

Flower preferences and constancy in four butterfly species

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Three butterfly species from the family Nymphalidae (*Speyeria mormonia*, *Cercyonis oetus*, and *Phyciodes campestris*) and one species of Lycaenidae (*Lycaena heteronea*) were observed visiting artificial arrays of cut flower heads, along the Brush Creek Trail in the Gunnison Valley, CO. It was found that the mechanism by which butterflies identify potentially nectar-rewarding flowers is species dependant. An array of cut flower heads was constructed to control for abundance and density, and to observe and record butterfly visitation patterns. Acrylic paint was used to manipulate color to determine if flower choice was dependent on morphology or color cues. Individual butterflies were observed during a foraging bout to the experimental arrays, and the proportion of total visits was used to describe preference while the transitions made between flowers was used to determine constancy. All observed butterflies preferred *Dugaldia hoopesii* flowers to *Wyethia amplexicus* in a natural array. Control arrays were used to assure that no butterfly had a preference for or against painted flowers. When the visual cues of color and morphology were decoupled using acrylic paint, each species of butterfly had widely different visitation patterns. *L. heteronea* prefers the color yellow and *W. amplexicus* morphology. *S. mormonia* prefers the color orange and *D. hoopesii* morphology. *P. campestris* prefers the original color of each flower species, and had no morphology preferences. *C. oetus* prefers the color yellow and shows no morphology preferences. This reveals that these four species are choosing based on a combination of visual cues. It is significantly more complicated to identify the primary cues and requires more research to illuminate these cues. Constancy was not found in any of the four butterfly species in any of the experiments conducted.

Key words: Flower preference, constancy, butterfly, *Dugaldia hoopesii*, *Wyethia amplexicus*, *Speyeria mormonia*, *Cercyonis oetus*, *Phyciodes campestris*, *Lycaena heteronea*.

Introduction:

Flower visitors that fly through wildflower meadows are observed to visit certain flowers while passing over, or skipping other potentially rewarding nectar sources. Darwin (1876) was the first to propose that this selective visitation was a result of behavioral constraints. He hypothesized that insects had limited memory capacities for how to handle a flower, and therefore would specialize on one species in order to reduce its handling time. Theories attempt to describe the phenomenon of constancy among pollinating insects. Constancy is defined as sequentially visiting flowers from one species even when other flowers are available, and can occur even if the individual insect shows no preference between flower species over a longer time period. Pollinating insects can exhibit flower constancy and or preference. An insect that sequentially visits one species could simply have a strong preference for that species. An insect that is constant makes long strings of visits to the same species even if it has no preference and over a longer time period may visit both species equally (Waser 1986). Flower constancy is beneficial for plants, since it increases the likelihood of receiving pollen from another member of their species, and therefore being successfully pollinated.

Butterfly pollinators use color as a cue over relatively long distances. Scent is used by foraging diurnal animals only at close range and is secondary to vision (Weiss 2001; Reddi and Bai 1984; find more sources). However, morphology also affects flower choice since nectar rewards from certain flowers are more easily harvested than from others. Morphology is extremely important for butterflies, as they cannot hover while

feeding and require a place to land. Therefore preferred flowers have landing platforms (e.g. Asteraceae).

Flower constancy has been observed in several genera of pollinating insects. Almost all of the studies of constancy have been on bees (Leebens-Mack and Milligan 1998). Constancy has already been identified in one skipper and two Pierid butterfly species. These studies use widely different experimental methods that have shown conflicting results and are difficult to compare with each other. In addition flower preferences and constancy have never been assessed on either Nymphalid or Lycaenid butterflies. One study of *Pieris rapae* showed that the time spent feeding on one flower was reduced after the animal had practice on a single flower morph, revealing a learning curve (Lewis 1986). When this species of butterfly switched plant species, a period of slower feeding occurred until the learning curve was achieved and the animal was efficient again. The implications of these results are that handling time decreases with experience; therefore an advantage for constancy is evident.

