

Floral herbivory: a possible mechanism maintaining a flower
color polymorphism in a selfing species, *Boechera stricta*

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Full Time Independent Research- REU
Summer 2015

Floral herbivory: a possible mechanism maintaining a flower color polymorphism in a selfing species, *Boechnera stricta*

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Abstract

Many studies have shown that pollinators are largely responsible for changing and maintaining flower color morph frequencies in nature. However, the preferences of pollinators do not always reflect observed frequencies in nature, especially in self-pollinating species. Thus, non-pollinator forces could be acting to change color morph frequencies. To test this hypothesis I used two species of Lepidopteran larvae and exposed them to floral and foliar tissue from white and purple *Boechnera stricta* plants using choice and no-choice tests. I also observed natural populations for damage to leaves and reproductive structures. In the choice and no-choice tests, I found that both generalist larvae and specialist on Brassicaceae larvae preferred tissue from white over purple color morphs. In nature there was no difference in damage to plants of either color across all sites. Observed natural damage did not depend on the frequency of the purple morph, nor did frequencies differ between sites. There was also no relationship between florivory and folivory. As specialist and generalists differed in their preferences, this suggests that herbivores may be able to alter color morph frequencies in nature if they have the ability to distinguish among a plant's secondary chemicals. These findings may provide insight into herbivore preference affecting observed color morph frequencies in nature of a selfing species.

Introduction

Plant-pollinator interactions have been well studied for over a century, beginning with, most notably, Darwin (1876). Pollinators have photoreceptors that enable them to distinguish between flowers of different color (Chittka et al., 1992). Individual plants of the same species may advertise to pollinators via polymorphisms in floral color, and, in return, many pollinators can then choose to visit the color that is preferred. For example, pollinators could then select the most preferential color of that species to them, altering frequencies of color morphs. The ability to distinguish color morphs of the same species may lead to selection for floral traits by pollinators. However, the preferences of pollinators do not always reflect the frequencies of different color morphs in natural populations (Chittka et al., 1992).

Irwin et al. (2003) proposed that in addition to pollinators, floral herbivores (florivores) could influence the selective pressures on floral phenotypes by preferentially feeding on reproductive structures of plants displaying particular color morphs. Specifically, they found that a wide variety of herbivores including Lepidopteran larvae as well as slugs, aphids and thrips caused more damage to white and yellow flowers (anthocyanin-recessive color morphs) than to purple or

bronze flowers (anthocyanin-dominant morphs) in *Raphanus sativus* (Irwin et al., 2003). Similarly, McCall et al. (2012) found that white was the preferred color morph in a study using choice tests, no choice tests and performance tests with *Spodoptera exigua* and *Plutella xylostella*, a specialist on Brassicaceae on *R. sativus*.

Studies like these have demonstrated the possibility of non-pollinators affecting selection for floral traits, although little is known about non-pollinator preferences. Recent studies suggest that florivores also have the ability to distinguish among flower color, like pollinators (Irwin et al., 2003, McCall et al., 2012). Traits that may be under selection by insects include secondary chemicals like glucosinolates and anthocyanins, which may influence florivore preference if florivores have the ability to distinguish among a plant's secondary chemicals. Anti-herbivore glucosinolates and anthocyanin floral pigments may share a biosynthetic pathway (Gachon et al., 2005), and some color morphs may be preferable over others because of their lower chemical content. Whether they are responding directly to floral color or indirectly to correlated traits (such as glucosinolates), florivores may reduce seed set by pruning on reproductive structures before fertilization (Honek and Martinkova, 2014), thus influencing plant fitness. This suggests that herbivores could play a role in altering selection for color morphs and thus determining the frequency of color morphs within populations in species where flower color is heritable.

