

**Effects of Floral Abundance, Pollinator Interactions, and Floral Morphology on Stigmatic
Pollen Deposition**

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Abstract

Pollination is a major driver of plant reproductive success and facilitates the growth of many of our major crops and is a foundation to the diversity of many ecosystems. Understanding the ecology of pollination will help preserve this important ecosystem service and predict the changes that will come with climate change. During pollination a pollinator transfers pollen to the stigma of a plant and the plant's reproductive success is determined by the receipt of conspecific pollen. Many factors can determine the ratio of conspecific to heterospecific pollen that is deposited on the stigma of an individual plant. The available pollinator community and their level of specialization, flower morphology, and the composition of the floral community all can interact in complex ways to influence the composition of pollen deposition. I hypothesize that in subalpine meadows of the Rocky Mountains the most significant driver of conspecific pollen deposition is relative floral abundance. My results show that both relative interaction abundance and relative floral abundance had insignificant effects on the deposition of conspecific pollen and that flower morphology could be the strongest driver.

Introduction

Importance of pollination

Pollination is a critical step in plant reproduction and is vital to the conservation of our agricultural systems and biodiversity (Kevan et al, 2003). It is estimated that 80-90% of flowering plants rely on an animal pollinator to transfer pollen between individuals (Ollerton et al, 2011). Reduction in pollination services can significantly reduce fruit set and quality in crop systems and reduce primary production in natural communities (Klein et al, 2009). Pollination by animals supports the production of 70% of leading global crops and many of these crops are effectively pollinated by native bees (Ricketts et al, 2008). Thus conserving habitat for wild pollinators can help maintain pollination ecosystem services within agricultural systems (Ricketts et al, 2008). In order to conserve these wild pollinators and consequently plant reproduction we need to understand the ecology of pollination networks.

Specialization and Generalization

Pollination ecology can be studied from many different scales as broad as network ecology to individual species' natural history. In ecological communities species are connected in complex networks of interactions (Hernández-Castellano et al, 2020). In pollination ecology, these interactions consist of an animal visitor and a flowering plant species. This involves the

transfer of pollen from flower to flower by an animal. The composition of the pollen that is deposited on a plant can be segregated between conspecific and heterospecific pollen. The more heterospecific pollen is transferred the less likely that flower is to be reproductively successful. Specialization of pollinators increases the likelihood of conspecific pollen transfer and supports plant reproduction (Brosi 2016). Therefore, from the plant perspective it would be most beneficial to attract pollinators that specialize during an individual foraging bout, to increase the transfer of conspecific pollen (Brosi 2016). However, most pollinator species are generalists and thus co-flowering plants tend to share pollinators (Brosi 2016). Generalization can be in the form of a generalist plant species or pollinator species. In both cases the degree of generalization is continuous with some species having very few partners to some having many. Generalization in plants provides many potential pollinators. This can be advantageous in providing alternative pollinators in the case of potential extinctions of pollinators or loss of phenological synchrony. This creates community resilience to linked extinctions. However, generalist pollinators are more likely to transfer heterospecific pollen which can reduce the fitness of plants with a high degree of pollinator sharing (Briggs 2016; Brosi 2016). As much as 25% of insects visiting flowers do not carry conspecific pollen on their bodies and can be considered a ‘cheater’ from the plant perspective because the insects obtain floral resources but do not transfer conspecific pollen (Alarcon, 2010). There are many drivers behind different levels of specialization and generalization within a pollination-network, which can have complex consequences for plant reproduction.

Morphology Effects on Reproductive Success

The fitness cost of receiving heterospecific pollen can drive the evolution of adaptations that minimize heterospecific pollen, such as modified pollen placement or divergence in flower color (Ashman, 2013). Complex flower morphology can constrain the visitor community that can potentially forage on a flower species (Stang 2006). If the visitor community is narrowed then the composition of the pollen deposited on the stigma may also be more selected for higher conspecific content. This is because floral visitor species vary in their ability to transfer pollen between plants (Adler et al 2006). Facilitation is particularly important to specialist plants that rely on one or few generalist pollinators (Ghazoul, 2006). Facilitation among species with morphologically highly distinctive flowers is driven by the availability of complementary rewards that can be discriminated between by pollinators (Ghazoul, 2006).