The questions addressed in this study are: what visual mechanisms of flower choice do butterflies primarily rely on? Do they choose where to land and feed based on color, morphology, or a combination of the two? In addition this study seeks to determine if flower constancy is a widespread phenomenon among butterfly species.

Methods:

Three species of Lepidoptera from the family Nymphalidae and one species from the family Lycaenidae were used for these experiments. *Speyeria mormonia* (Nymphalidae), the Mormon Fritillary is found in high elevation meadows in the North

West United States. *Cercyonis oetus* (Nymphalidae), the Small Wood Nymph, inhabits grasslands, sagebrush flats and open woodlands across the western montane United States. It has been found to nectar more fervently than most other butterflies in the subfamily Satyrinae (Glassberg, 174). *Phyciodes campestris* (Nymphalidae), the Field Crescent lives in diverse habitats including mountain meadows and open fields. *Lycaena heteronea* (Lycaenidae), the Blue Copper, lives in sagebrush meadows, mountain meadows and dry hillsides.

A series of experiments on the four species of Lepidoptera were conducted to examine the possibility of preference and constancy in flower visitation behavior between two plant species. *Dugaldia hoopseii* and *Wyethia amplexicaulis* (Asteraceae) were chosen for this study for their frequency of visitation by common butterflies in the area. The experimental sites were chosen based on where select species of plant and butterfly were abundant, and included meadows alongside the Brush Creek trail in Crested Butte, CO. Every visiting butterfly was recorded, however due to aggressive behaviors and limited visitation, a full data set was limited to the following four species: *S. mormonia*, *C. oetus*, *P. campestris*, and *L. heteronea*.

An artificial array of flowers set up in a wildflower meadow was used for experimentation to control of abundance and clustering. Plastic flower pics with 10 ml of Floralife solution (a commercially available flower preservative chemical mixture) were set in the ground to hold each flower placed 20 cm apart in a 6 by 8 grid. The cuttings were preserved in a solution of Floralife (concentration 5ml floralife/1L distilled water) to keep the flowers viable while in the array. This grid was set up within the normal visitation sites of the Lepidoptera and was constructed to minimize disturbance of the

wildflower meadow. All non-experimental flowers that were within the grid were removed to prevent any confounding effects on the experimental array. *D. hoopesii* and *W. amplexicus* flowers were randomly placed in an array in equal abundance and observed for visitation. Visitation was defined as contact with a flower within the experimental array. To control for the effects of height, each stem was cut to the same length. The nearest naturally occurring patch of *D. hoopesii* was approximately 600 meters away from the experimental site. It was removed 100 m from the nearby patch of *W. amplexicus*, however was separated by natural barriers. This meant that the experimental array was a unique food source for locally flying butterflies.

Butterfly behavior was observed and recorded for frequency of visitation to the flowers as well as pattern of visitation in terms of the specific sequence of flowers visited. Each array was observed at minimum two days and until at least 100 visits from the focus species were observed. This data was used to determine if these butterflies are flower constant, and/or have color/morphological preferences. To test for preference a one-sample t-test was used to compare the proportion of visits to one flower type during each individuals foraging bout to the null expectation of 0.5. To test for a pattern of flower constancy, a G-test of independence was used. Butterfly was not considered constant unless $P \leq 0.05$. Constancy would be indicated if the flower next visited is not independent of the flower last visited. Bateman's index of constancy (Waser 1986) was used to determine if the pattern of transitions was random ($=0$), inconstant ($=-1$) or constant($=+1$) on a continuum.

The initial array was composed of 24 flower heads of both *D. hoopesii* and *W. amplexicus* in their natural state randomly arranged in the 6x8 grid (Figure 2). Any

patterns of preference or constancy between the two species would reveal flower species preferences as a whole. Each individual that enters the experimental patch was recorded separately, and separated and analyzed by species. Each visit as well as the specific order of transitions between flowers within the patch was recorded. The proportion of total visits to each flower species was used to determine preference, while the transitions between flowers were counted to determine if the butterflies are flower constant.

