

Pollination Ecology and Natural History of

Pedicularis groenlandica

A Senior Thesis

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By

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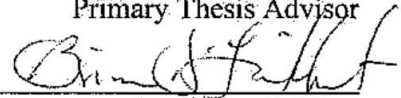
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Abstract

Arctic and alpine ecosystems have been found to be highly susceptible to changes caused by shifts in global climate trends. For this reason, it is important to study both the plants and the pollinators within these vulnerable ecosystems in order to best hypothesize how their mutualistic relationships may be affected by predicted, future climates. My study focuses on exploring the natural history and reproductive strategies of *Pedicularis groenlandica* (little elephanthead or elephantella). *P. groenlandica* relies on an evolved behavior in its pollinators whereby they buzz-pollinate the flowers. This dependency on such a specific behavior makes *P. groenlandica* a particularly interesting alpine plant to study. Through various pollination treatments, I found that pollen may be limiting to the seedset of these flowers. Flowers that I hand-pollinated with extra pollen tended to have slightly heavier seeds than those which were naturally pollinated by bees alone. I used microphones to record the acoustics of bumblebee flight buzzes in order to see how the number of buzzes within a patch of flowers related to the seedset of those flowers. When controlled for factors of visibility (such as the number of inflorescences within the patch), the number of recorded buzzes predicted the average seed weight of the flowers within that patch. Combined with past studies on *P. groenlandica*, my findings indicate the importance of bumblebees, and their evolved buzz-pollination behaviors, to populations of this species.

Introduction

Plant-interactions have been heavily studied around the world. While many studies have focused on the domestic honey bee (Apidae: *Apis mellifera*) due to its crucial role in the agriculture industry, there has also been a lot of work directed at studying various species of bumblebees (Apidae: *Bombus* sp.) and their roles in both crop production and natural plant communities (Potts et al. 2010). These studies are of great concern as various species of bumblebees have been found to be in decline in North America (Cameron et al. 2010), South America (Morales et al. 2013), and Europe (Goulson et al. 2008). Some of the primary causes for these population declines have been found to be habitat fragmentation and destruction (Cameron et al. 2010), pesticides (Potts et al. 2010), infection from parasites or pathogens (Cameron et al. 2010; Morales et al. 2013), and global climate change (Geib et al. 2015). It has been hypothesized that global climate change in particular can impact the abundances and local extinctions of different species of animals and plants due to biological interactions that are susceptible to changes in climates (Settele et al. 2014). Because of the momentum of global climate trends, it is important to study both the plants and the pollinators within vulnerable ecosystems in order to best hypothesize how their mutualistic relationships may be affected by predicted, future climates.

With rises in average temperatures and drops in average precipitation, alpine and arctic environments in particular are highly susceptible to climate change (Settele et al. 2014). Multiple studies with climate records of the Rocky Mountains have shown that in the past 50 - 60 years, either maximum or minimum average temperatures on various mountains have increased anywhere from 0.98° to 2° C (Miller-Struttmann et al 2015; McGuire et al 2012; Kopp and Cleland 2014). These changes in temperatures along with decreases in precipitation have been

found to be causing altitudinal shifts in wildflower populations as well as earlier flowering periods during the growing season (Kopp and Cleland 2014; Inouye 2008; Settele et al. 2014). With these climate-related changes, it is important to study the mutualisms that exist between alpine wildflowers and bumblebees. Many flowering plants depend on bumblebees for their reproductive success, and many bumblebees depend on wildflowers for pollen (a protein source in the colony) and/or nectar (an energy source for the individual workers) (Konzmann and Lunau 2014). Thus, climate-related changes in plant distribution and life-history may have impacts for pollinator communities and associated impacts on other plant species.

This study focuses on *Pedicularis groenlandica* Retz. (common names: little elephanthead, elephanthead lousewort, and elephantella), a member of the Scrophulariaceae family. There are numerous gaps in the knowledge concerning the natural history and life strategies of this species. *P. groenlandica* is a nectarless, buzz-pollinated flower that requires a pollinator in order to produce fruits (Macior 1968). As a member of alpine plant communities, this species provides an opportunity to examine the impacts of climate change on plant-pollinator interactions.

Buzz-pollination is a strategy that has evolved in approximately 20,000 species across numerous families of angiosperms (Buchmann 1983). Flowers that require buzz-pollination from a pollinator (most often bees) have specialized, poricidal anthers that contain pollen-filled pores on their tips (Macior 1964; Buchmann 1983). In order to dislodge pollen from these often hidden anthers, a bee must vibrate the flower using indirect flight muscles (Buchmann 1983; Macior 1964). Some aspects of this pollen dispersal strategy, and its co-evolution with pollinators, are not well understood, however there are several ideas as to how buzz-pollination evolved. Because nectarless flowers are often visited by bees that are efficient at removing large amounts of pollen, it is advantageous for plants to limit the amount of pollen that can be taken per

pollinator visit (De Luca and Vallejo-Marín 2013; De Luca et al. 2012). Also, when pollen is released via buzz-pollination, it is often ejected from the flower in a cloud that covers the pollinator, making it difficult to groom all of the pollen off of the body and into the corbiculae (Macior 1968). This is an effective way of ensuring there will still be pollen on portions of the pollinator's body that will later come into contact with the stigmas of other flowers that the pollinator visits (De Luca and Vallejo-Marín 2013).

