

Environmental Correlates and Fitness Consequences
of a Flower Color Polymorphism in *Boechera stricta*

Student: Ansley McDurmon

Mentor: Jill Anderson

Full Time Independent Research
Summer 2015

INTRODUCTION

Flowering color polymorphisms occur widely throughout angiosperms (Dick et al. 2011). Most commonly, these variations are the product of differential anthocyanin production (Holton and Cornish 1995). Anthocyanin, a flavonoid, causes flowers to vary from red to purple; in the absence of high levels of this chemical compound, petals may also be white (Dick et al. 2011; Holton and Cornish 1995). Pollinators exert significant selection on floral traits, including flower size and color (Gigord et al. 2001; Strauss and Whittall 2006; Waser and Price 1981, 1983). High levels of anthocyanins can confer a fitness advantage in outcrossing flowers like *Delphinium*, for pollinators prefer deeper colors over albino morphs (Waser and Price 1981, 1983).

The persistence of anthocyanin-rich polymorphisms may also be a consequence of frequency-dependent selection. In a study of the rewardless orchid *Dactylorhiza sambucina*, Gigord et al. (2001) observed that flowers with rare colors performed better than their more frequent counterparts. This negative frequency-dependent selection results from a behavioral response by pollinators; when pollinators recognize that the common morph does not provide nectar, the rare morph can “trick” pollinators into visiting because pollinators do not know that it also contains no nectar reward. (Gigord et al. 2001). However, color morphs also exist in species that primarily reproduce by self-pollination, negating the need for pollinator-attracting floral traits (Schemske and Bierzychudek 2001; Strauss and Whittall 2006; Dick et al. 2011). Because they are not largely subject to pollinator-driven selection, these color morphs present an interesting evolutionary paradox of selection without an apparent agent to maintain color-based diversity. This unusual polymorphism suggests that we must seek an alternate rationale for conserving a color polymorphism that is not associated with pollinator preference.

In the absence of pollinator-driven selective pressures, color-based polymorphisms may simply persist as a phylogenetic artifact. Thus, deeply colored flowers may remain in low frequencies simply because there is no agent selecting against coloration. However, flower color may not simply be a benign evolutionary artifact if other selective forces were to act upon the variable trait. Though they are not subject to selection by pollinator preference, color morphs could persist in self-pollinating species because of other biotic and abiotic factors, including herbivory, extreme temperatures, exposure to UV, water availability, or soil pH (Strauss and Wittall 2006). This polymorphism might vary in response to environmental stresses, which signal increased production along the anthocyanin biosynthetic pathway (ABP). Selective pressures may in turn maintain the rare color morph, for the role of anthocyanin in withstanding stress might ensure that the rare morph is more productive than its frequent counterpart during extreme environmental conditions. In this case, color morphs are an artifact of enhanced anthocyanin production throughout the stem of the plant (Asad et al. 2015; Pietrini et al. 2002; Strauss and Whittall 2006). When documenting the relationship between anthocyanin production and water-use efficiency, researchers identified purple-blue morphs of *Linthanus parryae* as more fit than their white counterpart during years of drought (Schemske and Bierzychudek 2001). Conversely, non-drought years produced greater white-morph yields than those with high anthocyanin content (Schemske and Bierzychudek 2001). These and other abiotic factors may maintain a specific anthocyanin response that indirectly affects flower color as well.

Biotic interactions may also select for high levels of anthocyanin production. Specifically, herbivory can exert selective pressure on flower color, for species that produce greater amounts of anthocyanin may also have greater resistance to herbivory, potentially increasing fecundity in darker-colored morphs (Asad et al. 2015; Irwin et al. 2003). Dick et al. (2011) found that anthocyanin production occurs in a series of six, well-defined steps; should a step be cut short in the anthocyanin

biosynthetic pathway, anthocyanin production (and, thus, coloration) would decrease first in petal color, then plant leaves and stem. With that decrease may come the loss of other flavonoids, which may act as herbivory deterrents as well as protection against damaging UV radiation (Holton and Cornish 1995).