In order to isolate the effect of color as a mechanism for flower choice, the petals of each flower were painted with acrylic paint. By matching different flower species in color, or by changing the color of one species with paint - the butterfly's method of flower choice could become apparent. We determined if they are choosing based on color or morphological search images. An Ocean Optics USB2000 spectrometer was used to ensure that the paint color matches the natural color of the petals (Figure 9, 10).

An array consisting of the most visited species was constructed in which flowers were painted the two different colors (Figure 3). *D. hoopesii* petals are orange in color while *W. amplexicus* are yellow. Each array contained one flower species, where half of the flowers had been painted yellow and half orange. All the flowers were painted to ensure acrylic paint is not a factor. Any preferences observed can then be attributed to preferences for particular flower colors. An array of the two flower species was constructed where all flowers are of the same color (Figure 4). An acrylic paint was applied to all flowers, so that both species were of equal abundance within the array and all were of the same color. This was repeated twice, once for each color, however the yellow morphology array did not receive sufficient visits before the flowers ceased to

bloom. Any preferences observed during this experiment would be attributed to preferences for a particular flower morph.

A control to determine the effects of acrylic paint on butterfly foraging was also conducted. An array of unpainted and painted flowers of each species was constructed with one half the flowers receiving no treatment, and one half being painted with acrylic paint to match the natural color of the petals. We did not anticipate the butterflies to be affected by the paint during foraging as the same technique has been used previously with no adverse affect on other foraging species (Waser and Price, 1985; Melendez-Ackerman et al. 1997).

Results:

After one season of observation and at least two days of visitation to each experimental array; 3,549 visits by 10 butterfly species were recorded and analyzed. A complete data set was limited to the following four species: *S. mormonia*, *C. oetus*, *P. campestris* and *L. heteronea*. As these four species had enough individual bouts of visitation to create a complete data set with ample visits to assure sufficient power for all experiments.

All of the visiting butterflies among these experimental arrays revealed no flower constancy, even when controlling for morphology or color (Table 1). Bateman's index of consistency was used to discover if the visitation patterns were completely random, constant or inconstant (Table 1). There was a wide range from random visitation patterns to a completely inconstant pattern of visitation across the butterflies in each array. There was no pattern of randomness verses inconstancy within species or even within

experimental arrays. These butterflies were therefore neither inconstant nor random but rather a mix of the two conditions.

Phyciodes campestris preferred *Dugaldia hoopesii* in the natural array. When choosing based solely on color, this species changed its preference. It preferred the familiar color morphology combination, where it over visited the yellow painted *W. amplexicus* (Figure 5), and the orange painted *D. hoopesii* (Figure 6). In the morphology array, *Phyciodes campestris* had no preference for morphology (Figure 7). *Lycaena heteronea* naturally preferred *D. hoopesii* (figure 1). However, its color preference was for yellow in both the *W. amplexicus* (Figure 5) and *D. hoopesii* color arrays (Figure 6). When choosing based on morphology, *Lycaena* significantly preferred the *W. amplexicus* morphology over the *D. hoopesii* morphology (Figure 7). This butterfly was the only species observed feeding from *W. amplexicus* flowers. *Cercyonis oetus* did not eclose until the last day of observation for the natural array, resulting in only 5 individuals that visited the array and a statistically insignificant preference. There is not sufficient data to determine this species natural preference for either *D. hoopesii* or *W. amplexicus*. Never the less, yellow was the preferred color for both flower species arrays (Figures 5, 6), but did not reveal any morphology preferences (Figure 7). *Speyeria mormonia* strongly preferred *Dugaldia hoopesii* in the natural array (Figure 1). It preferred the color orange regardless of which flower morphology created the array (Figures 5,6). When choosing based on morphology, *S. mormonia* preferred the morphology of *D. hoopesii* to *W. amplexicus*.

All species of butterflies equally visited the acrylic paint control arrays (Figure 8). A chi square analysis was used to determine if the expected visitation (proportion = 0.5)

was significantly different from the actual visitation. Regardless of which flower species was being tested, no butterfly over visited either the painted or unpainted flowers (all $P \geq 0.35$) by species and array.