In nature *Boechera stricta* (Brassicaceae) displays a flower color polymorphism. Most flowers are white, and across populations ~15% of individuals are purple-flowered (Anderson et al., unpubl.), although some populations have been documented with up to 50% purple individuals. *B. stricta* is a highly self-pollinating, or apomictic species (Schrantz et al., 2006). Species reproducing asexually via apomixis are also highly inbred and therefore useful in biological studies. Since pollinator visits to *B. stricta* are rare and unlikely to influence fitness (Anderson et al., unpubl.), this raises the question of which non-pollinator selective pressures might be maintaining the purple morph in *B. stricta* populations. My study tested the hypothesis that herbivore preference for tissue from white-flowered plants may be one of those forces.

While the possibility of herbivore color preference driving selection has been demonstrated in previous laboratory and greenhouse studies (McCall et al., 2012, Irwin et al., 2003), the ability of herbivores to drive selection on flower color in nature are largely uncharacterized. In *B. stricta*, herbivore color preferences remain unknown even in controlled laboratory settings. Thus, I used a combination of manipulative and observational techniques to address the following questions:

1. Do herbivores prefer to consume tissue from white or purple-flowered plants in choice tests?
2. Do specialist and generalist herbivores differ in their preferences of color morphs in no-choice tests?
3. Does the frequency of the rare color morph differ between populations?
4. Does damage to reproductive structures vary between native populations?

5. Is there a relationship between florivory and folivory?
6. Does damage vary with the frequency of the rare color morph?

Materials and Methods

Study system

I chose to use the ecological model species *Boechera stricta* (Brassicaceae) to address questions of herbivore selection upon floral color morphs. *B. stricta* is a mostly self-pollinating herbaceous 2-3 year perennial native to the Rocky Mountains (Anderson et al., 2014). It is found across a broad elevational gradient (1500-3700m) and occupies many different ecosystems in North America (Song et al., 2006, Rushworth et al., 2011). *B. stricta* develops a basal rosette in the spring, then bolts and flowers during June or July (Anderson et al., 2014). I conducted this experiment at the Rocky Mountain Biological Lab (RMBL) (Gothic, CO), enabling me to study diverse native populations which have experienced selection for local adaptation over thousands of years. (Rushworth et al., 2011). *B. stricta* also offers the ability to relate to other systems as a model organism, as it is closely related to *Arabidopsis thaliana*, a widely studied species with a fully sequenced and annotated genome (Song et al., 2006).

Choice tests

A choice test was performed to determine herbivore preference on tissues from different colored morphs. Stems of local *B. stricta* were harvested for their petals and leaves. The darkest purple phenotypes available were selected. All stems were harvested near Judd Falls in Gothic, CO and care was taken not to negatively impact these populations by removing too many individuals. As the herbivore community associated with *B. stricta* is currently being studied (Thomas Mitchell-Olds Lab, unpubl.), herbivores were obtained from a commercial retailer (Benzon Research, Carlisle, PA). This also ensured the larvae to all be first instars and have similar herbivory rates. Two species of Lepidopteran larvae were chosen, a generalist herbivore, *Tricoplusia ni* and *Plutella xylostella*, a specialist on Brassicaceae.

Tests were performed in a laboratory at the Gothic Research Center at RMBL in 48mm Petri dishes, the experimental arena. Each Petri dish contained one larva with both purple and white petals or leaves alternating by color and arranged in a circle. There were 25 arenas for each herbivore species (Table 1). After stopping the tests at 20hrs and 48hrs, respectively for petals and leaves, damage was quantified in percent using scans of the experimental arenas using a Canon image class scanner model: MF4570dn. Petal damage was quantified by visually estimating in 5% percent increments damage caused by herbivores using a visual scale due to the small size of *B. stricta* flowers and quality of the scans. Damage to leaves was quantified by dividing the damage by the total leaf

area using ImageJ software. A matched pairs t-test was used to determine species preference for flower color using the percent damage per leaf or petal. A post-hoc analysis, a Wilcoxon signed rank test, was also used, given the small sample size.