Anthocyanins provide pigmentation (Dick et al. 2011), yet these chemicals' roles in tolerating increased levels of herbivory, UV, drought, and temperature have spurred debate (Strauss and Whittall 2006). Anthocyanin production, a well-researched biochemical pathway that leads to the production of purple-red-blue flowers (Holton and Cornish 1995; Whittall et al. 2006), can vary continuously as a polymorphic trait. However, the biotic and abiotic interactions maintaining this trait (and many other polymorphisms) are not fully understood (Mitchell-Olds et al. 2007; Strauss and Whittall 2006). Studying the environmental factors that maintain color morphs can illuminate the underlying forces selecting for variations in anthocyanin production. Providing direct comparison between plants that do—and do not—exhibit high levels of anthocyanin allows researchers to measure the potential importance of anthocyanin coloration as it relates to fitness.

Boechera stricta maintains a flower color polymorphism, despite limited interactions with pollinators. This self-pollinating plant in the Brassicaceae family covers a large elevational gradient and geographic distribution throughout North America (Song et al. 2006; Rushworth et al. 2011), and color morphs exist in low levels among some of these populations. Flowers range from white to pink to purple, and stem color varies among white and purple morphs, for a white morph exhibits light green stem and purple morphs typically have a pink/purple stem (Embick, Mattoon, and Anderson, unpublished data). This variation may indicate differential cessation of the ABP, enabling us to assess whether environmental factors influence flower color, and to quantify the fitness consequences of flower color variation.

If the purple-colored flowers are an artifact of enhanced anthocyanin production throughout the stem of the plant, then it is not the purple flowers, but rather the associated anthocyanins that are under selection. Therefore, if anthocyanin production is closely related to protection against environmental stresses such as excessive UV radiation, drought, and herbivory, we expect more purple flowers in populations that experience greater environmental stress. Where environmental conditions are benign, white flowered morphs could have a similar fitness to purple morphs; conversely, white morphs would have higher fitness because there might be a cost associated with producing purple flowers. Data collected from this study cannot parse out whether biotic and abiotic factors induce a plastic response or select for the persistence of a heritable genotype related to color. Therefore, this study seeks to understand the environmental *correlates* of flower color polymorphisms in hopes of shedding light on future experiments related to heritability and plasticity within populations of *B. stricta*.

METHODS

Study System:

I studied the perennial mustard *Boechera stricta* along an elevation gradient (2700m-3400m) within the Gunnison National Forest and Rocky Mountain Biological Laboratory. Occurring in diverse areas such as deserts and alpine meadows (Rushworth et al. 2011), *B. stricta* exhibits polymorphic petal coloring that varies from white to light pink to purple across an elevation gradient (Embick, Mattoon, and Anderson, unpublished data). Pigmentation varies continuously within *B. stricta* populations. The environmental factors contributing to deregulation or mutation of enzymes within the anthocyanin biosynthetic pathway are not fully understood (Dick et al. 2011). Preliminary studies

have found that the alterations in anthocyanin production are correlated with the production of other flavonoids and herbivore resistance (Irwin et al. 2003; Asad et al. 2015), and variations in anthocyanin production may indicate a genetic or plastic response to abiotic agents of selection such as UV-light or extreme temperatures, and some biotic agents of selection such as herbivores (Winkel 2004).

I observed *B. stricta* at nine sites along an elevation gradient, establishing three 5m x 5m representative plots at each location. Using a color-categorization system established by Anderson and Embick, I assessed variations in flower pigmentation by comparing color morphs to a guide (Figure 1) and scoring each flower on a scale from 0-5 (white-dark purple). I attached labels to each individual indicating plant number and location.

Data Collection

Data collected from these plots illuminates the impact of slope, elevation, aspect, water level, soil pH, sun exposure, and plant diversity upon flower color, fecundity, and rates of herbivory within ten populations of *B. stricta*.

To quantify differences in fitness traits between purple and white color morphs, I measured herbivore damage, plant size (leaf number and plant height), number of flowers, and total fruit length on each plant at each plot. Herbivore damage for each individual plant was estimated as a function of the total number of leaves with herbivore damage $\times$ the % of leaf area removed by herbivores on those damaged leaves divided by the total number of leaves on a plant (Anderson et al. 2015). I censused each site twice: an initial census at the timing of flower (June and July), and a second census when fruits were mature (late July and early August). I censused higher elevations later in the season than lower elevations to produce data from plants near the same life stage.

To estimate potential correlations between environmental factors and flower color frequency, I measured soil pH, soil moisture, slope (H), aspect, elevation, and flowering plant diversity. To test soil pH, I collected two sets of soil samples at three locations within each plot to be analyzed by an outside laboratory. Slope (elevation gain/horizontal distance travelled), elevation, and aspect were analyzed as a proxy for UV radiation. To analyze species richness and abundance, I identified each flowering species present within a 1mx1m sub-section of each plot at maturity, scoring the area for the number of flowering plants as well as percent vegetative cover.