One way to study the impacts of pollinators on the reproductive success of plants is to consider whether the amount of pollen being deposited on a flower is limiting the fitness of that individual plant. There are many factors that influence the successful production of fruits with viable seeds. The pollinators of a flower may impact factors such as pollen quantity (i.e. pollen source) and quality (i.e. the amount of pollen deposited on a given stigma). Pollen limitation occurs in specific conditions where the distribution or population size of the pollinator is causing an insufficient amount of pollen to be deposited on the flowers, thereby limiting seed production (Knight et al. 2005). While some studies have found pollen limitation to be the primary cause of low seed production (Karrenberg and Jensen 2000), others have found that different factors (such as pollen source) have had more significant impacts on fitness (Wagner et al. 2016; Yang et al. 2004).

My research begins to examine the following questions:

Pollinator Ecology

- 1) Is pollen a limiting factor for seedset?
- 2) How does the number of recorded flight buzzes relate to the fitness of a patch?
- 3) To what extent are *P. groenlandica* self-compatible?

Reproductive Allocation

- 1) How is energy allocated for reproductive efforts? With augmented pollen?

Visibility/ Plant ecology/ Competition

- 1) How does the height of the plant affect the visibility of its flowers for pollinators?
- 2) How does the presence of neighboring flowering plants affect the pollination of *P. groenlandica*?
- 3) How does neighboring (encroaching) willows affect the visibility of *P. groenlandica*?

These questions focus on the natural history and life strategies of *P. groenlandica* in terms of its dependence on its pollinators for successful reproduction. This research was primarily conducted via various pollination treatments of the flowers and acoustic recordings of the bumblebees.

Materials and Methods

Study System:

Pedicularis groenlandica Retz. in the past has been considered a member of the Scrophulariaceae family. However, recently the entire genus *Pedicularis* has been argued to be part of the Orobanchaceae family (Yu et al. 2015). This may have to do with the hemiparasitic nature of many members of the genus (including *P. groenlandica*) which is a common characteristic of the Orobanchaceae family. *P. groenlandica* is an herbaceous, perennial plant that occurs in arctic, alpine, and sub-alpine environments. It is most often found in moist environments such as wet meadows, alpine swales, or alongside streams and lakes (Scott 1996; Macior 1968; personal obs. Z. Moffett). *P. groenlandica* is distributed across the western half of the United States and north into Canada (<https://plants.usda.gov/core/profile?symbol=PEGR2>).

The basal leaves of the plant grow close to the ground and are lanceolate and segmented. Individual plants often have one to three inflorescence spikes that can be 1-30 cm long (http://ucjeps.berkeley.edu/eflora/eflora_display.php?tid=36574). Within my study sites, I observed the flowering period of this species to be from about 9-24 July 2016.

Along the inflorescences of *P. groenlandica* are numerous light-pink to deep-purple flowers resembling small elephant heads. The anthers of four stamens are located within the main portion of the galea (fused petals that form the elephant-looking head), and when pollen is released from the anthers, it exits the flower through the base of the rostrum (the proboscis-like extension on the front of the flower). The style of each flower travels from within the galea and through the rostrum where the stigma slightly protrudes from the tip (Macior 1968). Pollen can only be released from the inner-anthers when vibrated by a pollinator (most often bumblebees) in what is called buzz-pollination. Bumblebees (Hymenoptera: Apidae: *Bombus*) forage on *P. groenlandica* by clasping the lateral petals of the flower as well as the galea with their legs before rapidly moving indirect flight muscles to vibrate the pollen out of the anthers (Macior 1964; Macior 1968). In this foraging position, the anterior abdomen of the bee is touching the stigma as it protrudes from the end of the rostrum, allowing for pollen to be transferred from the bee to the flower.

Historically, seven species of *Bombus* have been known to commonly pollinate *P. groenlandica* in Colorado: *B. bifarius*, *B. centralis*, *B. flavifrons*, *B. melanopyrgus*, *B. mixtus*, *B. occidentalis*, and *B. sylvicola* (Macior 1968). During the field season of 2016, *B. sylvicola* was almost exclusively observed on *P. groenlandica* with *B. bifarius* being the only other rare visitor in the study sites that were located on Pennsylvania Mountain (Park County, Colorado, USA). However, thorough surveys were not taken to observe all of the species visiting the *P.*

groenlandica.

