

Intraspecific flower color variation along elevation gradients

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Abstract

Flower color has key fitness implications for plants, and is under selection by both biotic and abiotic factors. Floral pigments can provide protection from herbivory and radiation, and can serve as essential mediators of plant-pollinator interactions. Where a plant resides on its elevation range determines the environment to which it is exposed—temperature, UV exposure, and pollinator community composition are just a few of the conditions that can significantly vary with altitude. Previous work in the East River Valley has revealed several significant changes in flower color at the community level with increased elevation: higher elevation species tend to reflect less blue and purple light, and more green, yellow, orange, and red light, compared to lower elevation species. Additionally, higher elevation species tend to be more saturated in bee and fly visual spaces. However, seeing these trends at the community level does not provide conclusive evidence for selection. Therefore, we looked at patterns at the species level to see if intraspecific color variation with elevation matches up with the community patterns, which would strengthen the evidence that selection is at play. We took reflectance spectra for 33 different species along a gradient from 8,000ft to 13,000ft, aiming to sample each species at intervals of 250ft. We found that each species varies differently with elevation, with no traits clearly predicting how a flower will change with higher altitudes. However, some trends frequently match the community-level patterns: when saturation in bee space showed significant changes within a species, in 85% of cases, it showed an increase with higher elevation. Similarly, violet reflectance decreased with elevation in 80% of variable species. However, many color components showed different intraspecific patterns than were observed in the community data: UVA was far more likely to decrease with elevation than to increase within a species. Additionally, green, yellow, orange, and red reflectance tended to decrease with elevation. Looking more closely at the variation at the species level can provide deeper insight into the selective mechanisms regulating flower color evolution.

I. Introduction

Floral pigments provide several key fitness benefits to plants—they contribute to pollinator attraction, herbivore resistance, and help guard against abiotic stressors like UV radiation (Chittka, 1992). For this reason, we might expect to see variation in flower color across different sets of biotic and abiotic conditions. This study seeks to quantify that variation by looking at it in pollinator visual spaces, with the goal of better understanding the selective mechanisms behind this variation.

As one ascends in elevation, the biotic and abiotic conditions that organisms face changes. Of particular relevance, UV exposure increases, temperature decreases, and pollinator community composition can change (Shrestha et al, 2014). In the West Elk Mountains where this study takes place, it has been observed that with increased elevation, the dominant pollinators transition from bees to flies (Irwin, Ranken, and Whitney; unpubl. data). We would expect these changes to create different selective pressures on flower color, potentially resulting in change in phenotype along the elevational gradient. Previous work at RMBL has in fact confirmed that we see these patterns. Gray et al. (2018) investigated overall floral color composition at the community level, and how it changed with elevation along six transects ascending from different valleys. They found that as altitude increased, the community-weighted mean color increased in saturation in both bee and fly visual spaces; that UVB, violet, and blue reflectance decreased; green, yellow, orange, and red reflectance increased; and UVA showed no significant change (Gray et al, 2018).

One caveat of this study, however, is that it only measured the reflectance of each species at one point in its range. Additionally, they observed high levels of species turnover, with very different species composition at high elevations compared to low elevations. This opens up the possibility that the observed patterns are not in fact created by natural selection, but by other biogeographic factors. It could be a result of historical effects; for example, it could be that higher elevations were originally colonized by species that had low blue reflectance, and the elevation pattern in blue has been retained over time despite no particular benefit to low blue reflectance at high elevation. Thus our study aims to clarify this question by looking at the intraspecific color variation of some of the species in the earlier paper along

their elevation ranges. We observe whether these species show the same patterns that appear in the community, or if they are fairly constant across their ranges. This will help clarify the mechanism behind the patterns we see at the community level. If we see the same patterns appearing at the species level, that would be much stronger evidence that natural selection is at play. This would expand our understanding of how plants adapt to different elevations, and how selection works to create variation across geographical space.

II. Methods

This study took place in the mountains surrounding the East River Valley in Gunnison County, CO, centered at the Rocky Mountain Biological Laboratory. Data were collected from June 1st to August 12th in the summer of 2019. We collected samples from 33 different native flower species along an elevation gradient from 8,000 ft to 13,000 ft. Figure 1 shows the collection locations for each species investigated. The lowest sites were down valley around the town of Almont. The highest sites were the peaks of Avery, Gothic, and Belleview. The most thoroughly sampled sites include Almont Triangle, Kebler Pass Road, and Virginia Basin. Species were sampled as fully as possible along their ranges, with the goal of collecting every 250ft. The average number of collections for a species was 11 and the average elevation range was 2,500ft.

For each collection, three flowers (each from a different individual) were picked in the field. When possible, the samples were placed in a cooler, and refrigerated for no more than five hours before being analyzed. Samples were scanned with an Avantes model 2048 spectrophotometer. The exact part of the flower that was scanned varied depending on the species. See table 1 for descriptions of the methods used to scan each species. We chose the regions to best represent the dominant color of the flower, and what a pollinator would be likely to see. In all cases, the petal or bract was shot at a 45° angle at a distance of 8mm. The petal or bract tip always faced away from the probe.

To examine color independently of particular pollinator visual spaces, we first calculated average reflectance in eight “raw” wavelength bands (UVB = 300–315 nm, UVA = 315–400 nm, violet = 400–440 nm, blue = 440–500 nm, green = 500–565 nm,

yellow = 565–590 nm, orange = 590–625, red = 625–700). We then analyzed the reflectance spectra using the R package “pavo,” (Maia et al., 2013) which allowed us to look at color components in fly and bee visual spaces (brightness, saturation). For each color component in each plant species, we compared three models: a null, color predicted by the linear effect of elevation, and color predicted by both linear and quadratic effects of elevation. The best model was chosen using AIC.

III. Results

For each of the color components analyzed, there were at least two species in the dataset that showed significant ($p < 0.05$) decreases with elevation and two that showed significant increases. Likewise, no species showed the same set of directional changes as any other (Table 2). There is no single trend that stands out as most consequential—as seen in Table 3, the color component with the highest proportion of nonzero slopes with elevation (bee brightness) only has 59% of species showing a significant change, with the rest of the species showing no change in brightness with elevation. This is fairly typical, with all but two of the components having within five percentage points of 50% showing patterns.

However, for each of the components, there is significant variation in how common the dominant patterns are in comparison to the counter-patterns. When a species is variable in saturation in bee space along its elevation gradient, in 85% of cases, it increases with elevation. See figure 2 for examples of each pattern for this color component. Likewise, violet reflectance decreases with elevation in 80% of species that vary in the violet range. Some color components did not show clear directional change—UVB and yellow reflectance both decreased in roughly 56% of species that were variable in those color ranges, indicating that these colors do not have any consistent response to elevation.