Floral Abundance Effects on Pollinator Visitation and Plant Reproductive Success

The composition of the floral community has very important effects on pollinator activity and significant impacts on the reproductive success of the individual plants. With a changing environment, plant species ranges are moving and nonnative species are increasingly being introduced to wild plant communities. These communities and networks are sensitive to subtle and dramatic changes in floral composition. The introduction of a regionally native plant in a new area in low relative abundance facilitates more pollinator interactions and creates more generalized networks (Hernández-Castellano et al, 2020). This results in the deposition of more heterospecific pollen which has contrasting negative and positive reproductive effects for the resident plant species (Hernández-Castellano et al, 2020). This has implications for native plant range shifts in response to climate change as novel plant communities are created. On the more extreme side, the introduction of non native species can result in more dramatic changes to plant and pollinator interactions. These can have both facultative and competitive effects that result in the reduction of seed set and reproductive success of the native plant community (Bruckman et al 2016, Bartomeus et al 2008, Morales et al 2009). Facilitation can occur due to the higher total amount of resources in the floral community attracting more pollinators to all the plants; however this does not necessarily bring about more native seed set (Bartomeus et al, 2008). Facilitation of pollinator visits reduces seed set in this context due to a high deposition of heterospecific pollen (Bruckman et al, 2016). A meta-analysis of over 40 studies shows that in general nonnative plants have a predominantly negative impact on the reproductive success of native plants by disrupting the pollination network via competition for pollination which ultimately reduces the number of pollination visits to native plants (Morales et al, 2009). Pollination networks are highly connected and sensitive to changes, thus it is important to study many different communities to understand the nuances of these sensitive interactions.

Looking at the addition of a novel plant species can be an easy way to understand the effects of floral community on pollination and plant reproductive success. However, it is also important to understand how a community varies throughout the season and examine how shifting floral abundance can contribute to floral reproductive success. Having data on this baseline community will be especially important so that future research can compare it to species invasions, range movements, and phenological shifts.

My Question and Hypothesis

This research aims to compare floral abundance and pollinator visits to the conspecific pollen deposition of seven focal species as a measure of floral reproductive success and visitor quality. Conducted in subalpine meadows of the Rocky Mountains, this data will provide insight on how plant reproduction changes along seasonal shifts in floral abundance and pollinator visitation. As seen in previous studies, pollination networks can be very complex and the specific context can drive the reproductive success of the different plant species. Thus uncovering the driver of conspecific pollen deposition in these subalpine meadows will reveal new insights. I hypothesize that the main driver of conspecific stigmatic pollen deposition will be relative floral abundance, with floral visitation and morphology playing a minor role.

Methods

Field Work

I sampled in five sites where floral abundance and visitor interactions are recorded as part of the Brosi lab's many projects at the Rocky Mountain Biological Laboratory (See Figure 1). Each site has three 10 meter transects that are 1 meter wide. The ten by one meter transects were divided into five segments lengthwise. This created five two meter by one meter segments in the transects (See Figure 2). Floral abundance was recorded by counting each blooming flower within all the transects and identifying it to species. In these segments we observed visitor interactions. Each time a pollination event (ie. an insect coming into contact with the reproductive organs of the flower) was observed, we captured the insect and assigned a gross identification (other fly, syrphid, bumble bee, solitary bee, hemipteran, beetle) and identified the corresponding flower to species. At these sites I choose nearby wilted flowers to sample. By collecting stigmas after wilting we did not affect plant reproduction. I left the remaining floral structure (including the ovary) undamaged. This minimizes the effect on the plant reproduction as collection was after pollen tube growth and the ovule fertilization. Sample species were: *Lathyrus leucanthus*, *Lupinus argenteus*, *Linum lewisii*, *Delphinium nuttallianum*, *Delphinium barbeyi*, *Geranium richardsonii*, and *Vicia americana*. The stigmas were mounted on microscope slides with fuchsin jelly in the field to avoid contamination and loss of pollen. In addition, I collected anthers of each target species and mounted them using the same method.

Lab Work

In the lab, I counted all the pollen on the slides (with the exception of *Vicia a.* and

Lathyrus l. samples, where I only counted the pollen on one half of the stigma) and categorized the pollen grains as either heterospecific or conspecific. Anther slides were used to identify conspecific pollen in conjunction with Brosi Lab reference images. I used a compound microscope with 40X to 400X magnification.

