

Nectar Variation in Simple and Compound Flowers; and its Effects on Plant Pollinator Interactions

Isaac Steinmann

Mentors: Dr. Paul CaraDonna and Dr. Jane Ogilvie

Collaborators: Gwen Kirschke

Abstract

Pollinators play an important role within a variety of ecosystems but are experiencing declines. In recent years, climate change has been affecting the mutualistic relationship between these organisms and the corresponding plants they interact with. It is important to closely study interactions such as visitation patterns to predict future behaviors more accurately. Visitation patterns vary greatly based on a variety of different factors such as availability of resources, competition, and morphology. In this study, the quantity and concentration of sugar in nectar across a variety of both simple and compound flower species was assessed by comparing umbellets to flowers. Upon analyzing the initial data, significant variance across all species studied was found with higher concentrations of nectar sugar appearing in compound umbellets (likely due to smaller flower size) and overall higher nectar volume appearing in simple flowers. Flower counts were also conducted, separately, to scale up the data and make an assessment regarding the population of each species sampled. This data was then analyzed to understand *Bombus flavifrons*'s plant visitation preferences from an energy cost/reward standpoint across both morphologies, in which it was found they prefer simple flowers likely due to too viscous nectar appearing in compound flowers, as well as the higher visitation frequency required in smaller flowers to fulfill the same nectar requirement. Further studies need to be done to support this theory.

Introduction

Bumble bees are involved in mutualistic pollinator relationships with plants in which a plant provides rewards for a bee usually in the form of floral nectar (a sugary solution containing sucrose, glucose, and fructose) and pollen (a powdery substance used for protein primarily during the larval stages) (Haydack 1970). In exchange, the bee assists in the reproductive process of the plant by distributing its pollen (male gametes) from the anthers of one flower to the stigma (female receptive surface) of another. (Haydack 1970; Nicole E. Miller- Struttmann). This relationship ultimately allows flowers to pass genetic material to the next generation at a small resource and energy expense. Unfortunately, climate change is having adverse effects on plant pollinator interactions and bumblebee populations are experiencing declines (Pradervand 2014). It is important to understand the various factors that affect these interactions such as availability of resources, competition, and morphology; to predict future trends.

In this study we will be assessing the concentration and quantity of nectar in flowers across simple flowers and composite umbellets within Gothic, Colorado; asking if nectar quality varies across both morphologies. In theory not all plants pay the same resource and energy expenses, different flower species may have evolved to reallocate materials and energy to satisfy distinctive roles within an environment thus ultimately leading to specialization and variation of floral resources as well as morphologies across different species to increase fitness within a specific niche (Blüthgen 2010). Thus, plants differ in how they present their flowers to potential pollinators. Compound species present their flowers in small clusters called umbels which sometimes are divided even further into smaller components called umbellets. Meanwhile, simple flowers follow the more typical morphology that most people are familiar with;

composed of stalks containing generally larger flowers with numerous sepals, petals, stamens and pistils. Due to the notable structural variance across both morphologies, it is hypothesized that significant variance exists when comparing nectar levels in simple and compound flower morphologies. Furthermore, it is also fair to speculate that the different energetic rewards in nectar across each species of flower will affect pollinator selectivity leading to varying visitation frequencies. Thus, within this study, pollinator plant visitations will be used to assess plant morphology preference within *Bombus flavifrons* a non-specialized bee species present within Gothic. It is hypothesized that this species of pollinator will prefer flowers of simple morphology to those that are compound due simple flowers being larger and thus more accessible which in change allows for the acquisition of comparable levels of nectar in fewer visitations.

Materials and Methods

Background:

The study was conducted across several different locations in within the Gothic townsite as well as Gothic Road and other surrounding field sites within Gunnison County, Colorado in collaboration with Dr. P. CaraDonna, Dr. J. Ogilvie, and Gwen Kirschke. Gothic is a high elevation (2891m above sea level) mountain site located at 38°57.5'N, 106°59.3'W (Kirschke 2019). The local microhabitats include aspen and conifer forests. (Kirschke 2019; Langenheim 1962). The collections were taken from both wet and dry sub alpine meadows. Six species of flowers were studied total, three simple and three compound: *Aconitum columbianum* (simple), *Delphinium babeyl* (simple), *Vicia americana* (simple), *Eriogonum* (compound) *subalpanium*, *Eriogonum umbelatum* (compound), and *Heracleum Sphondylium* (compound). These flowers were selected based on plant availability due to geographical proximity and phenology.