Discussion:

There was no evidence of constancy among the studied species of Lepidoptera. These butterflies were not observed making long strings of consistent visits to a particular flower, color or morphology. This foraging behavior could have implications for the effectiveness of butterflies as pollinators. Landing is the first choice that a foraging insect must make when visiting flowers. This study addresses this decision, however future research could address the time spent feeding, as well as the nature of pollen transferred by visiting butterflies. While constancy has not been identified as characteristic of foraging behavior in these four species, a similar experiment should be repeated for additional representatives of these two families as well as representatives of the families Papilionid, Pierid, and Riodinidae before any definite conclusions about butterfly constancy can be affirmed.

The results of this study differ from the previous study on the butterfly *Pieris rapae* (Pieridae). This could be explained by a phylogenetic pattern of constancy, as the butterflies in this study were from more recently diverged families. However an array of *W. amplexicus*, and *Agastache urticifolia* received a number of visits from *Colias eurytheme* (Pieridae) (Table 1). These experimental designs however are widely different. This looked at free flying adults that selectively visited an array, and followed their visitation patterns. It did not address time spent feeding on either flower. This is entirely

different from the study on *P. rapae* that looked at time spent foraging, as opposed to selective landings. However both aimed to measure and address the phenomenon of constancy in a butterfly species, and came to entirely different conclusions.

All butterflies showed a strong natural preference for *D. hoopesii* (Figure 1). When morphology was controlled for and butterflies were forced to choose based on color, widely different color preferences were revealed between species. Three species held their color preference between the two color arrays on either flower (Figure 5,6). However, in one species, *P. campestris*, it switched its color preference between the two flower species arrays. It had a preference for the familiar morphology color combination, meaning that for the arrays painted both yellow and orange, it preferred the yellow *W. amplexicus*, and likewise the orange *D. hoopesii*.

P. campestris and *C. oetus* appeared to be choosing based on color. *S. mormonia* and *L. heteronea* choose both for color and morphology, by showing preferences for both specific morphologies and colors. Due to these broad differences, no conclusions can be made about the mechanism that butterflies use to choose rewarding flowers. It is species specific when discussing color and morphological choices, and does not follow any sort of phylogenetic family patterns. Species were found to make decisions based on both color and/or morphology.

Lycaena heteronea visitation patterns changed through out the experiment. Learning is one possible explanation for this result. The species began the season with a strong preference for *D. hoopesii*, however when presented with the color arrays it consistently chose yellow, and later *W. amplexicus* in the morphology arrays. This species is the smallest butterfly, and is also the last to fly in the mornings. *L. heteronea*

was the only species observed to be successfully feeding off *W. amplexicus* flowers. The other species were observed attempting to feed unsuccessfully. *W. amplexicus* may have offered a better nectar reward due to its nectar content, or because it was receiving less visitation by the other butterfly species. This could result in the supply being less depleted by the time the *L. heteronea* had begun to fly and visit each morning.

The effect of acrylic paint on foraging butterflies is not significant. The individuals visited equally frequently both the unpainted and painted flowers in the paint control. It is known that butterflies have UV vision, and that *D. hoopesii* petals do have some UV transmittance. However, this is only at 10%, and we cannot conclude if the butterflies can detect that low level of UV light. However, if they could it did not change their foraging behavior as elucidated in the paint control. Scent has been shown to be a secondary cue in diurnal foraging butterflies, so the smell of acrylic paint would not be a large factor. The painted flowers however, were also allowed to dry before butterflies entered the experimental site.

There are several avenues of continued research that should be approached to better understand the mechanisms of butterfly flower visitation. This was just the tip of the iceberg in a widely unstudied field. The implications of this research can relate to the effectiveness of butterflies as pollinators, as well as the memory capacities of these species.