Data Analysis

After counting all the conspecific and heterospecific pollen grains, the percent of conspecific pollen was found in excel. For each site and date when stigmas were collected, the relative floral abundance and relative interaction abundance was calculated. This was done using the floral count and insect interaction data collected by the Brosi Lab and myself. relative interaction abundance was calculated as the number of visits to the target species divided by total visits observed at the site on the day of collection. Relative floral abundance was calculated as the number of flowers of each target species divided by the total flower count at the site on the day of collection.

A total of 104 stigma slides were included in the analysis. Table 1 shows how those stigma slides were distributed between site, day, and species. To test if there was a relationship between relative floral abundance and Percent of Conspecific Pollen deposited on a stigma, we made the scatter plot in Figure 3. Each of the points represents a separate stigma slide from one individual. The line is a linear regression to help visualize the trend. Similarly to Figure 3, in Figure 4 we wanted to explore if there was a relationship between relative interaction abundance and Percent Conspecific Pollen. To examine if there was a correlation between the two independent variables, relative floral abundance and relative interaction abundance, Figure 5 is a scatter plot showing the relationship relative interaction abundance with a linear regression.

The relationship of all these variables was further analyzed in a Linear Mixed Effects Model (LMM) to allow for both fixed and random effects, results in Table 2. The random effects included in the model were species nested within site and date. relative interaction abundance and relative floral abundance were included in the model as fixed effects. By including both variables in the model we were able to control for the effects of one while examining the effects of the other. The LMM was first run with relative floral abundance and relative interaction abundance as interacting variables due to the results from Figure 4. However, the model showed that the interaction was not significant. The model was run a second time with the variables not interacting with each other. The results in Table 2 are those of the LMM with the fixed effects

not interacting. The sample sizes were not large enough to include flower morphology in the model. Therefore, we ran a Welch Two Sample T-Test to compare the mean percent of conspecific pollen on complex and open flowers. The resulting box plot can be seen in Figure 6.