Finding Nectar Volume and Concentration:

Nectar was collected across all six species studied. Both morphologies required prior bagging of the flowers at least a before to prevent pollinators from accessing them. This tactic was necessary to achieve the most accurate readings of natural nectar production per flower possible. Nectar collection in simple flowers involved inserting capillary tube into the flower through the top opening to collect nectar from the nectaries which are a location at the base of the flower stamens where nectar generally collects. Compound flower nectar was collected similarly except it required centrifuging due to the small size of the flowers. During this process, a hole was made at the top of a centrifuge through which an umbellet was attached upside down with the stem going through the whole. The portion of the stem outside the tube was then taped down, and the tube was placed within the centrifuge so that the nectar could accumulate at the bottom and be collected with a capillary tube after. Based on the amount of nectar within a flower, different size capillary tubes were required. The sizes were 0.5, 1, 2, and 5 μ l; and were decided through trial and error. A ruler was then used to measure the distance filled by the nectar within the capillary tube for cataloguing. Nectar concentration was then found by transferring a drop of the collected nectar into a refractometer which was read and recorded in brix.

Calculating Sugar Amount per Flower:

To more accurately compare the quality of nectar within each species, a function that was acquired through Phillips et al. (2018) was used to calculate the amount of sugar in (mg) within a flower's nectar. This function uses concentration in brix gathered through the refractometer and combines it with volume converted from measurements gathered from the capillary tubes to deduce a rough estimate of how much sugar is present within each flower or umbellet.

Flower Counts:

Flower counts were used to scale up flower/umbel nectar sugar to overall plant nectar sugar across all six species. The flower counts on simple species were conducted by counting the number of stalks in a plant as well as the number of both flowers and fruit combined within each stalk. However, in compound plants, the number of umbels and umbellets per plant were counted instead. Counts took place within a series of locations around Gothic town, and Gothic Road in Gunnison County, Colorado (Figure 1).

Figure 1: (Larger image available in the appendix)



Flavifrons Visitation Data:

To assess *Bombus flavifrons* flower visitation preferences across simple and compound morphologies, long-term visitation data collected by Dr. Ogilvie across six sites from 2015-2021(present) will be used. This data is collected at weekly intervals across six sites in Gunnison County. Collections begin seasonally from the time of snowmelt until temperatures begin to cool again for a total of 16-20 weeks in the fall. Activity and resources including plant species

interacted with, and Bumble bee species as well as caste are monitored for 60 minutes across 3 sites, for a total of three 20-minute intervals.

Results

A graph for the volume (μl) of nectar collected from each flower across all six flower species was made (Figure 2). An ANOVA was conducted on this data giving an F value of 4.458 and a P-value 0.00104. A second graph for nectar concentration in brix versus plant species which can be seen in Figure 3 was also made. The third graph was for nectar sugar (mg) per flower versus plant species (Figure 4) and was also ran alongside an ANOVA which gave an F value of 4.77, and a P-value 0.000686. A histogram for the flower counts across all six species was made showing the distribution of structural components across each species (Figure 5). A graph for total nectar sugar (mg) per plant species was made by combining the flower count data with the sugar (mg) per flower data (Figure 6) and an ANOVA was conducted on it thus giving an F value of 22.89, and a P-value <0.0001.

Total average sugar values per plant were calculated showing that on average *Delphinium barbeyi* had 154.466463 mg, *Eriogonum subalpinum* had 53.711539, *Eriogonum umbellatum* had 43.959735, *Aconitum columbianum* had 17.487671, *Vicia americana* had 12.431247, and *Heracleum sphondylium* had 4.361477. A Tukey HSD was ran (Figure 9; grouping in Figure 7) showing that *Eriogonum umbellatum* (vs) *Eriogonum subalpinum* had -9.751804 difference, *Heracleum sphondylium* (vs) *Eriogonum subalpinum* had -49.350061 difference, *Aconitum columbianum* (vs) *Eriogonum subalpinum* had -36.223868 difference, *Delphinium barbeyi* (vs) *Eriogonum subalpinum* had 100.754924 difference, *Vicia americana* (vs) *Eriogonum subalpinum* had a -41.280292 difference, *Heracleum sphondylium* (vs) *Eriogonum umbellatum*