Data Analysis

To tease apart environmental covariates of flower color, I performed a multinomial logistic regression of flower color on abiotic conditions and foliar herbivory with a random effect for plot nested within site. I then regressed total fruit length (a proxy for seed production) on flower color, site ID, elevation, and interactions between flower color and site ID with covariates for plant size and foliar damage using R statistical software to test if having the purple polymorphism confers a fitness advantage.

A significant interaction between site ID and flower color on fitness could indicate local adaptation if purple flowered plants have higher fitness in sites where purple flowers predominate. I calculated the exact proportion of the population at each site with white vs. pink or purple flowers.

RESULTS

In the nine sites that I sampled, the percentage of plants with white flowers varied from 0.0-63.63%. My analyses revealed that flower color varies significantly with environmental conditions, but I did not find significant relationships between flower color and fecundity. I detail the results of these analyses below.

Environmental Correlates of Flower Color: elevation and aspect and percent cover

Flower color varied significantly with aspect, elevation, and percent cover varied significantly (Table 2, Figures 3, 4, and 5), as color can be expected to increase at higher elevations, southerly facing slopes, and when grown higher percent vegetative cover. Plant height, slope, and soil moisture did not vary significantly with flower color.

Agents of environmental stress covary with flower color: aspect and elevation both positively predict greater percentages of purple color morphs. Plants on south-facing slopes and those at higher elevation are more likely to be purple than are those at lower elevation sites. These findings match in part the studies of correlations between UV and anthocyanin production. Ultraviolet radiation increases as a function of slope, aspect and elevation (Bennie et al. 2008); studies of UV and flower pigmentation have shown that anthocyanins increase in fruits and flowers exposed to UV-light, suggesting that UV-B rays may cause a plastic response in flower color among *Boechera stricta* at different elevations (Arakawa 1988).

I found a surprising relationship between flower color and plant density, suggesting that there are more purple flowered plants in areas with greater vegetation cover (Table 2). This novel result suggests that it is possible that interspecific competition could influence this flower color polymorphism.

Fitness Consequences of Flower Color: elevation, height, percov, slope

Flower Color did not vary significantly with silique (fruit) length (Figure 6, Table 3). Silique length is tightly correlated with total number of seeds {Anderson, 2015 #7240}. I found more purple flowers at higher elevations, south facing slopes, and when plants were grown in a denser populations. Other environmental factors showed a stronger relationship with fitness. Height varied significantly with silique length (Table 3), suggesting that larger plants produce more siliques than do small plants. Slope and aspect show marginally significant variance with silique length, suggesting that plants experience greater fecundity at softer slopes and southerly-facing sites (Table 3).

We found that fecundity does not vary with flower color in natural populations. The lack of significant difference between silique lengths and color polymorphisms suggests that differential color production does not confer a fitness advantage in natural *Boechera stricta* populations during an abnormally rainy summer.

In observing environmental predictors of fitness traits of flower color polymorphisms, I found that flower color is one of several variables affecting herbivory in *Boechera stricta*, though it does not show significant impact on plant fecundity (Table 4).

Purple-flowered plants may experience less herbivory than their white counterparts, suggesting that purple coloration is associated with the production of anti-herbivory agents throughout the stem and leaves of the plant. This finding pairs well with a choice test carried out on purple and white *Boechera* taken from sites near observational plots. Michelea Keefe found that the mustard herbivore specialist *Plutella xylostella*, and a generalist *Tricoplusia ni* selectively herbivore the petals

and leaves of white morphs in a controlled choice test between *Boecheera* of both color morphs (unpublished data, 2015). However, increased rates of herbivory did not appear to affect plant fecundity ($p=.8599$), suggesting that *Boecheera stricta* may be adapted to withstand high rates of herbivory without significant fitness consequences. Similarly, a study of fecundity and rates of herbivory in *Chaetocnema confinis* found no evidence for a cost (or benefit) of resistance in herbivorized plants, postulating that herbivore resistance may have been costly when it first arose, but that selection has reduced those costs by modifying the pleiotropic effects of resistance (Simms and Rausher 1987). Herbivory did not vary significantly with silique length (Figure 7, Table 3), contradicting theories that a “cost” of herbivore resistance (defined by Rhoades 1979 as amount of resources allocated to resistance) may reduce fitness of herbivore-resistant morphs due to misappropriation of valuable resources (Rhoades 1979).