Stigma Collection Site Map

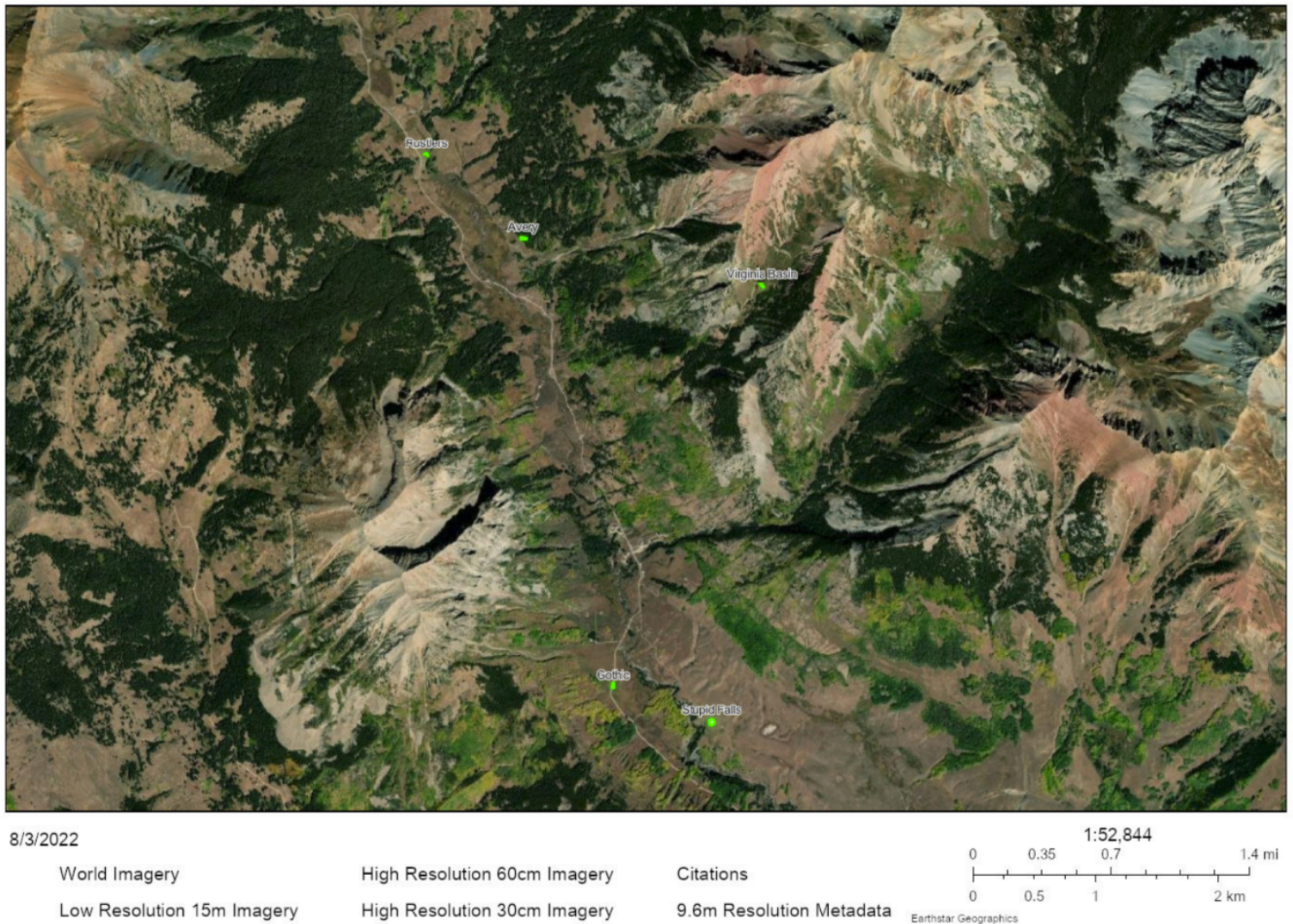


Figure 1: Map of five sites located in the surrounding area of the Rocky Mountain Biological Laboratory.

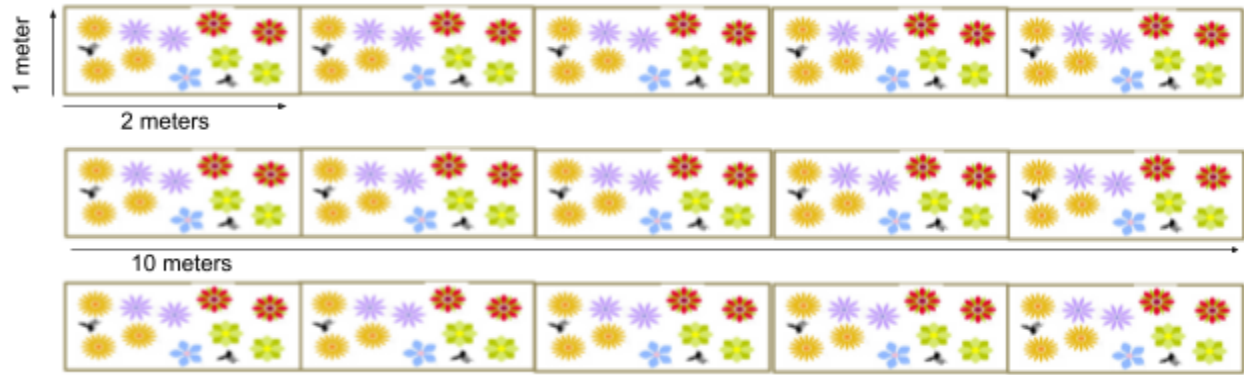


Figure 2: Representation of transects at sites

Results

The relative floral abundance was positively correlated with the percent of conspecific pollen deposited on stigmas (linear regression, Figure 3). Similarly, there is a positive correlation between the relative interaction abundance and the percent of conspecific pollen deposited on stigmas (linear regression, Figure 4). There is also a positive correlation between relative floral abundance and relative interaction abundance (linear regression, Figure 5). The Linear Mixed Effects Model (LMM) shows that the effect of Floral Abundance (value: 0.94495) was much stronger than the relative interaction abundance effect (value: 0.04844) on the percent of conspecific pollen deposited on stigmas (LMM values, Table 2). However, the p-values show that the relationships between relative floral abundance and the percent of conspecific pollen ($p = 0.1135$) and the relative interaction abundance and the percent of conspecific pollen ($p = 0.9745$) were insignificant (P-Values, Table 2). There was a significant difference in conspecific pollen deposition between flowers with complex and open morphologies, as seen in the box plot in Figure 6. The mean percent of conspecific pollen on the stigmas of open flowers is 16.8% and in complex flowers the mean is 77.3% with a $p\text{-value} < 2.2e-16$.