had a -39.598257 difference, *Aconitum columbianum* (vs) *Eriogonum umbellatum* had a -26.472064 difference, *Delphinium barbeyi* (vs) *Eriogonum umbellatum* had a 110.506728 difference, *Vicia americana* (vs) *Eriogonum umbellatum* had a -31.528487 difference, *Aconitum columbianum* (vs) *Heracleum sphondylium* had a 13.126193 difference, *Delphinium barbeyi* (vs) *Heracleum sphondylium* had a 150.104985 difference, *Vicia americana* (vs) *Heracleum sphondylium* had a 8.069770 difference, *Delphinium barbeyi* (vs) *Aconitum columbianum* had a 136.978792 difference, *Vicia americana* (vs) *Aconitum columbianum* had a -5.056423 difference, and *Vicia americana* (vs) *Delphinium barbeyi* had a -142.035215 difference.

Figure 6: Graph of total nectar sugar in mg versus different plant species. (*Larger image available in the appendix*)

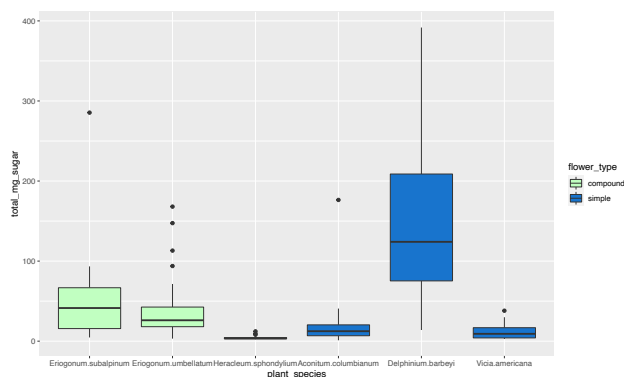


Figure 7: Tukey HSD chart with grouping based on average nectar sugar (mg) per plant. (*Larger image available in the appendix*)

Comparing nectar sugar in mg per plant:

SPECIES	TOTAL (mg) SUGAR	GROUPS
Delphinium barbeyi	154.466463	A
Eriogonum subalpinum	53.711539	B
Eriogonum umbellatum	43.959735	BC
Aconitum columbianum	17.487671	BC
Vicia americana	12.431247	BC
Heracleum sphondylium	4.361477	C

Discussion

In this study, nectar was collected and assessed to see if simple and compound morphologies vary in nectar sugar levels or if they provide similar rewards to incoming pollinators. When comparing nectar concentration in brix across species it was found that nectar seems to appear in slightly higher concentrations among compound species likely due to the smaller size of its structural components. However, conversely, the data for sugar volume (μl) per flower shows that volume tends to be slightly higher among simple species likely due to larger structural components within the morphology. After running an ANOVA for the volume (μl) of nectar collected it was found that the P-value was 0.00104 which signifies that there is enough evidence to reject the null hypothesis. Furthermore, the F value came out to 4.458 meaning that variance is significant due to the existence of independent variables across the categories examined. Ultimately, what this means is that nectar volume likely varies across species. A similar deduction was made when an ANOVA was conducted for nectar sugar (mg) per flower with the F value being 4.77 also showing that variance likely exists in nectar levels per flower across plant species studied. Furthermore, the null was also rejected in this instance, shown by the P-value 0.000686.

In the histogram performed for the flower counts there was no noticeable distribution across any of the species except for in *Aconitum columbianum* where the data seemed to skew right. As for the data for total nectar sugar (mg) per plant, the graph showed that *Delphinium barbeyi*, a simple plant, had the highest nectar sugar per plant while *Heracleum sphondylium* a compound plant had the lowest sugar per plant. Furthermore, when comparing sugar levels with a Tukey HSD; *Eriogonum umbellatum*, *Aconitum columbianum*, and *Vicia americana* all seemed to have similar sugar nectar levels per plant which is interesting considering that there is both

compound and simple morphologies in this grouping. After running an ANOVA, the P-value for total nectar sugar per plant came out to less than 0.0001 thus rejecting the null. The F value came out to 22.89 which shows that significant variance in total sugar likely exists across all species.