Alignment of the most common patterns within species to the community patterns was idiosyncratic. Six of the twelve color components analyzed showed evidence for a similar linear effect of elevation, while the other six did not. The components that lined up include short wavelengths of light (UVB, violet, and blue), saturation (in bee and fly visual space), and brightness in fly spaces. Those that did not include longer

wavelengths of light (green, yellow, orange, and red), bee brightness, and UVA (the only component found not to change in the community data, which decreased in 78% of variable species in the intraspecific data).

It is worth noting, however, that many of the changes in color components with elevation in both datasets had more significant quadratic relationships than linear ones. Thus, for example, while the linear coefficients for change in brightness in bee space are opposite in the two datasets, the linear change was very small to begin with—the quadratic relationship was stronger than the linear model in the community data, and the same is true for the majority of variable species. Figure 3 shows a graph of one of these quadratic relationships in brightness to bees for *Lathyrus lanzwertii*.

IV. Discussion

Perhaps the most straightforward takeaway from this study is that every species varies differently across its elevation gradient. Each species has a different relationship with the myriad of changing pressures associated with higher elevations, and thus have adapted in different ways. We have seen that flower color does have a significant reaction to elevation—only three species in the dataset showed no significant changes with elevation (Table 2). However, our results complicate the community patterns by revealing that there are no universal trends in how color changes at higher altitudes.

Despite that, our data do show that some color components are more likely to change in one direction than others. The strongest such relationship we observed was in saturation in bee visual space (where strength is defined as the proportion of species variable in a given color component followed the dominant pattern). Saturation was much more likely to increase than decrease, as out of 13 variable species, only two were seen to significantly decrease. The reason for this may be related to pollinator attraction. In the alpine, vegetation is often more sparse, and individuals may have to stand out more in order to attract pollinators (Gray et al., 2018). Having a higher saturation is associated with being more distinctive, and more attractive to bees (Lunau, 1990).

The next two strongest patterns were the decrease of UVA and violet reflectance. A potential abiotic reason for this is the increase of UV exposure at higher elevations. Having pigments that absorb these higher frequency forms of radiation may allow for plants to be more easily damaged when the amount of total radiation is strong. UV and violet light have more energy than other parts of the spectrum, and thus have the greatest potential to be destructive to organisms. Therefore, it might be advantageous to have pigments that absorb these frequencies so the tissue is not damaged. There could also be biotic reasons for the shift. Bees have innate preference for blue and UV flowers (Giurfa et al., 1995). So, as the dominant insects turn from bees to flies, having these higher frequency pigments may be less important for pollinator attraction.

A particularly surprising result is the prevalence of counter patterns in reflectance of green, yellow, orange, and red light with elevation. While the community data showed that species found at higher elevations tend to reflect more of these colors, we do not observe the same trends within species. This could be evidence that the patterns observed at the community level are not in fact caused by a selective gradient. The increase in species reflecting these colors at higher elevations could be a random historical effect, or a phylogenetic artifact.

Given the diversity of how the species in our dataset react to conditions along elevational gradients, the next step will be to see if we can build a model to predict how the different color components for a given flower will change. Examples of inputs for this model include: the dominant color of the flower in human visual space, the primary pollinators, and phylogenetic groupings. Such a model, which will be improved as we get data on more species, will help illuminate what factors are affecting each color component. For example, if sorting by primary pollinators produces stronger patterns for changes in saturation with elevation, that would be evidence that pollinator community shift is the primary driver of saturation change with elevation. Other color components may likewise be more likely to be selected by the abiotic changes associated with elevation gain. Sorting through these different color components and factors will provide insight into the evolution of flower color, and how it is balanced within the various benefits it may provide to plants.