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Species	Natural array			Wyethia color			Dugaldia color			Orange Morphology		
	G	P	B	G	P	B	G	P	B	G	P	B
<i>Phyciodes c.</i>	0	1		0.680	0.410	-1	3.596	0.058	0.396	0.345	0.557	-0.172
<i>Lycaena h.</i>	1.552	0.213	0.090	3.506	0.061	0.451	0.170	0.680	0.127	1.828	0.176	-1
<i>Cercyonis o.</i>				1.495	0.222	-0.160	1.854	0.173	-0.342	0.176	0.675	-0.044
<i>Speyeria m.</i>	1.642	0.200	-1	0.109	0.742	-0.092	2.392	0.122	-0.086	0.604	0.437	0.057
<i>Colias e.</i>	0.341	0.559	0.127									

Table 1: Constancy of butterflies, by species and then by array, tested with a G test of independence. G values, P values are given. Bateman's index of constancy where (-1) completely inconstant, (0) random, (+1) constant is represented by the column "B"

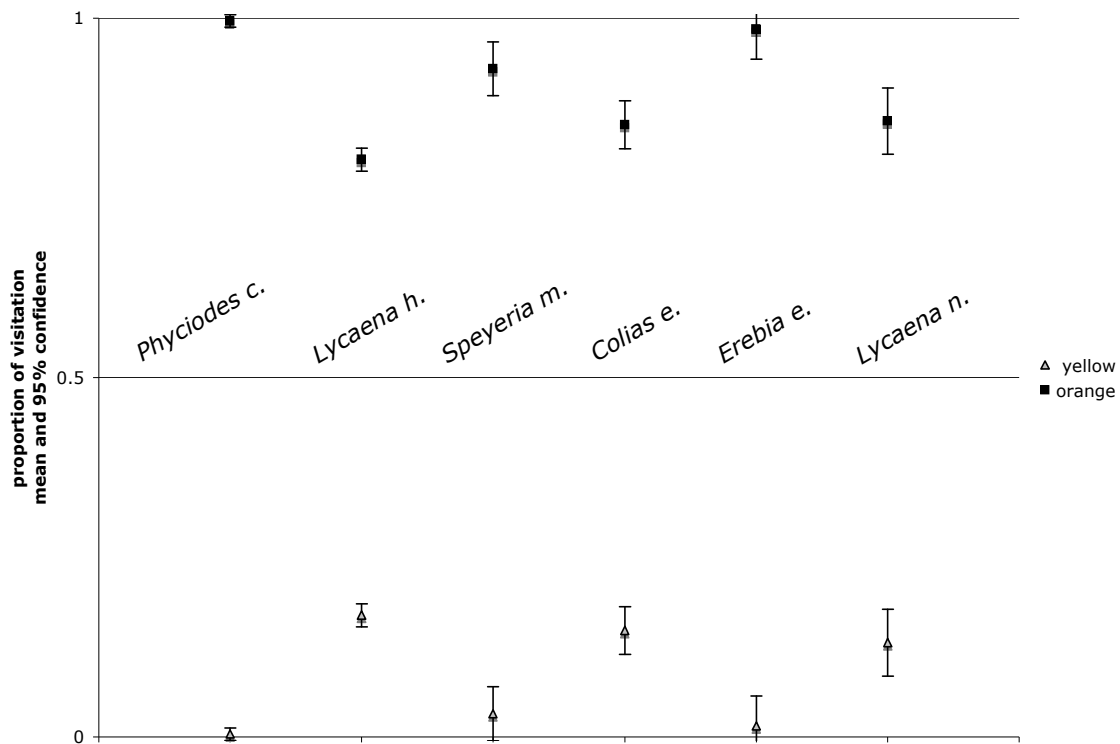


Figure 1: Natural preference between *Wyethia amplexicus* and *Dugaldia hoopesii*

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Figure 2: Natural array of two flower species, *Wyethia amplexicus* and *Dugaldia hoopesii*. Shape represents the morphology of flowers while white vs. grey represents the color of the flowers.

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Figure 3: Color array of one flower species, where morphology is kept constant but individual flower heads are painted both yellow and orange.

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Figure 4: Morphology array of two flower species, where color is kept constant by painting all flowers the same color acrylic paint.