Table 1: Table of Stigma Collections

This table shows how many individuals of each species were sampled for stigma collection at each site and date.

site	Date	Species	Count
AP	7/14/2022	Geranium richardsonii	8

Site	Date	Species	Count
KP	7/1/2022	Linum lewisii	2

site	Date	Species	Count
AP	7/6/2022	Geranium richardsonii	5
AP	7/6/2022	Lupinus sp.	5
AP	8/1/2022	Geranium richardsonii	5
GT	7/12/2022	Linum lewisii	6
GT	7/12/2022	Vicia americana	2
GT	7/18/2022	Geranium richardsonii	1
GT	7/18/2022	Linum lewisii	3
GT	7/18/2022	Vicia americana	5
GT	7/25/2022	Delphinium barbeyi	3
GT	7/25/2022	Linum lewisii	3
KP	7/1/2022	Delphinium nuttallianum	3
KP	7/1/2022	Lathyrus leucanthus	1

Site	Date	Species	Count
RG	6/28/2022	Delphinium nuttallianum	4
RG	6/28/2022	Linum lewisii	3
RG	7/8/2022	Delphinium barbeyi	5
RG	7/8/2022	Linum lewisii	5
SF	7/14/2022	Delphinium barbeyi	4
SF	7/22/2022	Delphinium barbeyi	9
SF	7/26/2022	Delphinium barbeyi	7
VB	6/27/2022	Lathyrus leucanthus	5
VB	7/19/2022	Geranium richardsonii	6
VB	7/19/2022	Lathyrus leucanthus	7
VB	7/19/2022	Linum lewisii	4
VB	7/19/2022	Vicia americana	2