When observing *Bombus flavifrons* visitations it became apparent that this species of pollinator disliked compound flowers thus ultimately having zero visitations to any compound morphology plants. One theory is that due to the higher concentration of sugar present in compound plants the nectar is far more viscous therefore making it less favorable to the *Bombus flavifrons*. Another theory as to why simple plants were visited more frequently has to do with the nectar reward per flower across both types of morphology. In the compound morphology there is a far greater number of much smaller flowers grouped together versus just a few large flowers as is the norm within simple morphologies. This ultimately means that it would require *Bombus flavifrons* a far greater number of visits to all the flowers within a compound umbellet to acquire the same energetic reward versus a simple flower. This in change makes compound flower visitations far more inconvenient even if sugar nectar levels are comparable across an umbellet and a flower such as with *Aconitum columbianum*, and *Vicia americana* which are both simple, versus *Delphinium Barbeyi* which is compound (Figure 7). Another likely theory for *Bombus flavifrons*'s simple morphology preference comes to physiological incompatibility. Although *Bombus flavifrons* is a generalized species it might still possess physiological features such as tongue size or just overall weight that make it far more difficult for them to access the nectar in compound flowers thus causing the species to avoid this sort of recourse unless in dire need. But since there is such a high abundance of simple flower resources currently, there is no need for this species to venture out into inconvenient endeavors.

Ultimately, as predicted, the study shows that significant variance in nectar quality exists across all species, though there are certain trends that are loosely reflected across morphologies such as higher concentration in compound flowers and higher volume in simple flowers. However, these trends aren't fully conclusive being that there is overlap in nectar sugar levels across both morphologies. Furthermore, *Bombus flavifrons* has shown a visitation preference to simple flowers as predicted, though a larger variety of sampled species should be studied to further support this theory. Future studies should focus on other non-specialized pollinators to see if this preference is unique to *Bombus flavifrons*. Another future research idea would be to study other simple floral species with high nectar concentrations that are congruent to those found in compound species so that the viscous nectar theory can be examined further.

It is important to continue collecting data for this project to increase data significance. Furthermore, it would be interesting to venture out and collect data from other locations to test whether plant competition is a significant factor within the plant selection process of *Bombus flavifrons*. At the end of the day, it is important to study pollinator plant interactions such as visitation patterns to predict future behaviors and better understand the mutualistic plant pollinator relationship and how it affects surrounding ecosystems.

Appendix

Figure 1: Map of sites used during plant counts.