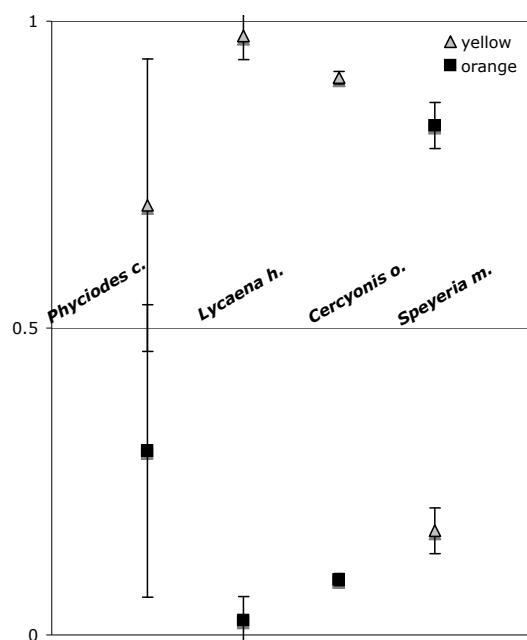


Figure 5: Color array for *Wyethia amplexicus* with mean proportion of visitation and 95% confidence intervals shown for four species of butterfly.

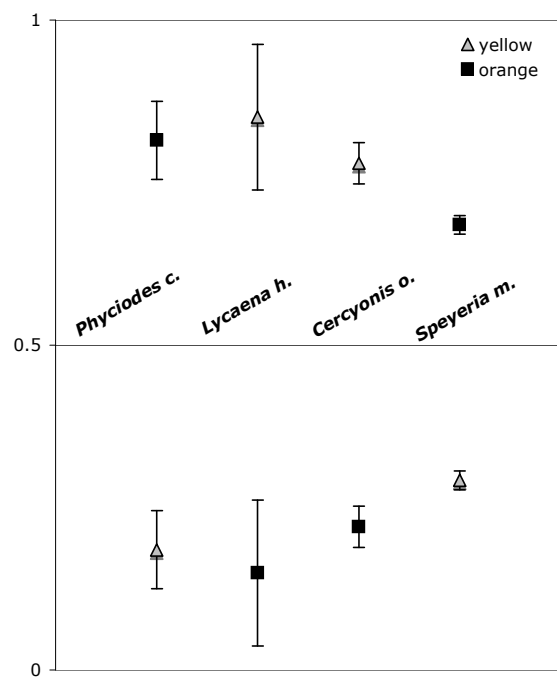


Figure 6: Color array for *Dugaldia hoopesii* with mean proportion of visitation and 95% confidence intervals shown for four species of butterfly.

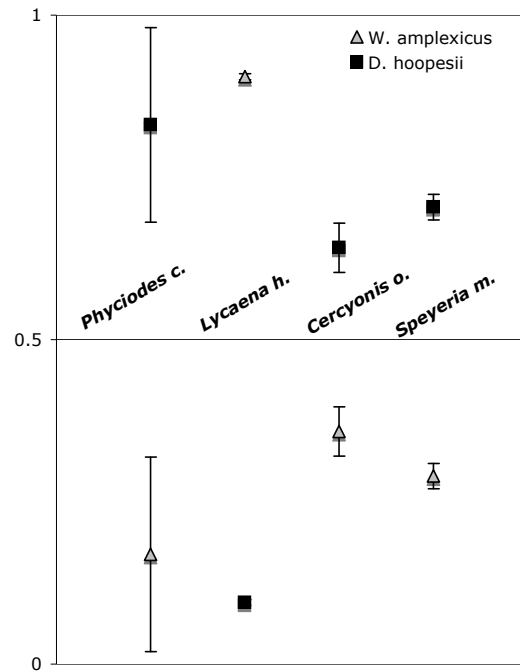


Figure 7: Morphology array of both flower species painted orange, showing mean proportion of visitation and 95% confidence interval.