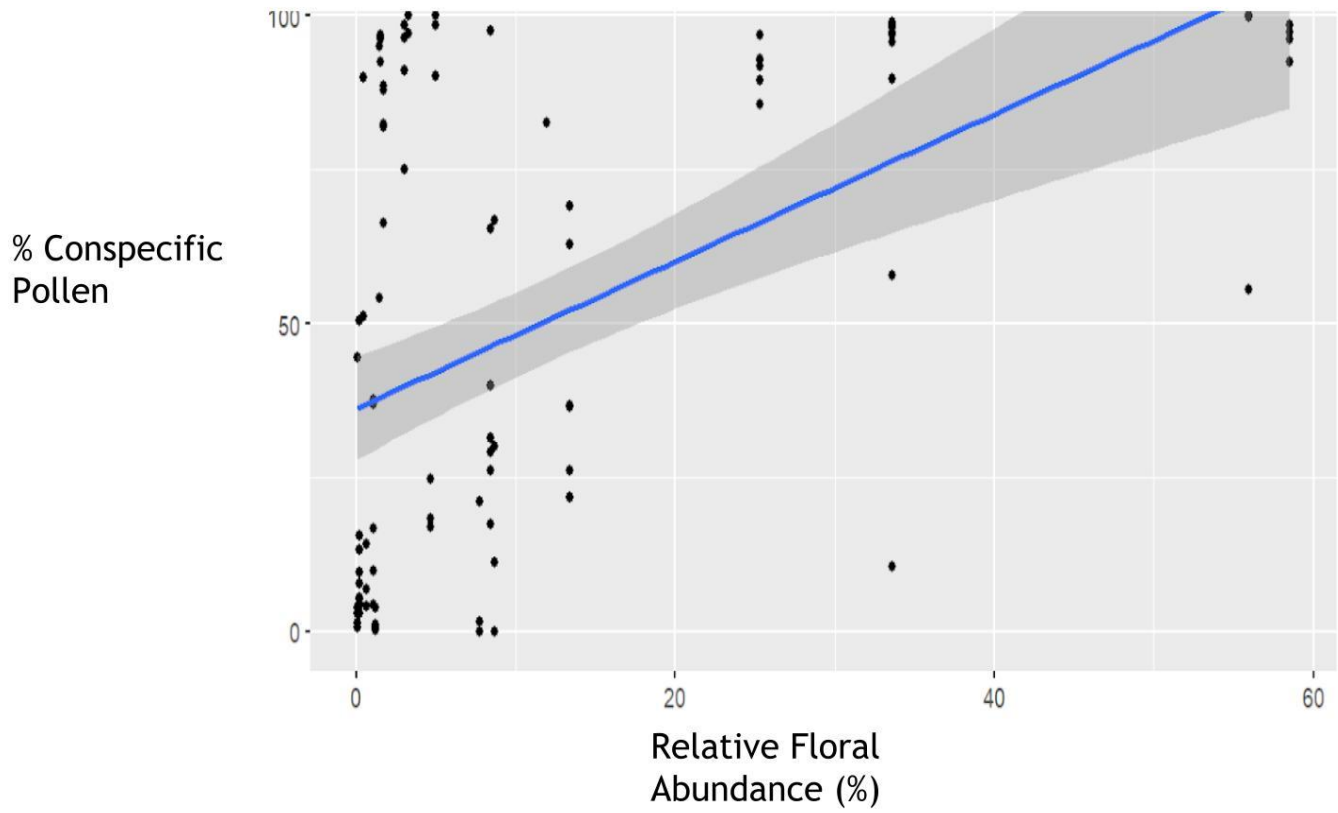


Figure 3: Relationship between Relative Floral Abundance and Percent of Conspecific Pollen on Stigma

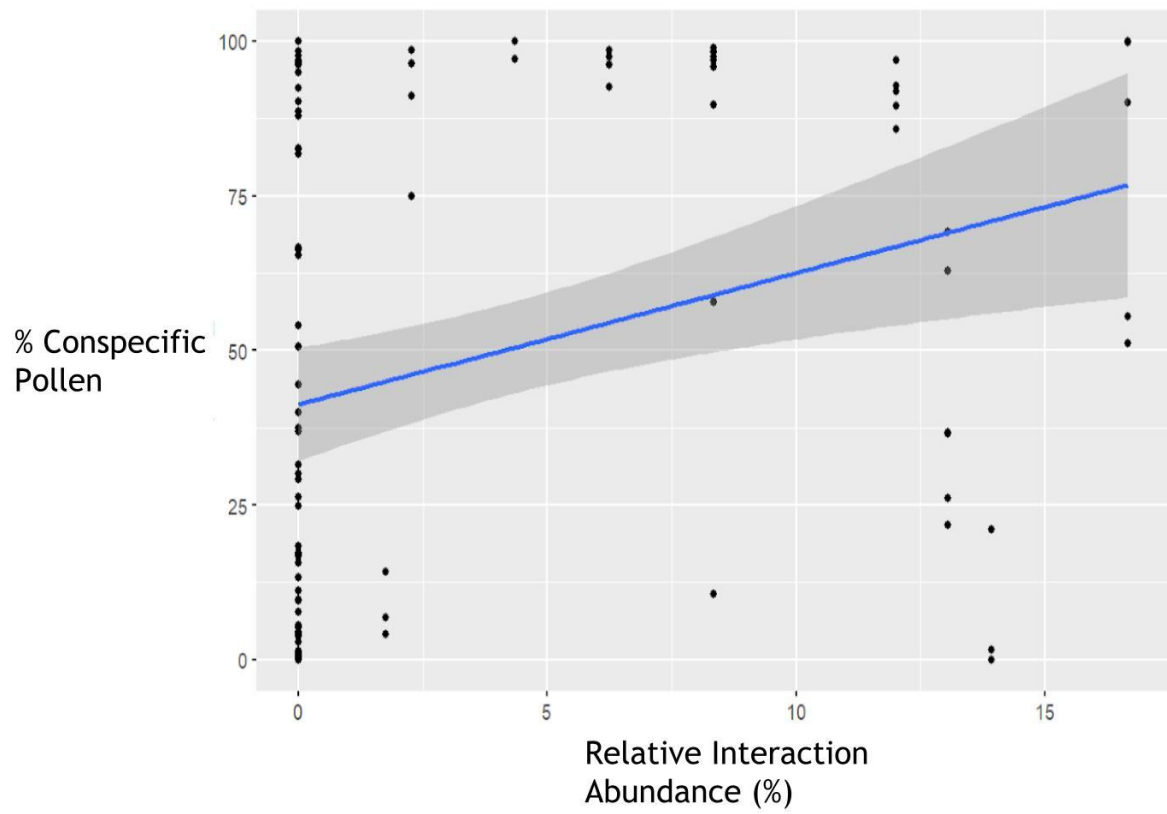


Figure 4: Relationship between Relative Interaction Abundance and Percent of Conspecific Pollen on Stigma