V. Literature Cited

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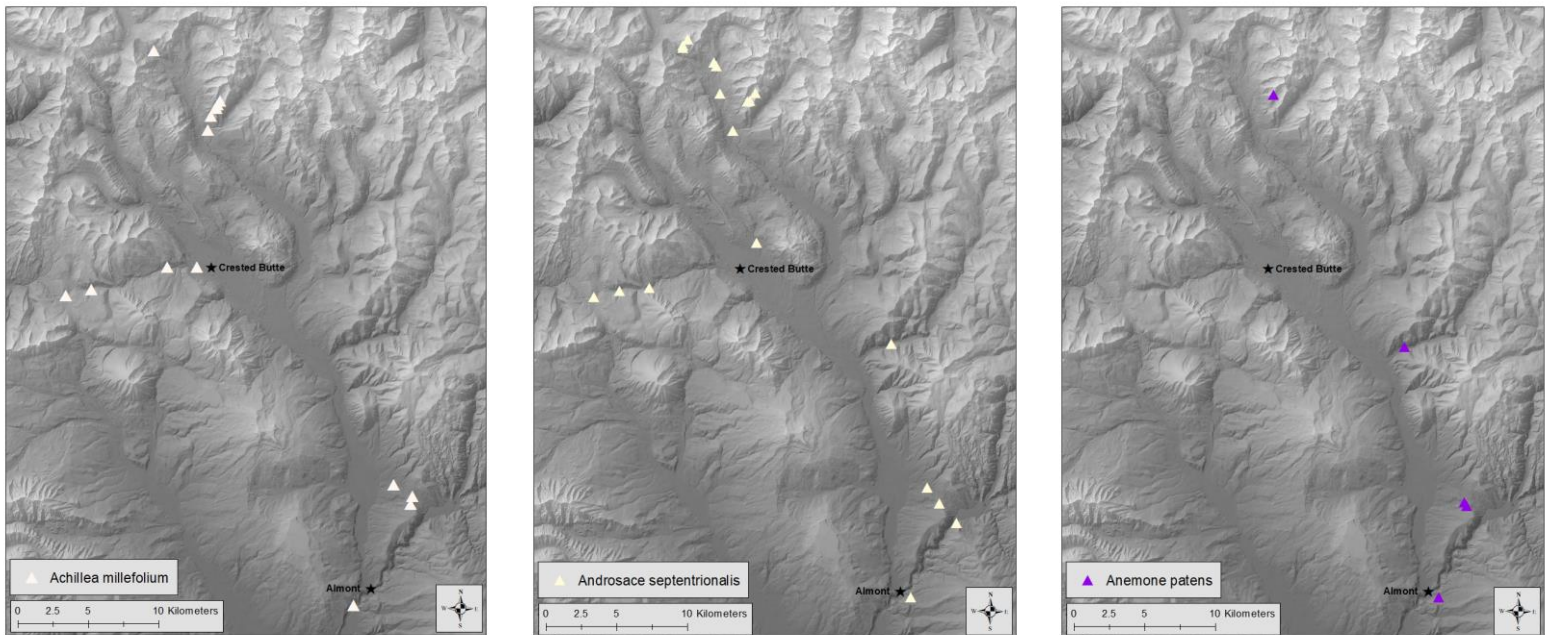
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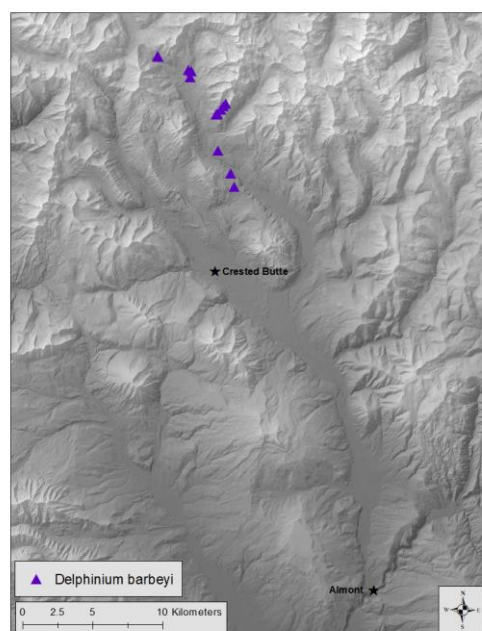
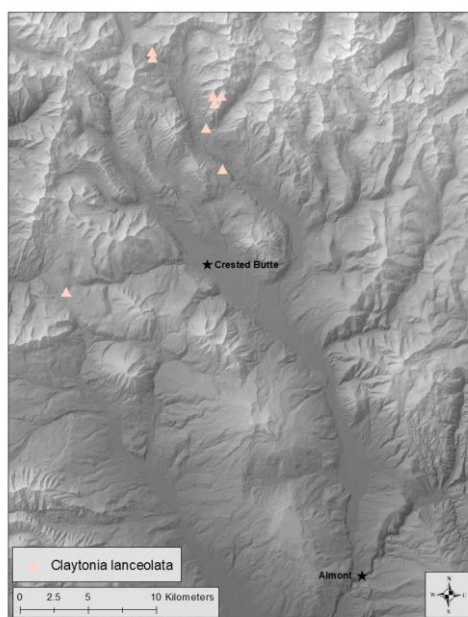
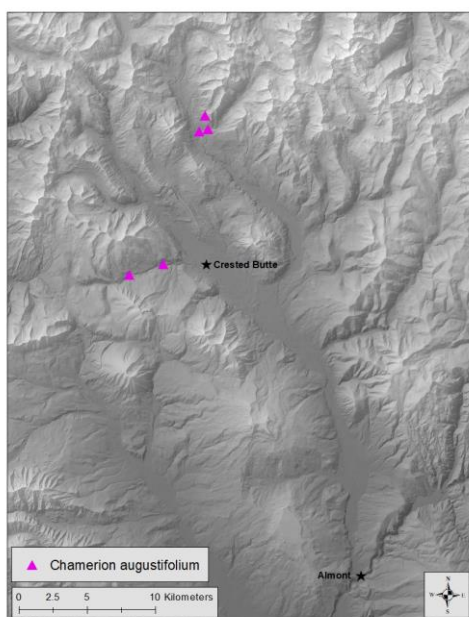
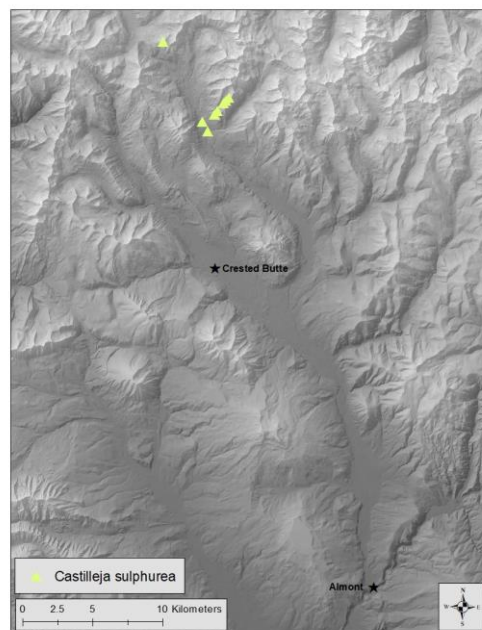
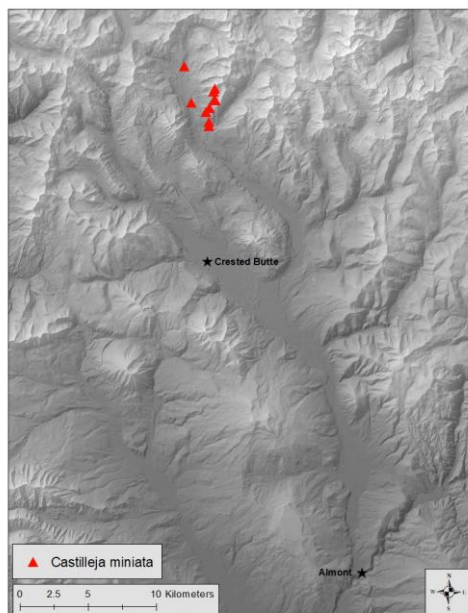
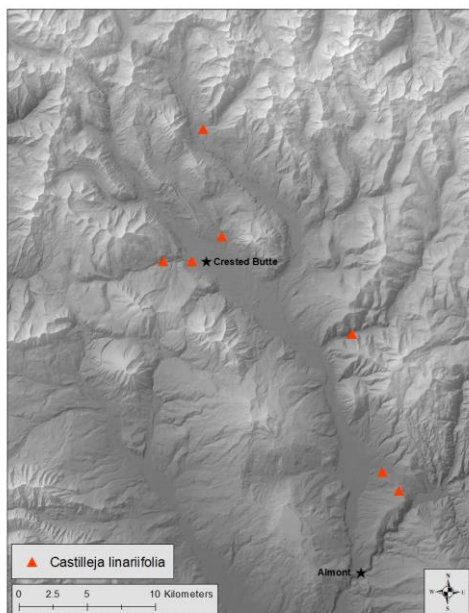
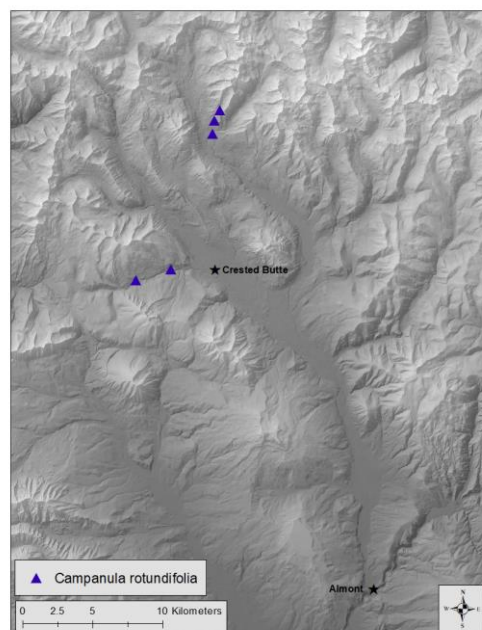