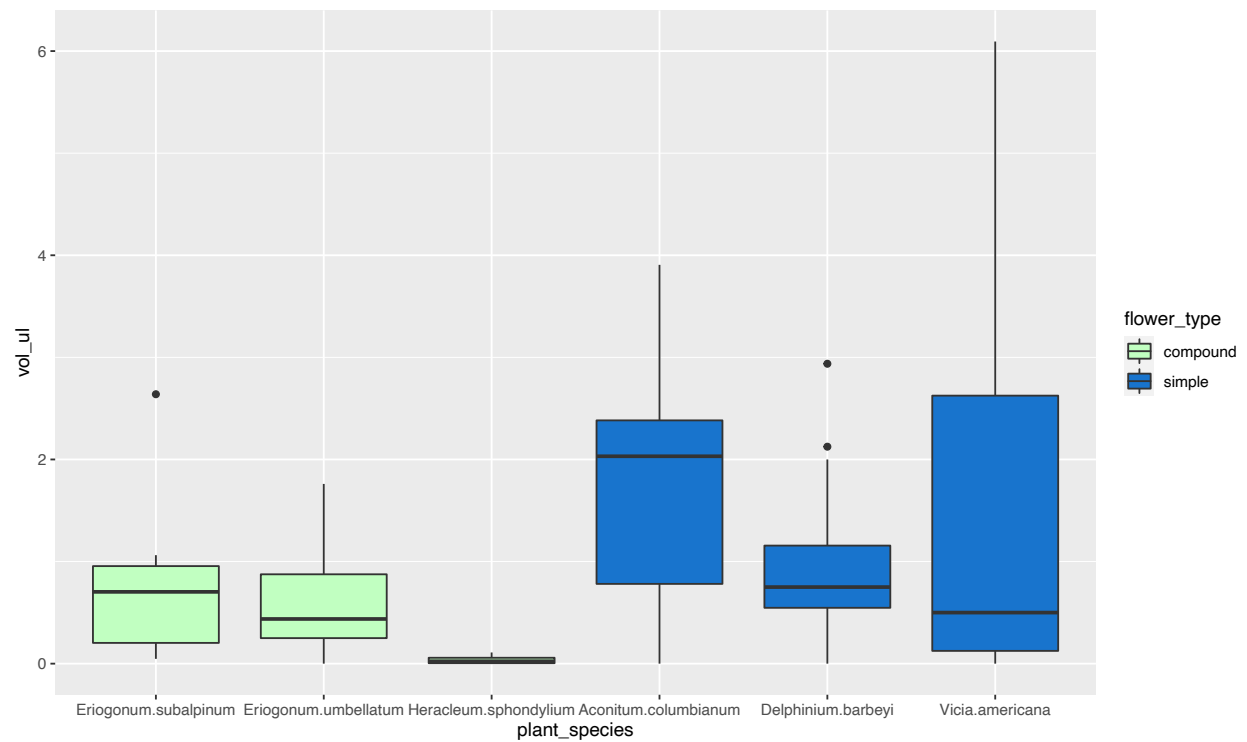
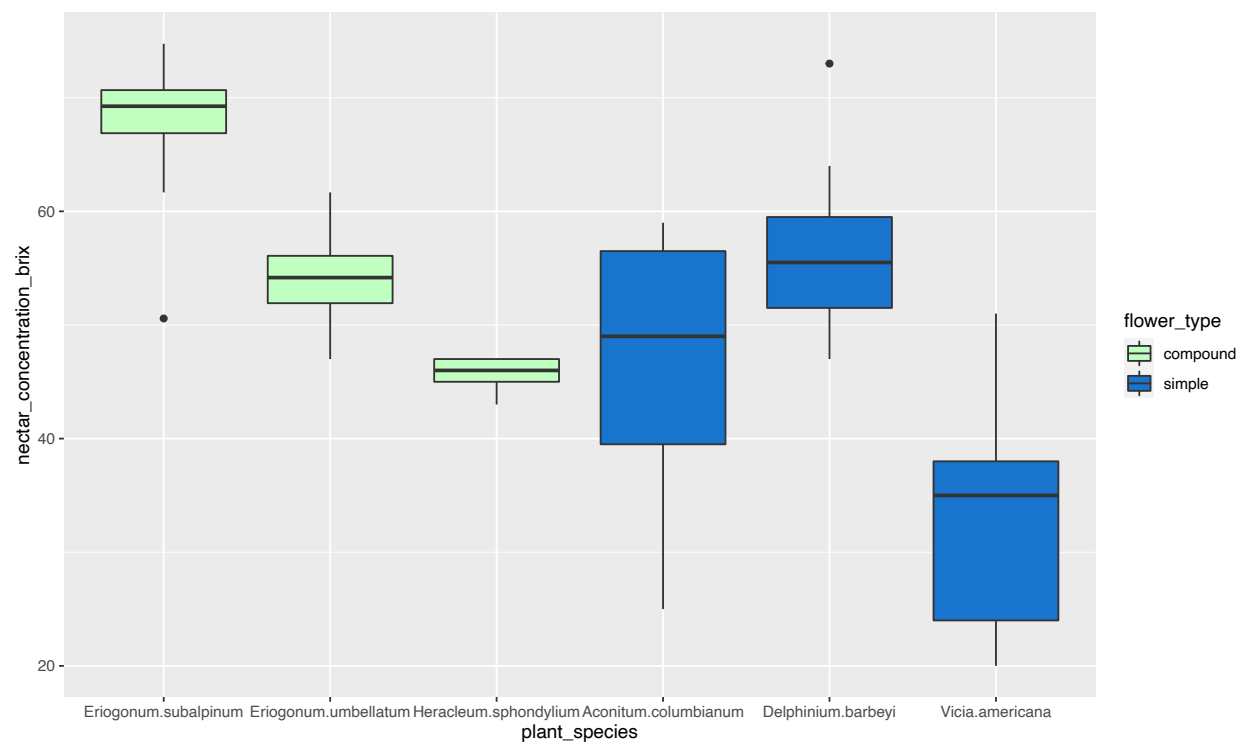
Figure 2: Graph of Vol (μ l) vs plant species**Figure 3:** Graph of nectar concentration in brix vs plant species.