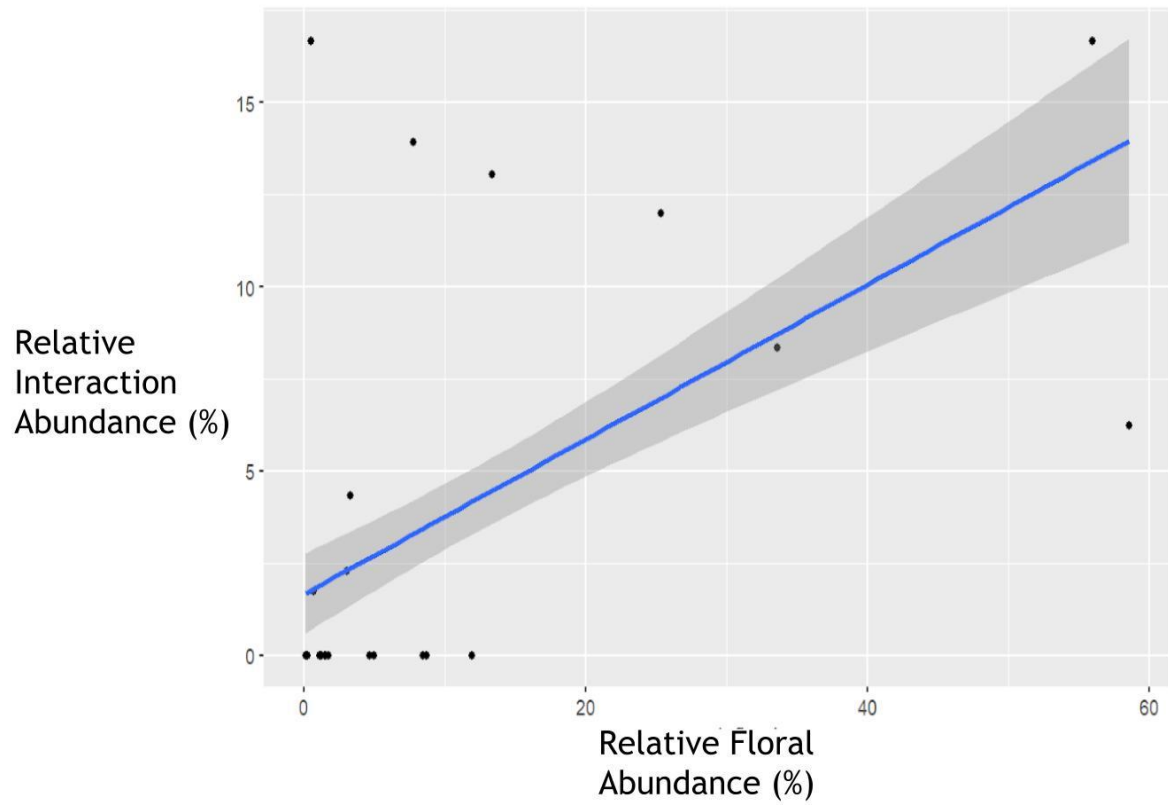


Figure 5: Relationship between Relative Floral Abundance and Relative Interaction Abundance

Table 2: Results from LMM Model

	Value	Standard Error	P-Value
Relative Floral Abundance	0.94495	0.522585	0.1135
Relative Interaction Abundance	0.04844	1.463807	0.9745