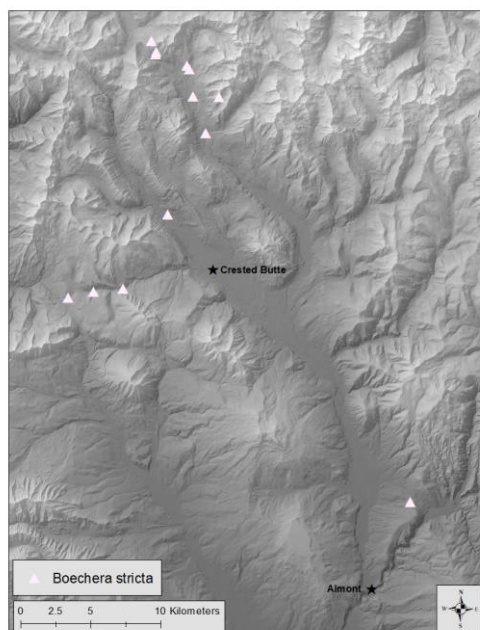
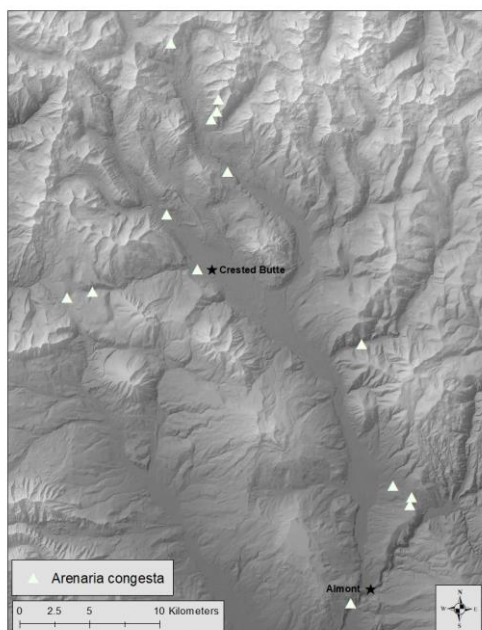
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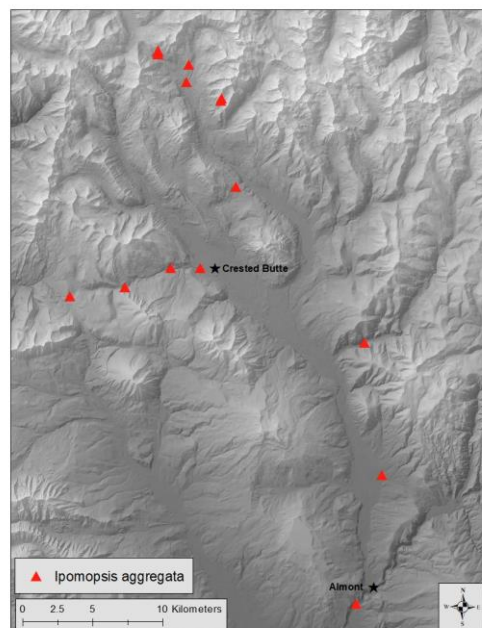
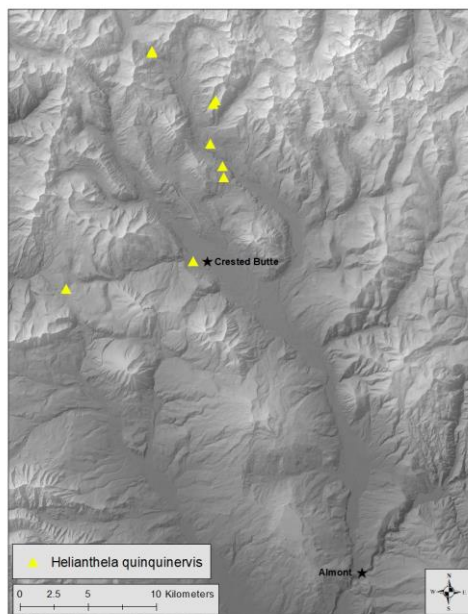
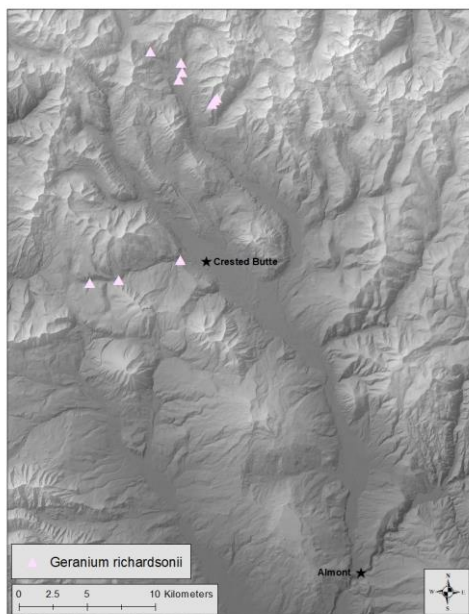
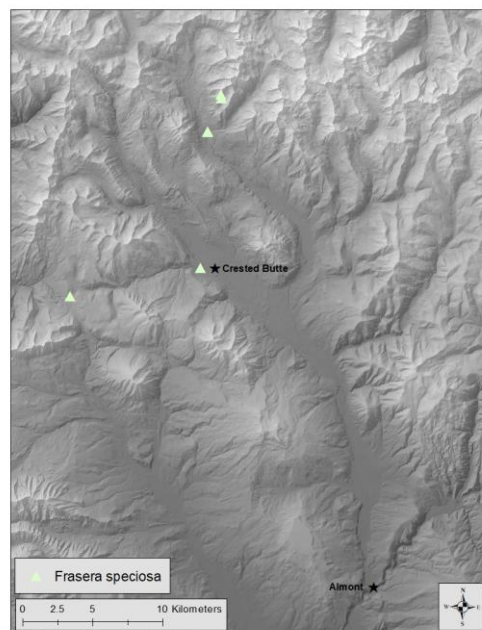
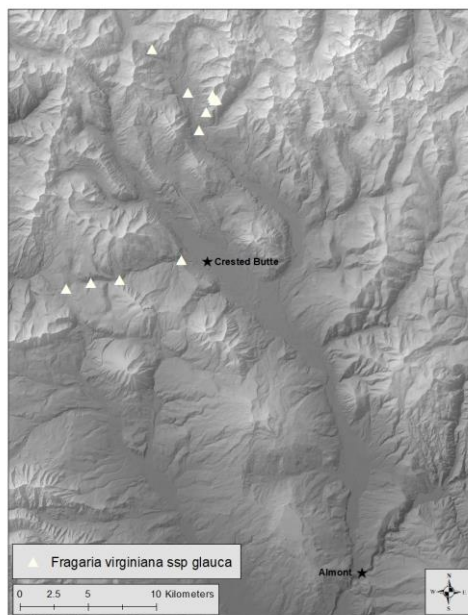
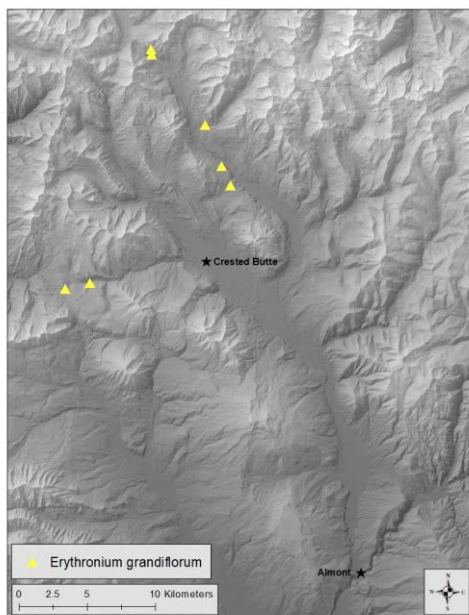
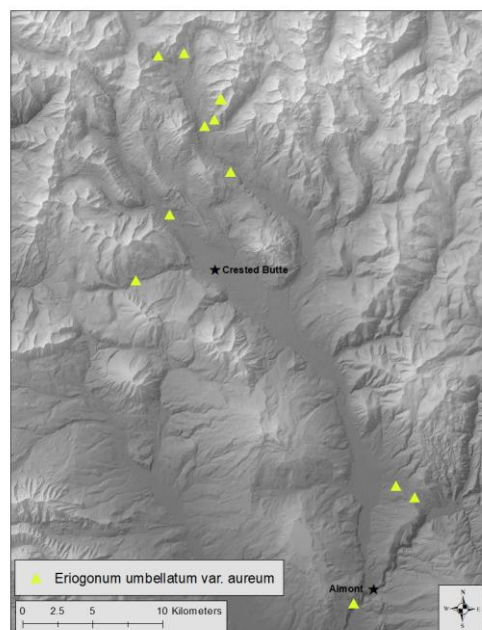
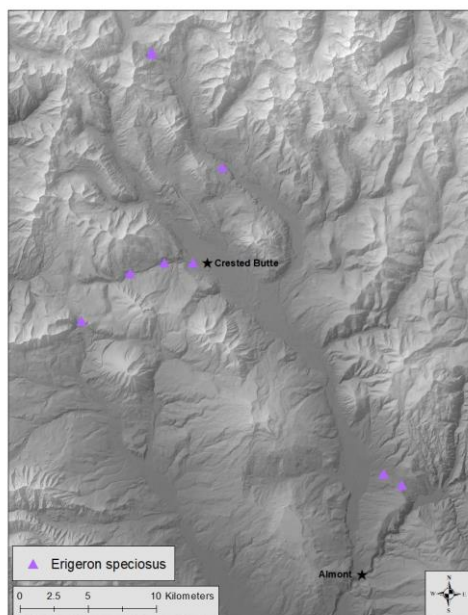