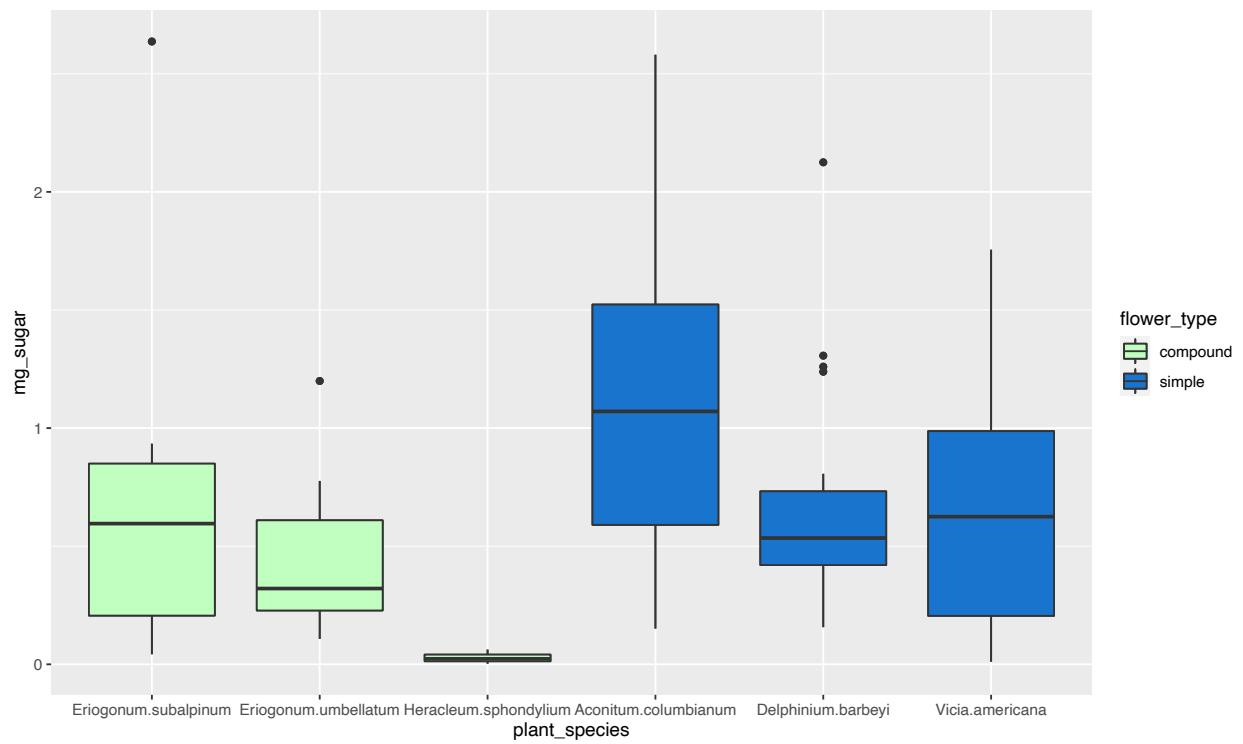
Figure 4: Graph of nectar sugar (mg) vs plant species.

Figure 5: Histogram of distribution in structural morphology within different species of simple and compound flowers.