No-choice tests

No-choice tests were performed to determine individual preferences of generalist and specialist larvae. 50 individuals of each species of herbivore (Table 1) were used in these assays, but were presented with petals or leaves from only one color morph. Damage to leaves and petals was quantified using the methods described above. I used a two-way ANOVA with color and species as effects and damage to leaves or petals as the response variables.

Table 1. Layout of experimental arena designs

Lepidopteran Species	Choice Tests	No Choice Tests
<i>Plutella xylostella</i>	25 petal tests 25 leaf tests N=50	25 white petal tests 25 purple petal tests 25 white leaf tests 25 purple leaf tests N=100
<i>Tricoplusia ni</i>	25 petal tests 25 leaf tests N=50	25 white petal tests 25 purple petal tests 25 white leaf tests 25 purple leaf tests N=100

Correlations between florivory, folivory and rare morph frequency in natural populations

Native populations were censused at three sites for damage to leaves and reproductive structures. This was done to compare damage in nature caused by native herbivores to the damage quantified in the choice and no-choice tests, using model herbivores. Populations were censused at Judd Falls (JF), (2985m), Emerald Lake (EL) and North Pole Basin (NPB) near Gothic, Colorado (Fig. 3). These sites were chosen for their abundance of *B. stricta* and the presence of its rare purple morph. Three 5x5m quadrats were established at EL and NPB, and two 6x6m quadrats were used at JF. All open flowers and leaves were examined for damage. Plants were classified as purple or white shortly after flowering. All damage was quantified using by counting the number of total leaves/petals, the number of damaged leaves/petals and visually estimating the average percent damage on damaged structures (Schranz et al., 2009). All *B. stricta* plants in each quadrat were identified and given an individual ID number using biodegradable jewelry tags to continue observing damage to reproductive structures after flowers had been shed. The quadrats were censused for number of total *B. stricta* plants and the frequency of the purple color morph was calculated by dividing the number of purple-flowered individuals by the number of total *B. stricta* plants per quadrat. Mixed model tests were used to determine the effect of site and quadrat on the purple color morph.

Results

Choice tests

In choice tests, both species of herbivore larvae preferred tissue from white plants. (Petals: $p < .0001$, Fig. 1A; Leaves: $p = 0.0404$, Fig. 1B, Table 2).