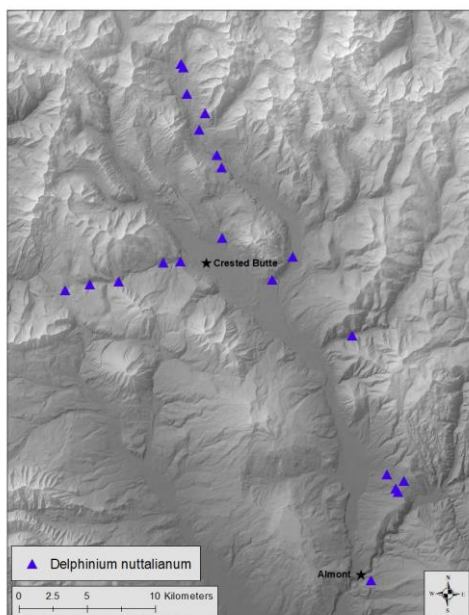
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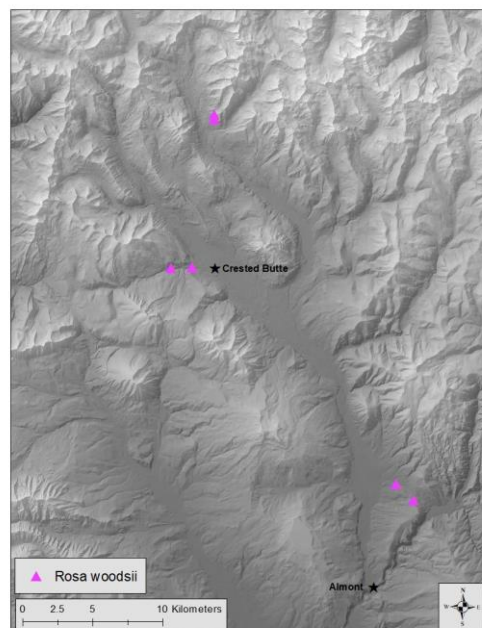
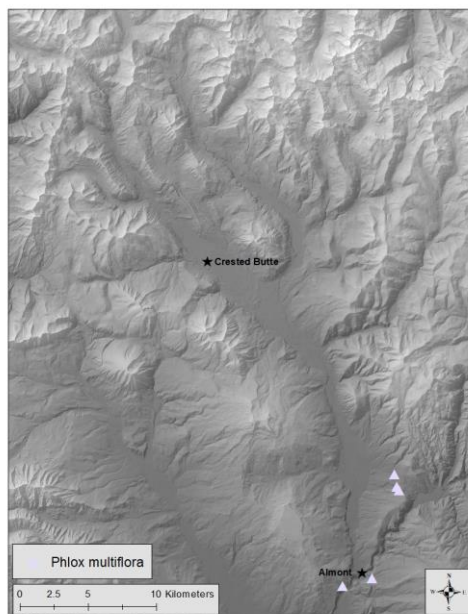
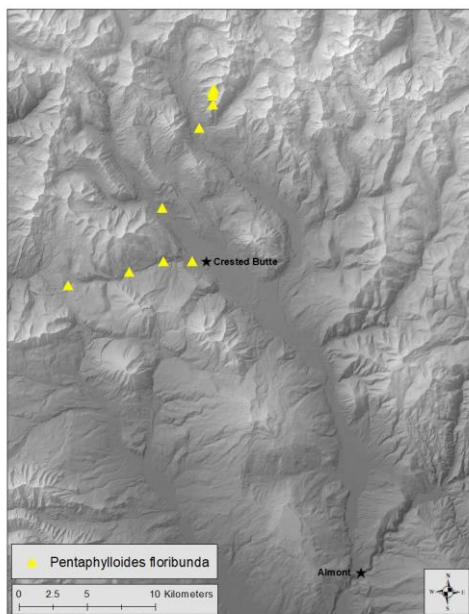
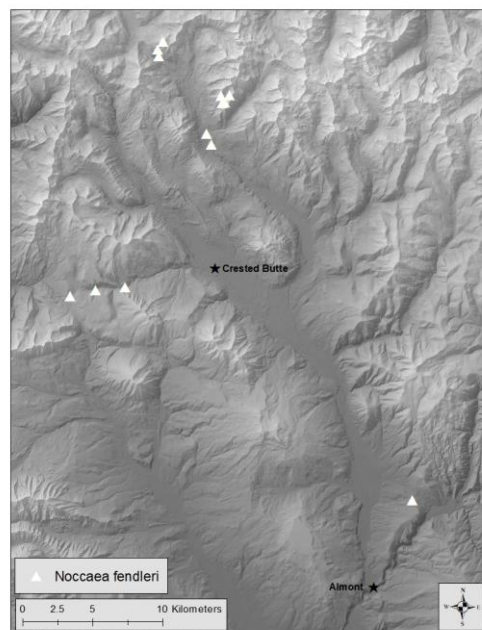
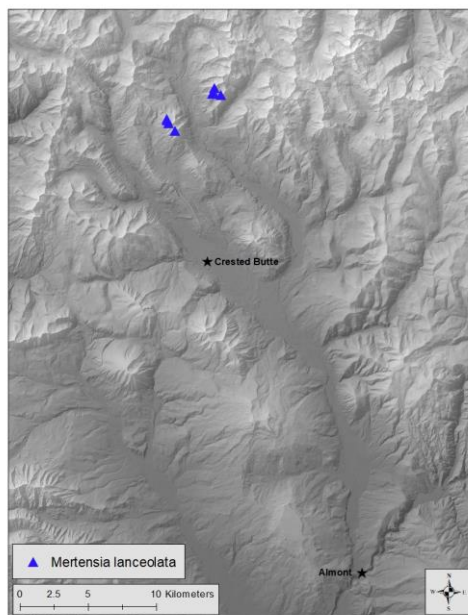
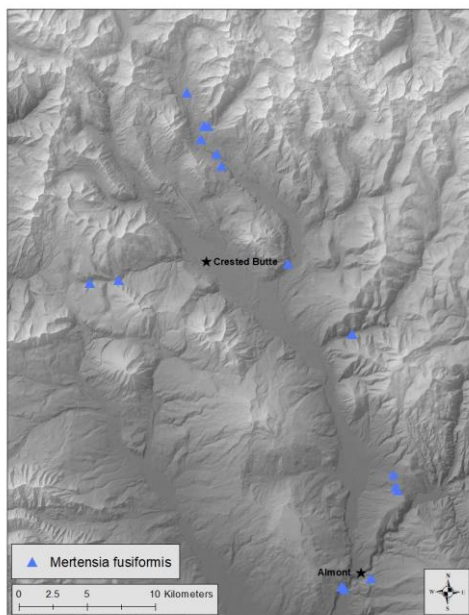
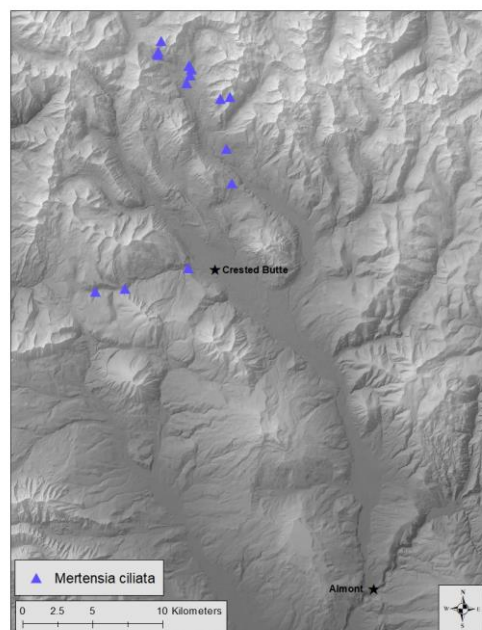
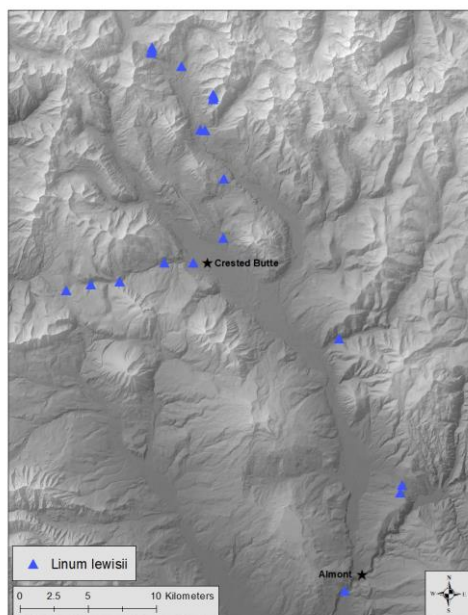
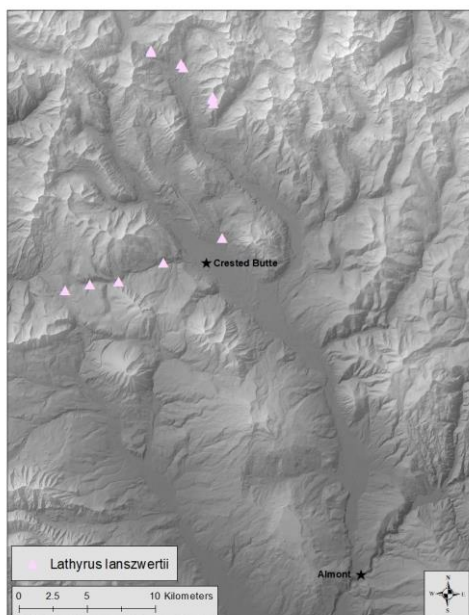
Tables and Figures