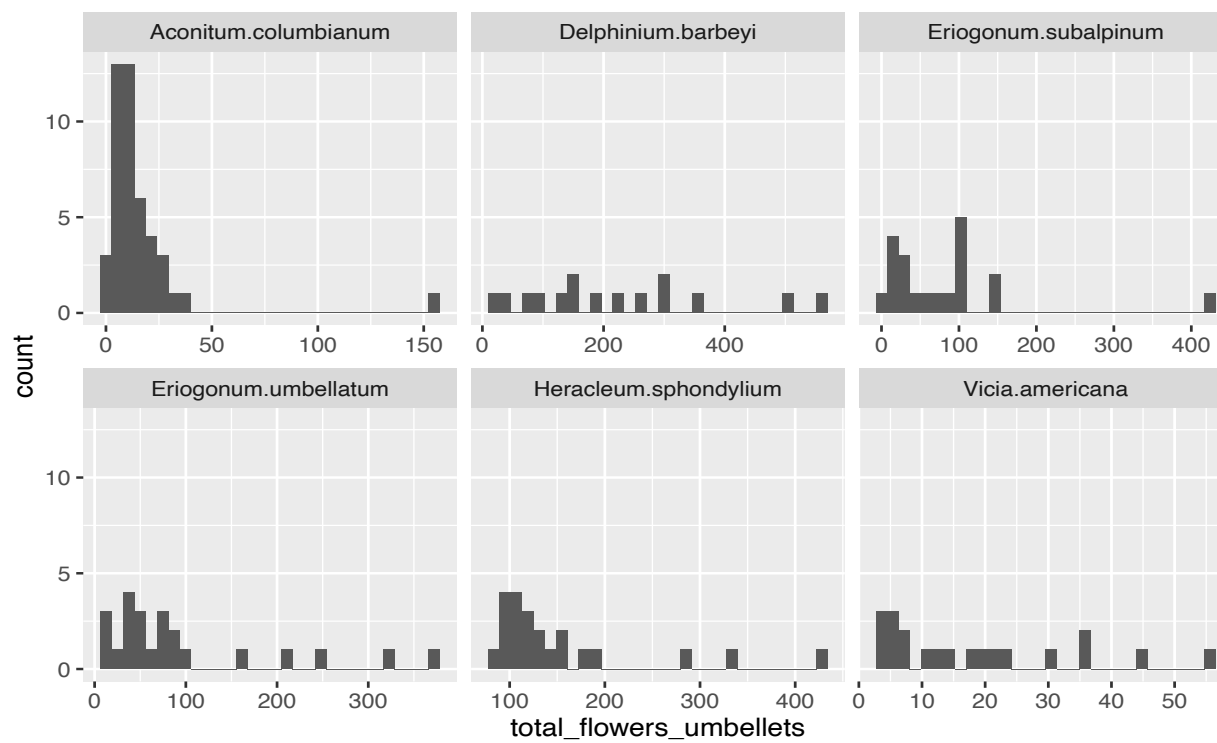
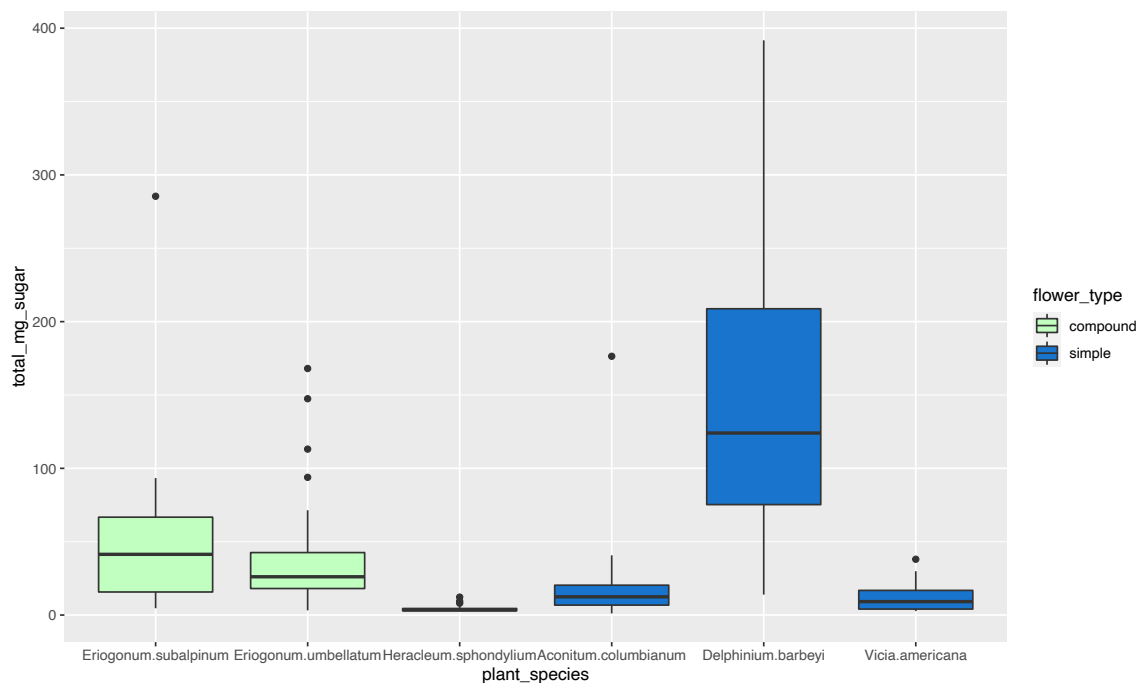