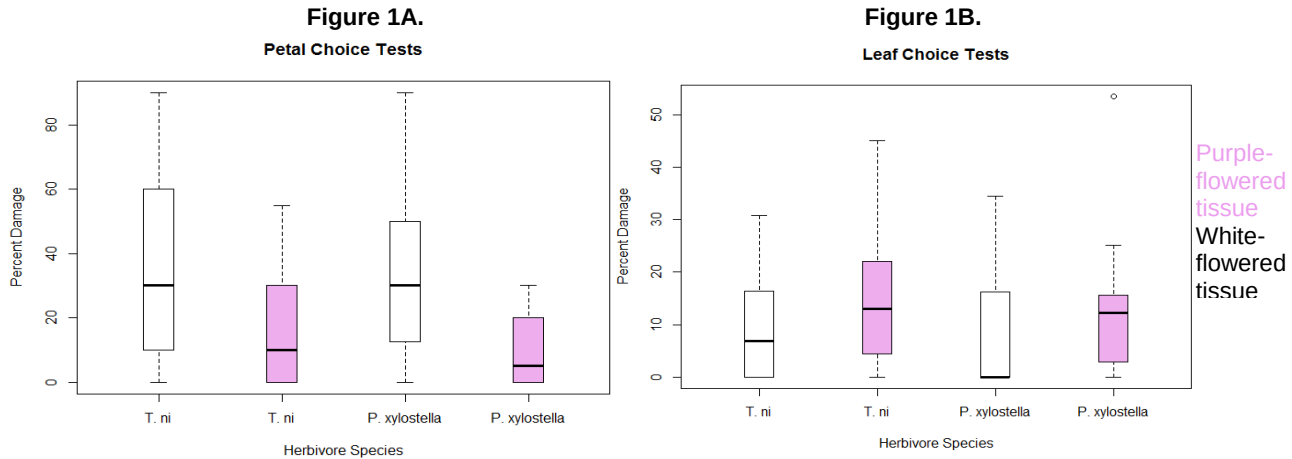


Figure 1A. Petal damage scores from 20hr choice tests between white and purple petals for the specialist *P. xylostella* (N=25) and generalist *T. ni* (N=25). **Figure 1B.** Leaf damage scores from 48hr choice tests between leaves from white and purple plants for the specialist *P. xylostella* (N=25) and generalist *T. ni* (N=25).

Table 2. Choice tests	Leaves	Petals
t-ratio	2.104989	-7.12845
DF	49	49
p-value	0.0404*	<.0001*
n	50	50
Wilcoxon Signed Rank	p=0.0478*	p=<.0001*

No-choice tests

Specialist and generalist Lepidopteran herbivore larvae both preferred tissue from white plants in the no-choice tests. The specialist, *P. xylostella* did not vary in its preference for either color, while the generalist, *T. ni* largely preferred consuming tissue from white plants. There was a significant interaction between plant color and herbivore species both tests. There was no difference in preference for the specialist (*P. xylostella*), the generalist (*T. ni*) showed a significant preference to consume tissue from white plants (Fig. 2A, 2B, Table 3A, 3B).

Table 3A. No-choice test- Leaves	Color	Species	Color*Species
F Ratio	10.0313	3.2605	5.3968
p-value	0.0021	0.0741	0.0223**

Table 3B. No-choice test- Petals	Color	Species	Color*Species
F Ratio	17.7733	1.3316	7.7558
p-value	<.0001*	0.2514	<0.0065**

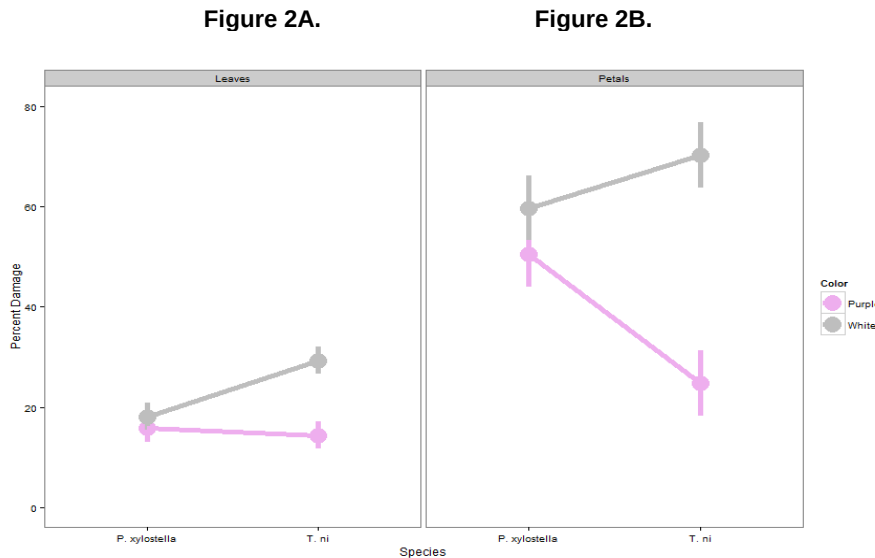


Figure 2. Least squares means plots from 48hr no-choice tests using leaves **(A)** and petals **(B)** from purple or white plants between specialist *P. xylostella* and generalist, *T. ni*.

Correlations between florivory, folivory and rare morph frequency in natural populations

The frequency of the rare color morph differed significantly among natural populations of *B. stricta*, but at the quadrat level rather than the site level (Fig. 3). Mean damage to petals and leaves did not vary depending on plant color (Table 5, 6). This was consistent across all sites [Fig. 4A, 4B].