Figure 1. Maps of collection sites for each species analyzed.









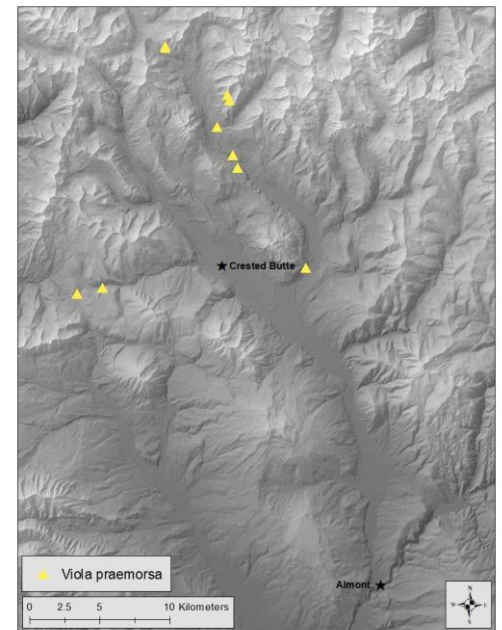
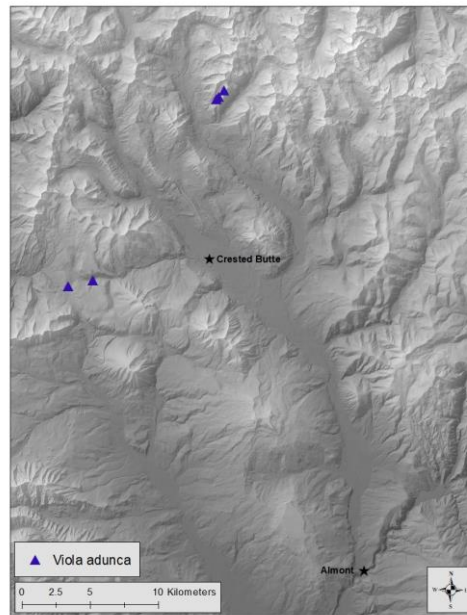
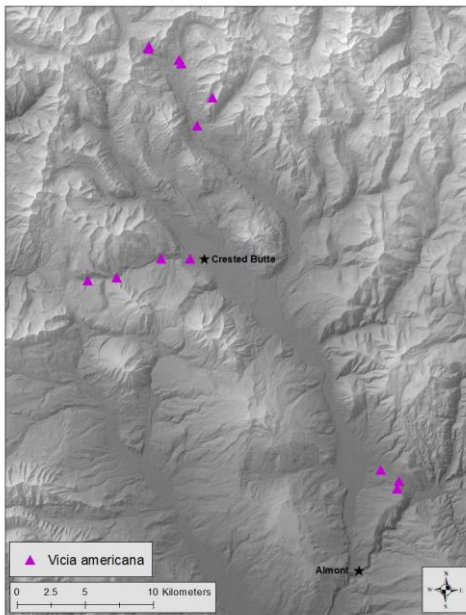