Figure 6: Graph of total nectar sugar (mg) per plant across all plant species studied.**Figure 7:** Tukey HSD chart with grouping based on congruent average nectar sugar (mg) per flower species.**Comparing nectar sugar in mg per plant:**

SPECIES	TOTAL (mg) SUGAR	GROUPS
Delphinium barbeyi	154.466463	A
Eriogonum subalpinum	53.711539	B
Eriogonum umbellatum	43.959735	BC
Aconitum columbianum	17.487671	BC
Vicia americana	12.431247	BC
Heracleum sphondylium	4.361477	C

Figure 8: *Bombus flavifrons* plant visitation data.

species	plant_species	total_visits
flavifrons	Aconitum.columbianum	344
flavifrons	Delphinium.barbeyi	1320
flavifrons	Vicia.americana	258
flavifrons	Eriogonum.subalpinum	0
flavifrons	Eriogonum.umbellatum	0
flavifrons	Heracleum.sphondylium	0

Figure 9: Tukey HSD data.

Plant Species Compared	Diff	Lwr	Upr	P adj
Eriogonum.umbe llatum(vs)Eriogon um.subalpinum	-9.751804	-52.36131	32.857697	0.9857872
Heracleum.spho ndylium- Eriogonum.subal pinum	-49.350061	-92.40762	-6.292498	0.0146903
Aconitum.colum bianum- Eriogonum.subal pinum	-36.223868	-73.67686	1.229128	0.0642056
Delphinium.barb eyi- Eriogonum.subal pinum	100.754924	53.15300	148.356851	0.0000001
Vicia.americana- Eriogonum.subal pinum	-41.280292	-85.35111	2.790529	0.0803032
Heracleum.spho ndylium- Eriogonum.umbe llatum	-39.598257	-81.15889	1.962374	0.0714332
Aconitum.colum bianum- Eriogonum.umbe llatum	-26.472064	-62.19404	9.249908	0.2723688
Delphinium.barb eyi- Eriogonum.umbe llatum	110.506728	64.25442	156.759034	0.0000000
Vicia.americana- Eriogonum.umbe llatum	-31.528487	-74.13799	11.081014	0.2739687
Aconitum.colum bianum- Heracleum.spho ndylium	13.126193	-23.12906	49.381446	0.9012971
Delphinium.barb eyi- Heracleum.spho ndylium	150.104985	103.43958	196.770389	0.0000000

Figure 9 continued: Tukey HSD data.

Plant Species Compared	Diff	Lwr	Upr	P adj
Vicia.americana-Heracleum.sphondylium	8.069770	-34.98779	51.127333	0.9943271
Delphinium.barbeyi-Aconitum.columbianum	136.978792	95.42842	178.529161	0.0000000
Vicia.americana-Aconitum.columbianum	-5.056423	-42.50942	32.396573	0.9988083
Vicia.americana-Delphinium.barbeyi	-142.035215	-189.63714	-94.433288	0.0000000

Works Cited

- Blüthgen N, Klein AM. 2011. Functional complementarity and specialization: The role of biodiversity in plant-pollinator interactions. *Basic and Applied Ecology*. 12(4), 282-291.
- Haydack MH. 1970. Honey Bee Nutrition. *Annual Review of Entomology*. 15(1), 143-156.
- Miller-Struttmann NE, Geib JC, Franklin JD, Kevan PG, Holdo RM, Ebert-May D, Lynn AM, Kettenback JA, Hedrick E, Galen C. 2015. Functional mismatch in a bumble bee pollination mutualism under climate change. *Science*. 349(6255), 1541-1544
- Pradervand JN, Pellissier L, Randin CF, Guisan A. 2014. Functional homogenization of bumblebee communities in alpine landscapes under projected climate change. *Climate Change Responses*. 1(1)