There was no relationship between the amount of damage that plants received to leaves or petals, regardless of whether they were purple or white, this was found across all sites. To determine whether foliar and floral damage levels were correlated, I performed a regression of percent damage to leaves on percent damage to petals for all plants individuals in all quadrats, using a mixed model with flower color as a fixed effect and site and quadrat nested within site as random effects. This test used a regression between percent damage to petals and percent damage to leaves [Fig. 5, Table 6].

To test whether the preference of herbivores for native *B. stricta* color morphs might be frequency-dependent, I analyzed the average level of damage to leaves and petals in response to the frequency of the purple morph in each quadrat. The amount of herbivore damage on both leaves and petals of white and purple flowers did not depend on the frequency of the purple morph at the quadrat level [Fig. 6A-D, Table 7].

Members of the Thomas Mitchell-Olds lab also used plants from another study that were scored for glucosinolate levels, herbivory and color. It was found

that there is no correlation between color and herbivory (Mitchell-Olds, unpubl.data).

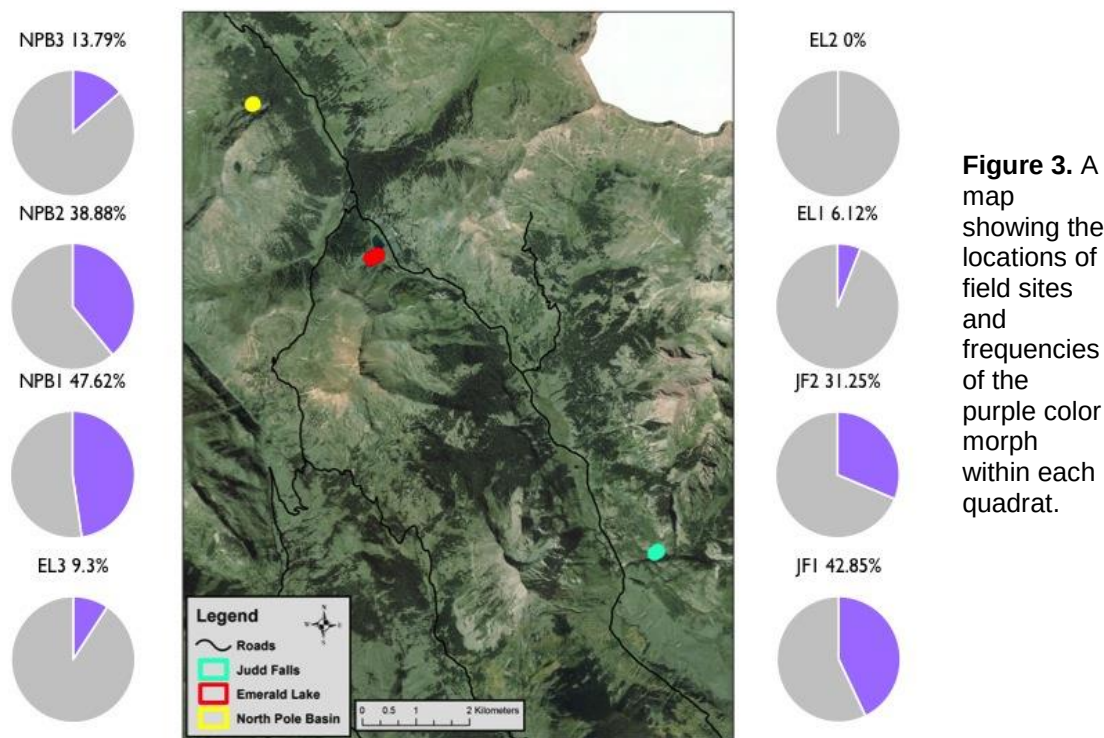


Table 4. Frequency of purple morph in native populations	Leaves	Petals
Site p-value	0.8023	0.6385
Quadrat [Site] p-value	0.6849	0.1137