Because neither flower color nor its covariant herbivory predict fecundity, it is unlikely that the color polymorphism experiences selective pressure due to herbivores. Though this relationship has been observed in some local species (Irwin et al. 2003), these occasional biotic agents of selection do not maintain or reduce colored morphs in local populations of *Boecheera stricta*.

Understanding the relationship between environments under stress and phenotypic variation may illuminate heritable plant defenses under selection. Results suggest that flower color may vary positively in response high percent vegetative cover (a sign of increased competition), elevation, and aspect (correlates of UV radiation). Though flower color is highly heritable, this study cannot differentiate between a plastic response to environmental variables or selection for the persistence of a heritable genotype related to color. Though some environmental factors are associated with increasing amounts of non-white morphs, color polymorphisms do not translate into differential fitness. This lack of a relationship suggests that the environmental stress contributing to flower color polymorphisms do not pose direct selective pressure upon *Boecheera stricta*.

The results of this study call for increased study of this color polymorphism, specifically in identifying and quantifying the chemical underlying color variations. A greater understanding of flavanoid production and coloration may create an increasingly more important variable of study, allowing scientists to study minute differences in flower color as an indicator of the rapidly changing environments of our warming climate.

LITERATURE CITED

- Anderson, J. T., C-R. Lee, and T. Mitchell-Olds. 2010. Life-history QTLs and natural selection on flowering time in *Boecheera stricta*, a perennial relative of *Arabidopsis*. *Evolution* 65: 771-787.
- Anderson, J., N. Perera, B. Chowdhury, and T. Mitchell-Olds. 2015. Microgeographic patterns of genetic divergence and adaptation across environmental gradients in *Boecheera stricta* (Brassicaceae). *The American Naturalist*.
- Arakawa, O. 1988. Photoregulation of anthocyanin synthesis in apple fruit under UV-B and red light. *Plant and cell physiology*, 29.8: 1385-1389.
- Asad, S.A., S Muhammad, M Farooq, A Afzal, M Broadley, S Young, H West. 2015. Anthocyanin production in the hyperaccumulator plant *Noccaea caerulea* in response to herbivory and zinc stress. *Acta Physiologiae Plantarum* 37.1: 1-9.

- Dick, C.A., J. Buenrostro, T. Butler, M.L. Carlson, D.J. Kliebenstein and J.B. Whittall. 2011. Arctic mustard flower color polymorphism controlled by petal-specific downregulation at the threshold of the anthocyanin biosynthetic pathway. *PLoS ONE* 6.4
- Gigord, Luc DB, MR Macnair, A Smithson. Negative frequency-dependent selection maintains a dramatic flower color polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soo. 2001. *Proceedings of the National Academy of Sciences* 98.11: 6253-6255.
- Holton, TA and EC Cornish. 1995. Genetics and biochemistry of anthocyanin biosynthesis. *The Plant Cell* 7.7:1071.
- Irwin, RE., S.Y. Strauss, S. Storz, A. Emerson, G. Guibert. 2003. The role of herbivores in the maintenance of a flower color polymorphism in wild radish. *Ecology* 84.7: 1733-1743.
- Mitchell-Olds, T., J. H. Willis and D. B. Goldstein. 2007. Which evolutionary processes influence natural genetic variation for phenotypic traits? *Nature Rev Genet.* 8: 845-856.
- Pietrini, F., M. A. Iannelli, and A. Massacci. 2002. Anthocyanin accumulation in the illuminated surface of maize leaves enhances protection from photo-inhibitory risks at low temperature, without further limitation to photosynthesis. *Plant, Cell & Environment* 25.10: 1251-1259.
- Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores. *Herbivores: their interaction with secondary plant metabolites.* Academic Press, New York, 3-54.
- Rushworth, C., B-H Song, C-R Lee, and T. Mitchell-Olds. 2011. *Boechera*, a model system of ecological genomics. *Molecular Ecology* 20.23: 4843-4857.
- Simms, E. L., & Rausher, M. D. 1987. Costs and benefits of plant resistance to herbivory. *American Naturalist* 130.4: 570-581.
- Schemske, D.W., and P. Bierzychudek. 2001. Perspective: evolution of flower color in the desert annual *Linanthus parryae*: Wright revisited. *Evolution* 55: 1269–82.
- Song, B. H., M. J. Clauss, A. Pepper and T. Mitchell- Olds. 2006. Geographic patterns of microsatellite variation in *Boechera stricta*, a close relative of *Arabidopsis*. *Mol. Ecol.* 15: 357-369.
- Strauss, Sharon Y., and Justen B. Whittall. 2006. Non-pollinator agents of selection on floral traits. *Ecology and Evolution of Flowers* 120-138.
- Waser, N. M. and M. V. Price. 1981. Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*. *Evolution* 35: 376-390.
- Waser, N. M. and M. V. Price. 1983. Pollinator behaviour and natural selection for flower color in *Delphinium nelsonii*. *Nature* 302: 422-424.