Table 1. List of the spectrophotometry methods for each species, and what part of the flower was scanned.

Species	Shooting notes
<i>Achillea millefolium</i>	Mount petal on tape, shoot face
<i>Androsace septentrionalis</i>	Cut of corolla, mount on tape
<i>Anemone patens</i>	Shoot inside and outside of petal
<i>Arenaria congesta</i>	Mount petal on tape, shoot face
<i>Boechera stricta</i>	Mount petal on tape, shoot face
<i>Campanula rotundifolia</i>	Place probe on outside of corolla
<i>Castilleja linariifolia</i>	Mount bract on tape, shoot inside
<i>Castilleja miniata</i>	Mount bract on tape, shoot inside
<i>Castilleja sulphurea</i>	Mount bract on tape, shoot inside
<i>Chamerion augustifolium</i>	Shoot face of lower petal
<i>Claytonia lanceolata</i>	Mount petal on tape, shoot face
<i>Delphinium barbeyi</i>	Shoot lower petal
<i>Delphinium nuttalianum</i>	Shoot lower petal
<i>Erigeron speciosus</i>	Create mat of ray flowers on tape
<i>Eriogonum umbellatum var aureum</i>	Mount flower on tape, shoot side
<i>Erythronium grandiflorum</i>	Mount top petal on tape, shoot widest part
<i>Fragaria virginiana ssp glauca</i>	Shoot petal face
<i>Geranium richardsonii</i>	Shoot petal face
<i>Helianthella quinquinervis</i>	Shoot widest part of random ray flower
<i>Ipomopsis aggregata</i>	Shoot top of tube, mounted on tape
<i>Lathyrus lanzwertii</i>	Shoot bottom of banner
<i>Linum lewisii</i>	Shoot petal face
<i>Mertensia ciliata</i>	Mount flower on tape, shoot side of tube
<i>Mertensia fusiformis</i>	Mount flower on tape, shoot side of tube
<i>Mertensia lanceolata</i>	Mount flower on tape, shoot side of tube
<i>Noccaea fendleri</i>	Mount petal on tape, shoot face
<i>Rosa woodsii</i>	Shoot face of petal
<i>Vicia americana</i>	Shoot outside of keel, mounted on tape
<i>Viola adunca</i>	Mount bottom petal on tape, shoot middle
<i>Viola praemorsa</i>	Mount bottom petal on tape, shoot middle