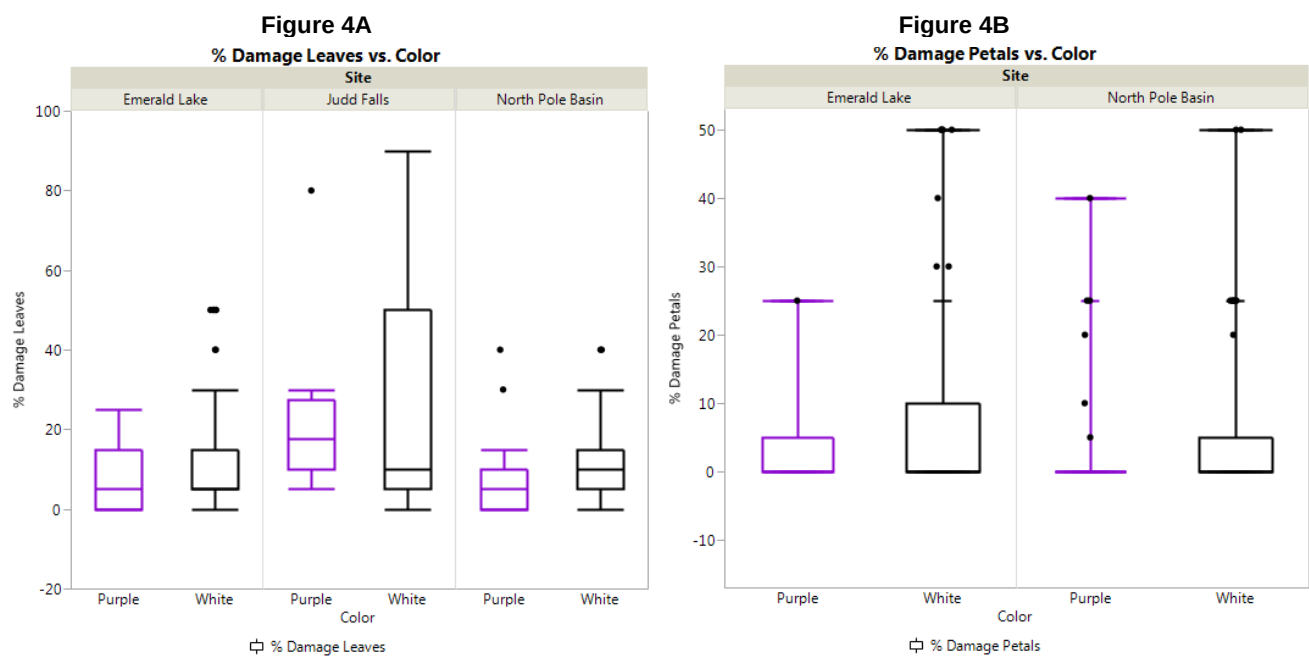


Figure 4A, Figure 4B: Box and whisker plots showing mean damage to reproductive structures and leaves did not vary depending on plant color, or quadrat.

Table 5. % Damage leaves vs. color	Leaves	Color
DF	69.43	75.62
F-Ratio	0.9127	0.8521
p-value	0.3427	0.3589