Whittall, JB, C Voelckel, DJ Kliebenstein and SA Hodges. 2006. Convergence, constraint, and the role of gene expression during adaptive radiation: floral anthocyanins in *Aquilegia*. *Mol. Ecol.* 15:4645-4657.

Winkel S.B. 2004. It takes a garden. How work on diverse plant species has contributed to an understanding of flavonoid metabolism. *Plant Physiology* 127:1399–1404.al

FIGURES and TABLES



Figure 1: Categorized flower color variations of *Boechera stricta*
(Embick and Anderson, Final RMBL Paper 2015)

Table 1: Environmental Conditions by Site						
Site	Percent Non-White Morph	Slope	Elevation (ft)	Aspect	Percent Soil Moisture	Percent Vegetative Cover
Peanut Mine	0					
Snodgrass	0					
Gothic Townsite	14.01					
Judd Falls	63.63					
Rustler's Gulch	17.02					
Emerald Lake	20.68					
Kebler Pass	13.75					
Schofield Pass	13.63					
North Pole Basin	45.65					

Table 2: Environmental Correlates of Color Polymorphisms: General Linear Mixed Model Fit by Maximum Likelihood				
Analysis via logistic regression with a binomial (or binary?) distribution was preformed to test whether flower color varies in response to environmental stress. ($\chi^2 = \dots$, p-value < 0.0001).				
	Estimate	Std. Error	z value	Pr(> z)
Height	-1.387e-02	7.650e-03	-1.813	0.069813 .
Aspect	7.258e-03	1.877e-03	3.867	0.000110 ***
Slope	6.287e-01	1.568e+00	0.401	0.688482

Elevation	1.696e-03	5.369e-05	31.594	< 2e-16 ***
Percent Cover	-2.410e+00	6.203e-01	-3.885	0.000102 ***
Soil Moisture	5.567e+00	3.332e+00	1.670	0.094824 .

Table 3: Environmental Correlates of Fitness: General Linear Mixed Model Fit by Maximum Likelihood Analysis via logistic regression determines whether environmental factors may significantly predict whether environmental conditions significantly influence plant fitness ($\chi^2=$, p-value < 0.0001).

	Estimate	Std. Error	t value	Pr(> z)
Plant Height	4.498e-02	6.228e-03	7.221	5.14e-13 ***
Herbivory	1.391e-03	4.797e-03	0.290	0.7718
Slope	-1.766e+00	1.064e+00	-1.660	0.0969 *
Flower Color	2.017e-01	2.062e-01	0.978	0.3279
Percent Cover	-6.669e-01	3.575e-01	-1.866	0.0621 *
Aspect	1.064e-03	1.065e-03	0.998	0.3181
Elevation	-1.312e-04	1.438e-05	-9.124	< 2e-16 ***

Table 4: Environmental Correlates of Herbivory: Linear Mixed Model

An ANOVA was performed to determine whether environmental factors may affect rates of herbivory among individuals ($\chi^2=$, p-value < 0.026).

	Value	Std.Error	DF	t-value	p-value
Plant Height	-0.18928	0.07475	266	-2.532135	0.0119
Slope	23.41003	14.58523	266	1.605050	0.1097
Flower Color	-4.81505	2.86769	266	-1.679069	0.0943
Percent Cover	-8.90150	4.94328	266	-1.800726	0.0729
Elevation	-0.00558	0.00304	266	-1.836973	0.0673
Aspect	0.02610	0.01542	266	1.692302	0.0918

