84$, $P<0.05$; Fig 2). Male *S. mormonia* chose the Carmelized orange hue over the Gusto orange hue significantly more often than expected (98:11 observed vs. 55:55 expected, Chi-square test: $\chi^2= 68.81$, $P<0.05$; Fig 3). Male *S. mormonia* chose the Cornucopia orange hue over the Gusto orange hue significantly more often than expected (37:8 observed vs. 23: 23 expected, Chi-square test: $\chi^2= 18.3$, $P<0.05$; Fig 1). Male *S. mormonia* however, did not significantly chose between Cornucopia orange hue and Osage orange hue (36:46 observed vs. 41:41 expected, Chi-square test: $\chi^2= 1.218$, $P<0.05$; Fig 1).

iv. **Discussion:**

v. Literature Cited:

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