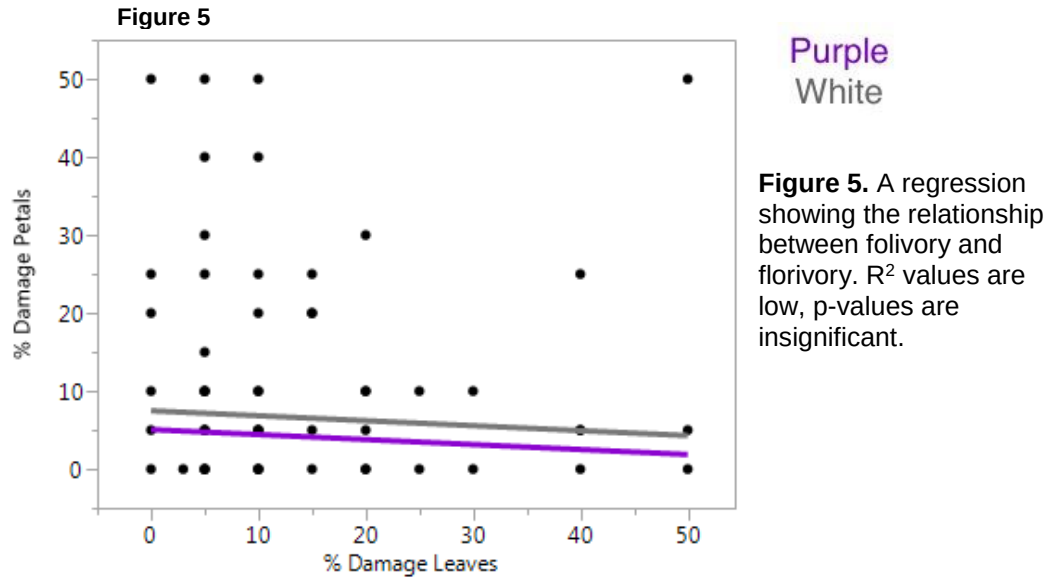
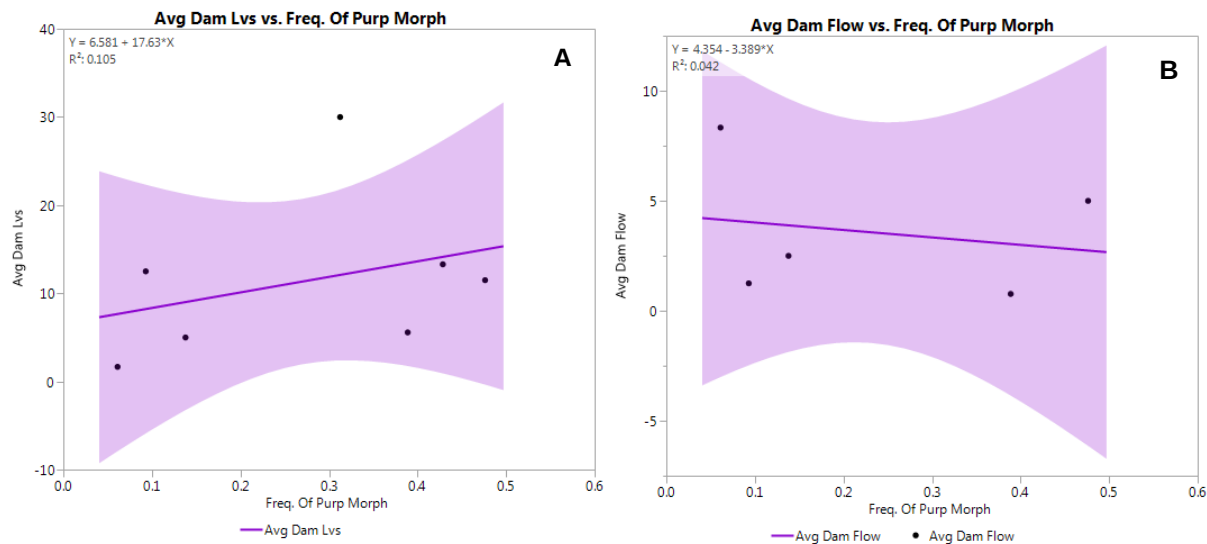


Table 6. Regression between florivory and folivory	
R^2	0.009105
N	220
Damaged leaves p-value	0.4422
Color p-value	0.2865