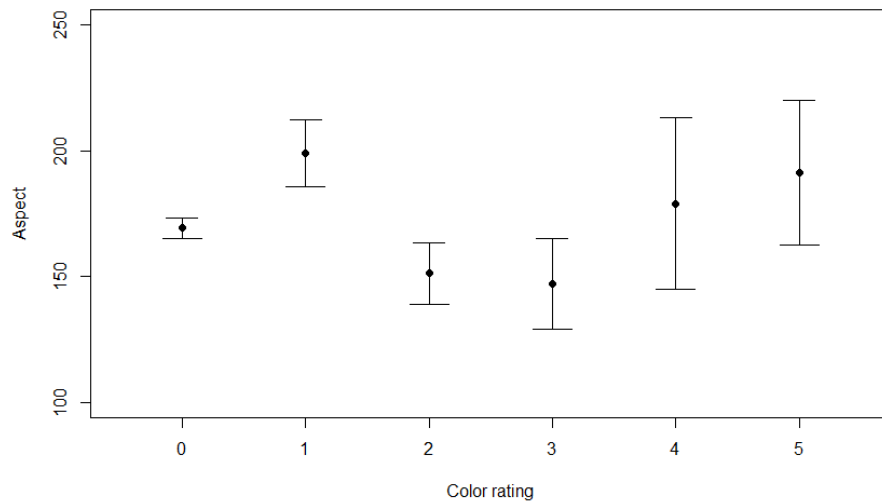


Figure 3: Mean Aspect of Flower Colors. Flower color varies significantly with aspect (p -value=0.000110). Each data point represents mean aspect at an individual flower color classification.

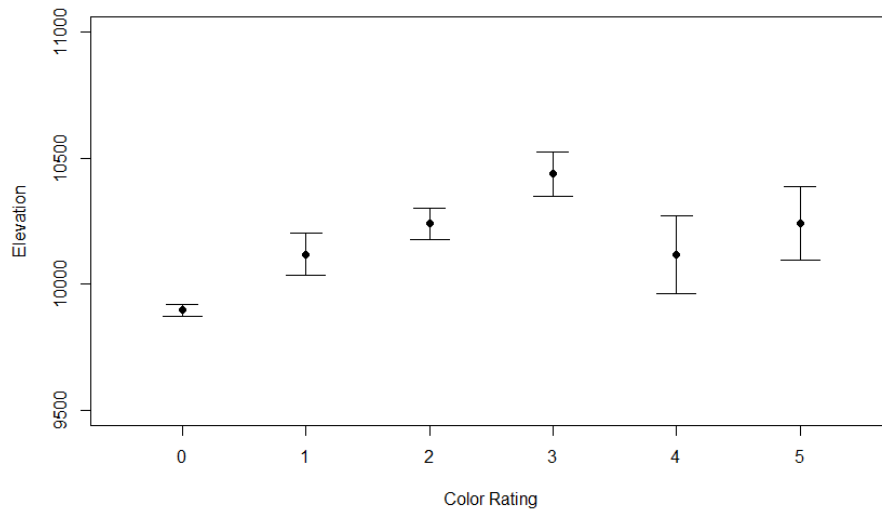


Figure 4: Mean Elevation (ft) of Flower Colors Flower color varies significantly with elevation (p -value< $2e-16$). Each data point represents mean elevation at an individual flower color classification.