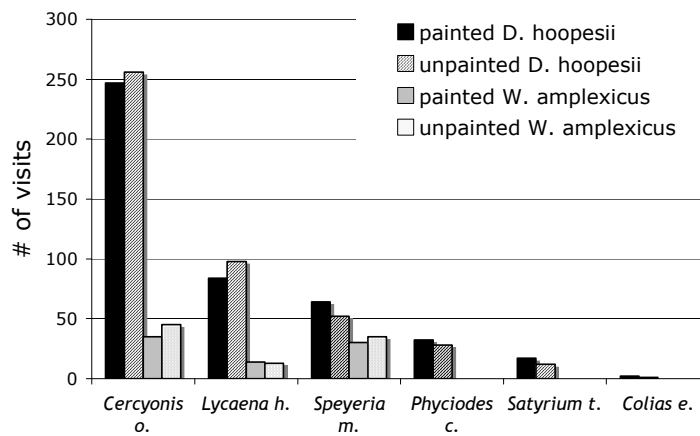


Figure 8: Acrylic paint control array showing total visits to unpainted and painted flowers by species.

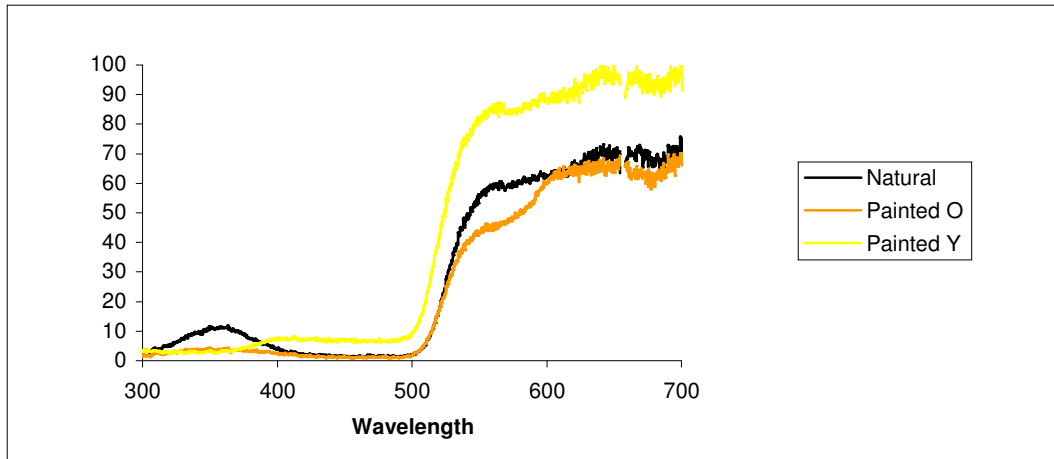


Figure 9: Color spectrum of the petals of *Dugaldia hoopesii* and painted orange and yellow with acrylic paint.

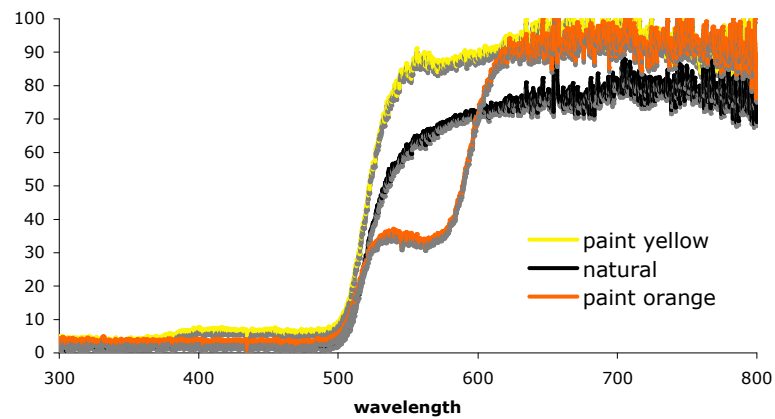


Figure 10: Color spectrum of the petals of *Wyethia amplexicus*, naturally, painted yellow, and painted orange with acrylic paint.