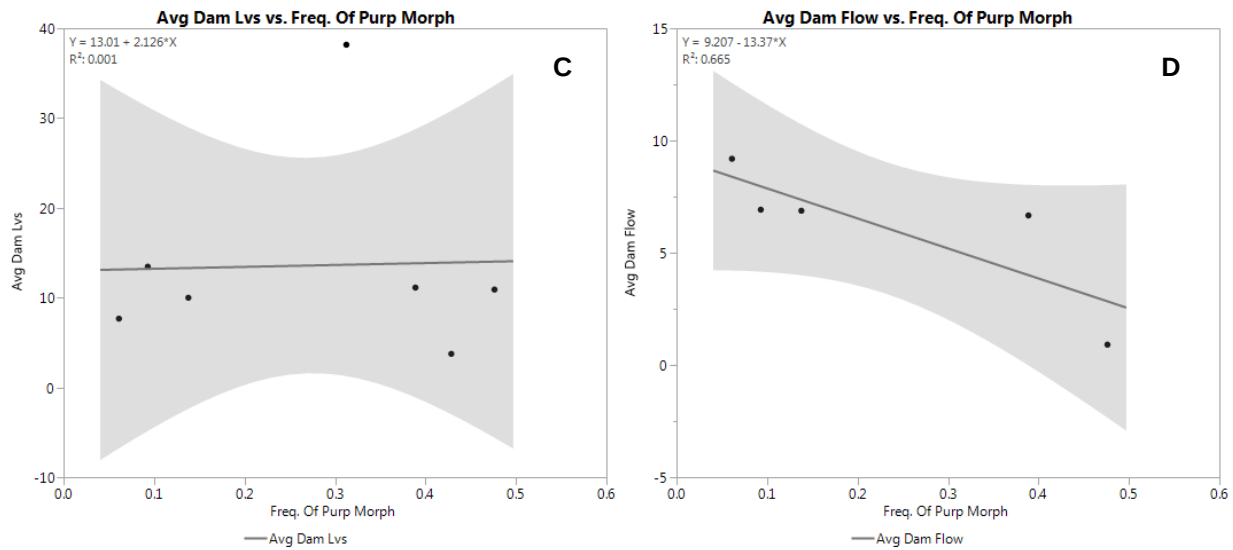


Figure 6A-D. Regressions showing the relationships between damage to leaves and petals on both white and purple plants and the frequency of the purple color morph.

Table 7. Damage to tissue vs. the frequency of the purple morph	Purple Leaves, A	Purple Flowers, B	White Leaves, C	White Flowers, D
R^2	0.105	0.0042	0.001	0.665
p-value	0.478	0.7421	0.9452	0.0925
N	8	8	8	8

Discussion

The results from the lab experiments did not reflect the preferences of herbivores at the field sites. This could be due to many reasons. Similar studies have shown that there is no relationship between leaf anthocyanin content and herbivore performance (Strauss et al., 2004, Irwin et al., 2003). However, it is possible that there are pleiotropic effects between the pathways of anthocyanins and glucosinolates. Meaning that the genes controlling color are able to influence the plant's resistance to herbivores. It is also possible that the presence of other secondary compounds, for example, other flavanoids could be affecting herbivore preference. Another hypothesis could be the effect of the herbivore community differing at each site. Perhaps there are only glucosinolate specialist herbivores at the observed sites and they have no color preference. If specialists and generalists are present, it could be that the non-preferences of the specialists overwhelm the preferences of the generalists, and therefore no pattern is detectable at the coarse level at which damage was estimated visually. This study could inspire future investigation into the genes or pathways that control the polymorphism, which would be more difficult in a non-model species. This study was also a first glance into the possibility of herbivores affecting selection for color frequencies in nature of *B. strica*.

Conclusion

This work shows that in lab experiments using *B. stricta* and Lepidopteran larvae, tissue from white plants received significantly more herbivore damage than tissue from purple plants. In field observations there was no significant difference in damage to plants of either color morph. These findings may help to provide insight into herbivore preferences on the maintenance of a color polymorphism in a largely selfing species.

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