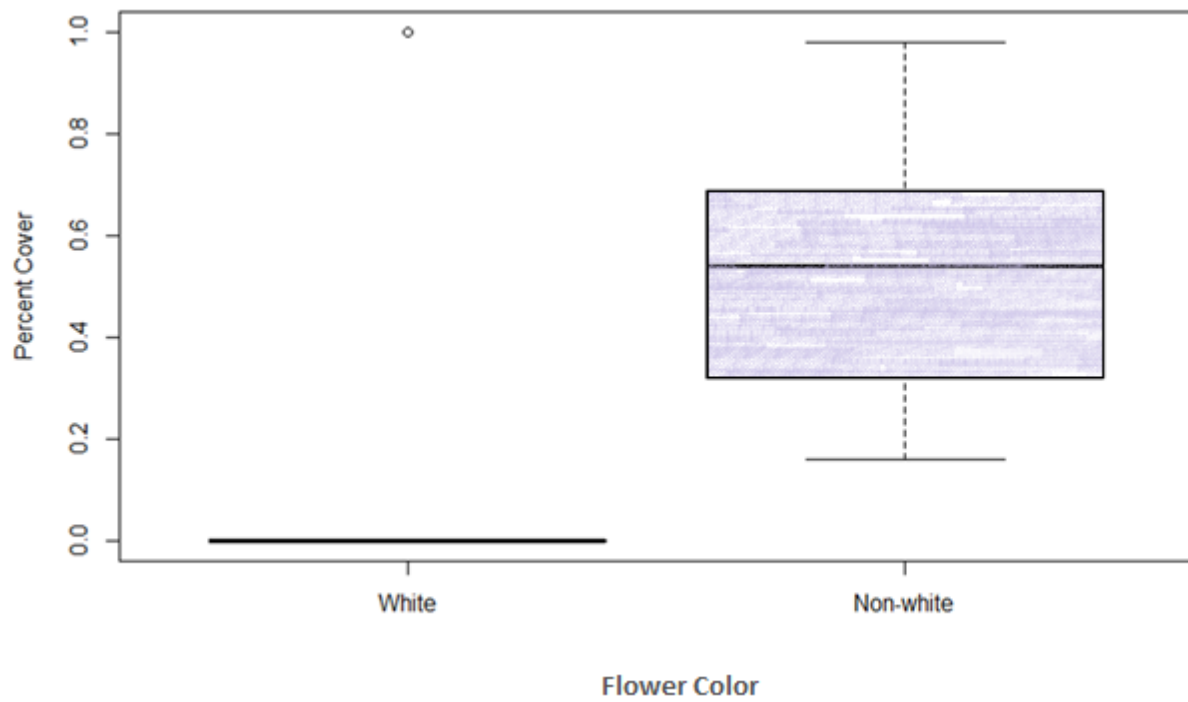


Figure 5: Mean Percent Cover between white and non-white morphs (+/- s.e. bars) Flower color varies significantly with percent vegetative cover (p-value=0.000102).

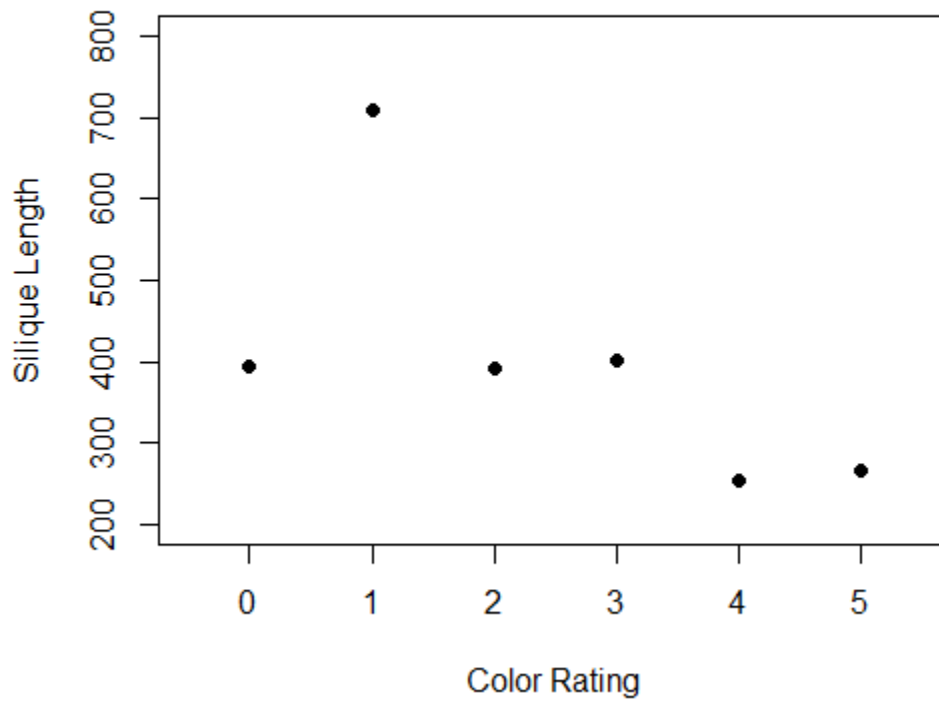


Figure 6: Silique length (mm) as a function of Flower Color Flower color does not vary significantly with silique length ($p=0.3279$). Each data point represents mean silique length (mm)

Figure 7: Silique Length (mm) as a function of Herbivory Silique Length does not vary significantly with herbivory ($p=0.7718$).

Cannot create graph

Figure 8: Elevation and Silique Length

Cannot create graph

Figure 9: Aspect and Silique Length

Cannot create graph

Figure 2: GIS map of study sites near Gothic and Crested Butte, Colorado