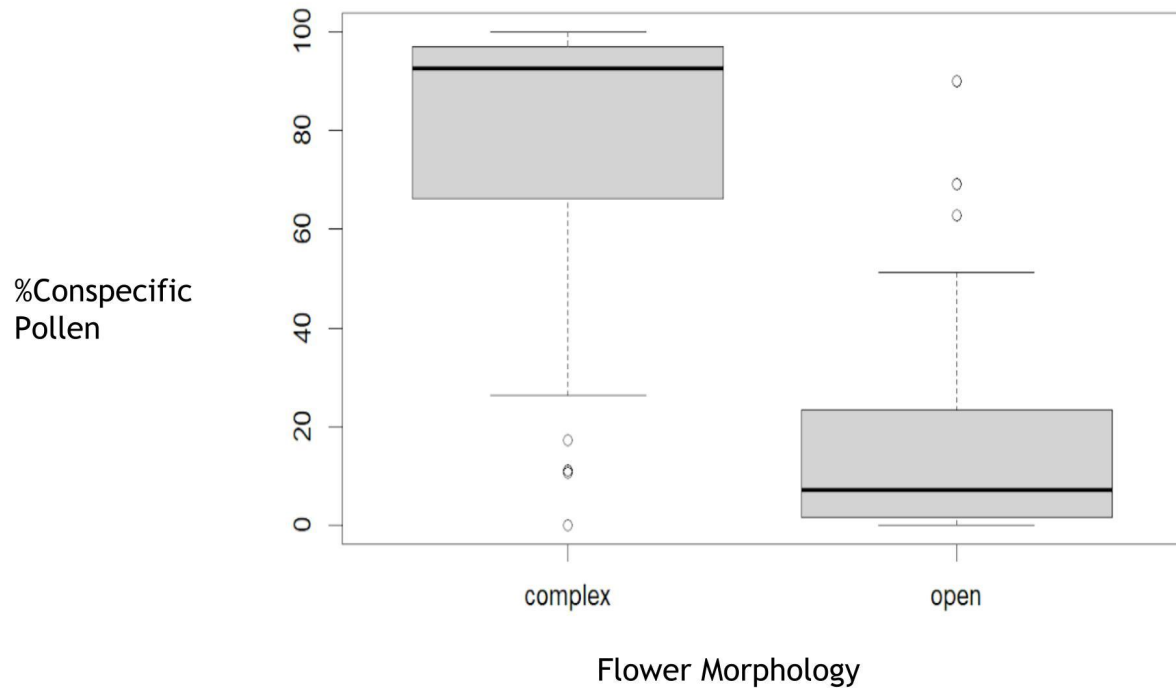


Figure 6: Comparison of conspecific pollen deposition on flowers with complex and open morphologies

Discussion

The positive trend between relative floral abundance and conspecific pollen deposition is consistent with other studies (Duffy et al 2011). However, the lack of significance in this relationship and that of relative interaction abundance to the percent of conspecific pollen indicates that there were most likely other factors driving the composition of stigmatic pollen. The taxonomic identity of the pollinators can also play a key role in structuring the transfer of conspecific pollen (Cullen et al. 2021, Alarcon 2010). Although the data set included in this study did not include the pollinator identity, it is possible that complex flower morphology was limiting the visitor community. The flower species in this study with complex floral morphologies were *Lathyrus leucanthus*, *Lupinus argenteus*, *Delphinium nuttallianum*, *Delphinium barbeyi*, and *Vicia americana*. Complex flower morphologies can be an evolutionary adaptation that minimizes the deposition of heterospecific pollen (Ashman, 2013). This is done by constraining the visitor community that can access the stigma of the flower (Stang 2006). All these flower species have petals that are structured to cover the stigma. From observations in the field, it has been noted that it takes a large pollinator to move the petals of these species and

deposit pollen on the stigma (M. Cathcart, personal observation). *Delphinium nuttallianum* and *Delphinium barbeyi* are known to be pollinated by bumble bees and hummingbirds (Williams et al. 2001). *Lathyrus leucanthus*, *Lupinus argenteus*, and *Vicia americana* are visited by large bodied bee species of *Megachile*, *Osmia*, and *Bombus*, which are likely the only effective pollinators of these plants (Forrest et al. 2010). Compared to beetles, butterflies, flies, and wasps, bees carry the most conspecific pollen grains (Alarcon, 2010). This is because bees actively forage for pollen, tend to have floral constancy during a foraging trip, have hairy bodies, and have evolved specialized structures for carrying pollen (Michener, 2000). This all could be contributing to the exceptionally high proportion of conspecific pollen on the stigmas of flowers with complex flower morphologies.

These results show that flower morphology is most likely the main factor determining the percent of conspecific pollen deposited on the stigma of a flower in the subalpine meadows surrounding the Rocky Mountain Biological Laboratory. The initial hypothesis that the most significant driver of conspecific pollen deposition is relative floral abundance is not supported by these results. In addition, the relative interaction abundance was insignificant in determining the percent of conspecific pollen on a stigma. Further research should look at larger samples of open faced and complex flowers to identify the most significant factor in the transfer of conspecific pollen within each group. Understanding the transfer of conspecific pollen within meadows furthers our knowledge of the reproductive success of our native plants and may give us the tools to protect biodiversity in the future.

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