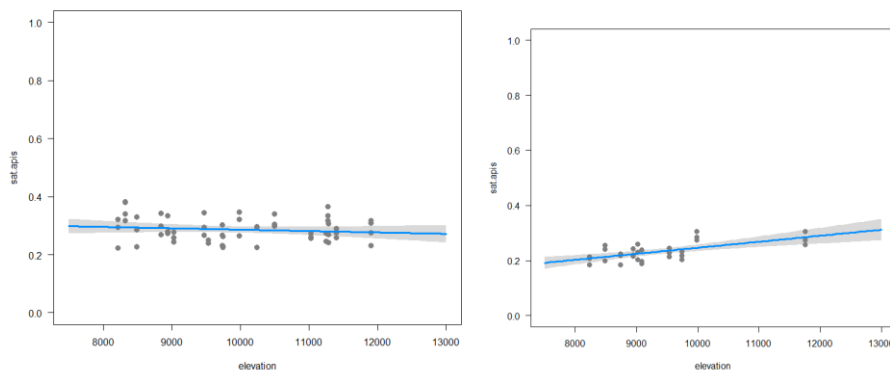
Table 2. A matrix showing the directions of the linear effects of all color components on each species. Three show no change in any components, but the other 26 species all have a unique set of changes.

Species	UVB	UVA	Violet	Blue	Green	Yellow	Orange	Red	Bee Brightness	Fly Brightness	Bee Saturation	Fly Saturation
<i>Geranium richardsonii</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Helianthella quinquinervis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Viola adunca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Boechnera stricta</i>	0	0	0	0	0	0	0	0	0	0	0	-
<i>Frasera speciosa</i>	0	0	0	0	0	0	0	0	0	0	-	0
<i>Castilleja miniata</i>	0	0	0	+	+	+	+	0	+	+	0	0
<i>Castilleja linariifolia</i>	0	0	-	-	0	-	-	0	-	-	0	0
<i>Fragaria virginiana</i>	0	0	-	-	-	-	-	-	-	-	0	0
<i>Mertensia fusiformis</i>	0	0	-	-	-	-	-	-	-	-	0	-
<i>Vicia americana</i>	0	0	+	+	+	0	+	+	+	+	0	0
<i>Mertensia ciliata</i>	0	-	0	0	-	-	-	0	-	0	0	0
<i>Phlox multiflora</i>	0	-	-	-	-	0	-	-	-	-	+	+
<i>Mertensia lanceolata</i>	-	0	0	0	0	0	0	0	0	0	+	+
<i>Delphinium barbeyi</i>	-	0	0	-	-	0	0	0	-	-	+	0
<i>Arenaria congesta</i>	-	-	0	0	0	0	0	0	0	0	+	+
<i>Achillea millefolium</i>	-	-	0	0	-	-	0	0	-	0	+	+
<i>Erigeron speciosus</i>	-	-	0	-	-	-	0	0	-	-	+	0
<i>Anemone patens</i>	-	-	-	0	0	0	0	0	0	0	+	0
<i>Pentaphylloides floribunda</i>	-	-	-	0	0	0	0	0	0	0	+	+
<i>Linum lewisii</i>	-	-	-	-	0	0	0	0	-	-	+	+
<i>Erythronium grandiflorum</i>	-	-	-	-	-	0	0	0	-	-	+	+
<i>Lathyrus lanzwertii</i>	-	-	-	-	-	-	-	-	-	-	0	0
<i>Ipomopsis aggregata</i>	-	-	-	-	-	-	-	-	-	-	+	+
<i>Delphinium nuttalianum</i>	+	0	0	0	0	0	0	0	0	0	0	0
<i>Claytonia lanceolata</i>	+	0	0	+	+	+	+	+	+	+	0	0
<i>Viola praemorsa</i>	+	0	-	-	-	-	-	-	-	-	0	0
<i>Androsace septentrionalis</i>	+	+	-	0	0	+	0	0	0	0	0	+
<i>Eriogonum umbellatum</i> var. <i>aureum</i>	+	+	+	0	+	+	+	0	+	+	0	0
<i>Noccaea fendleri</i>	+	+	+	+	0	0	0	0	0	+	-	-

Table 3. A summary of the elevation trends seen across all of the species investigated, as well comparison to the patterns seen in the community level data. Table reports the fraction of the species in the dataset with a significant ($p < 0.05$) non-zero linear slope. Also depicted is the ratio within the variable species of those that show the most common pattern to those that show a significant counter pattern. Of those showing the most common directional trend, the average slope is reported. Then there is the linear coefficient from the community study as well as a comparison of both the magnitude and direction of those slopes to the averages from the intraspecific data for each color component.

Color Component	Fraction of species showing significant change	Ratio of dominant pattern to counter pattern	Average linear coefficient for dominant pattern	Linear coefficient from community data	Ratio of intraspecific slopes to community slope
Bee saturation	0.45	5.50	0.00040	0.00012	3.33
Raw violet	0.52	4.00	-0.00322	-0.00781	0.41
UVA	0.48	3.67	-0.00175	0	—
Bee brightness	0.59	3.25	-0.00007	0.00005	-1.40
Fly saturation	0.41	3.00	0.01476	0.00011	134.18
Raw red	0.28	3.00	-0.00650	0.00940	-0.69
Raw blue	0.52	2.75	-0.00469	-0.0106	0.44
Raw green	0.52	2.75	-0.0571	0.00584	-9.78
Fly brightness	0.55	2.20	-3.70E-06	-0.00047	0.0079
Raw orange	0.41	2.00	-0.006	0.0124	-0.48
UVB	0.59	1.83	-0.00101	-0.00085	1.19
Raw yellow	0.48	1.80	-0.00498	0.0132	-0.38

Figure 2. Graphs of the three elevation patterns observed in saturation in bee space. First graph shows the *Androsace septentrionalis* which has no significant nonzero slope. Second graph shows *Arenaria congesta* which has a significant increase ($p < 0.001$). The third shows *Noccaea fendleri*, which is one of two species that showed a significant decrease with elevation ($p = 0.006$).



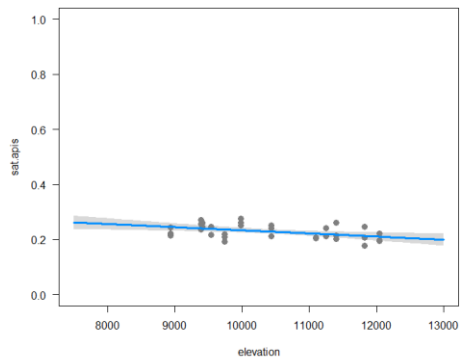


Figure 3. *Lathyrus lanzwertii* brightness in bee visual space with elevation. This species shows a quadratic relationship with ascending elevation ($p = 0.002$).