Experimental Design:

Site and Plant Selection

The study sites for this project were within the krummholz of Pennsylvania Mountain at about 3530 m elevation. Pennsylvania Mountain is located within the Mosquito Range in Park County, Colorado. The vegetation of the krummholz is a mosaic of willows (*Salix* sp.) and spruce (*Picea* sp.) just below tree line. *P. groenlandica* can be found in the moist, open spaces formed between *Salix* bushes. The observed neighboring flowering species were *Vaccinium* sp., *Trifolium parryi*, *Bistorta bistortoides*, and *Pedicularis parryi*.

The field portion of this study took place from 4-19 July, 2016. However I returned to the sites on 4 September, 2016 in order to harvest the mature fruits. During the month of July, 2016, the average high temperature in Fairplay, CO was 21.66°C (71°F) and the average low temperature was 6.11°C (43°F). During this month there was also a total recorded precipitation of 6.1 cm (<http://www.usclimatedata.com/climate/fairplay/colorado/united-states/usco0530/2016/7>).

Twelve study patches containing *P. groenlandica* were selected in early July, 2016. Eight of these patches were designated for pollination treatments, fruit collection, and audio recordings while four patches were used exclusively for audio recordings and fruit collection from naturally pollinated plants. Study patches were selected based on the presence of around 100 inflorescences growing in a relatively small area (patch sizes ranged from 6m² to 25²) among *Salix* sp.

Acoustic Recording

Audio recordings in the field took place 10-24 July, 2016. Recordings were initially taken with six iPads and microphones, but by the end of the season, as many as eleven iPads/mics were in use on a given day. Electret microphone capsules were mounted on thin metal poles at plant height near the center of the patch. These poles were rotated around the center of the patch for each new recording. The Awesome Voice Recorder (AVR version 3.5; Newkline Co., Ltd) application was used to record the acoustic signals at a sampling rate of 11,025 kHz/48 kbps. Recordings started at approximately 0800 hr and lasted approximately six to nine hours. At the start of the flowering period, iPads and microphones were only set up in patches where multiple open flowers had been observed. iPads and microphones were rotated daily based on the stages of the flowers as well as in an effort to have the patches recorded an equal number of days. All available mics/iPads were used on most days, however, there were a couple of days where only a few were set up due to inclement weather conditions and a limited amount weather-proof boxes for the iPads. By the end of the flowering period, each of the twelve patches had between 5 and 7 days of recordings.

Pollination Treatments

For each patch, an inflorescence count was made. In patches 1-8, inflorescences were randomly chosen and marked for the following treatments: naturally outcrossed, augmented pollen, control (naturally outcrossed), hand-pollinated outcrossed, and hand-pollinated selfed. In each of these patches there were three paired treatments of the hand-pollinated outcrossed and selfed plants and five paired treatments of augmented pollen and control (naturally outcrossed).

Once the hand-pollinated plants were randomly selected, they were placed in pollinator exclusion bags before the flowers were open. All treatment plants were hand-pollinated from 9-14 July as soon as they were mature. A *P. groenlandica* flower was deemed mature when the stigma was visibly protruding from the rostrum. Flowers on a single inflorescence mature from the bottom upwards. In order to hand-pollinate these flowers, a VegiBee (VegiBee LLC, Saint Louis, MO) was used to vibrate the anthers into releasing pollen. A VegiBee is an electric, battery powered, pollinating device that is used in gardens to collect pollen from buzz-pollinated plants. When *P. groenlandica* flowers were vibrated, pollen was released in a cloud and was collected onto a black plastic spoon. Pollen could then be transferred onto the stigmas of flowers by touching the spoon to the stigmas or by use of a paint brush. Due to the varied timing of flowers on a single inflorescence, enamel paint was used to mark which flowers had already been hand-pollinated. Light-weight colored wire was later used to mark which flowers on an inflorescence had been hand pollinated. Pollen for the augmented pollen and bagged outcrossed treatments was collected from nearby patches of *P. groenlandica*. Pollen for the bagged selfed plants was collected from multiple flowers at once on a single inflorescence, and that pollen was then applied to all of the mature flowers on that inflorescence.

The number of naturally outcrossed plants, other than the five control plants, was determined by the number of inflorescences in the patch. Patches having fewer than 100 inflorescences had 5 naturally outcrossed plants that were marked for future collection; the number of naturally outcrossed plants in patches having greater than 100 inflorescences was determined as 5% of the total inflorescences within the patch. In patches 9-12, inflorescences were randomly chosen and marked for the naturally outcrossed treatment alone.

Flower counts and inflorescence heights were measured for all augmented pollen plants as

well as all of the naturally outcrossed plants. Five random naturally outcrossed plants from each patch were selected to measure the number of nearby flowering plants as well as willow cover. In order to measure these two factors, I established a 5m² circle centered on each plant using a nail fixed with a piece of measured string. The number of flowering plants within the radius of this string was counted. Willow cover was estimated as the percent of the area of the circle that was covered by willow in a top down view.

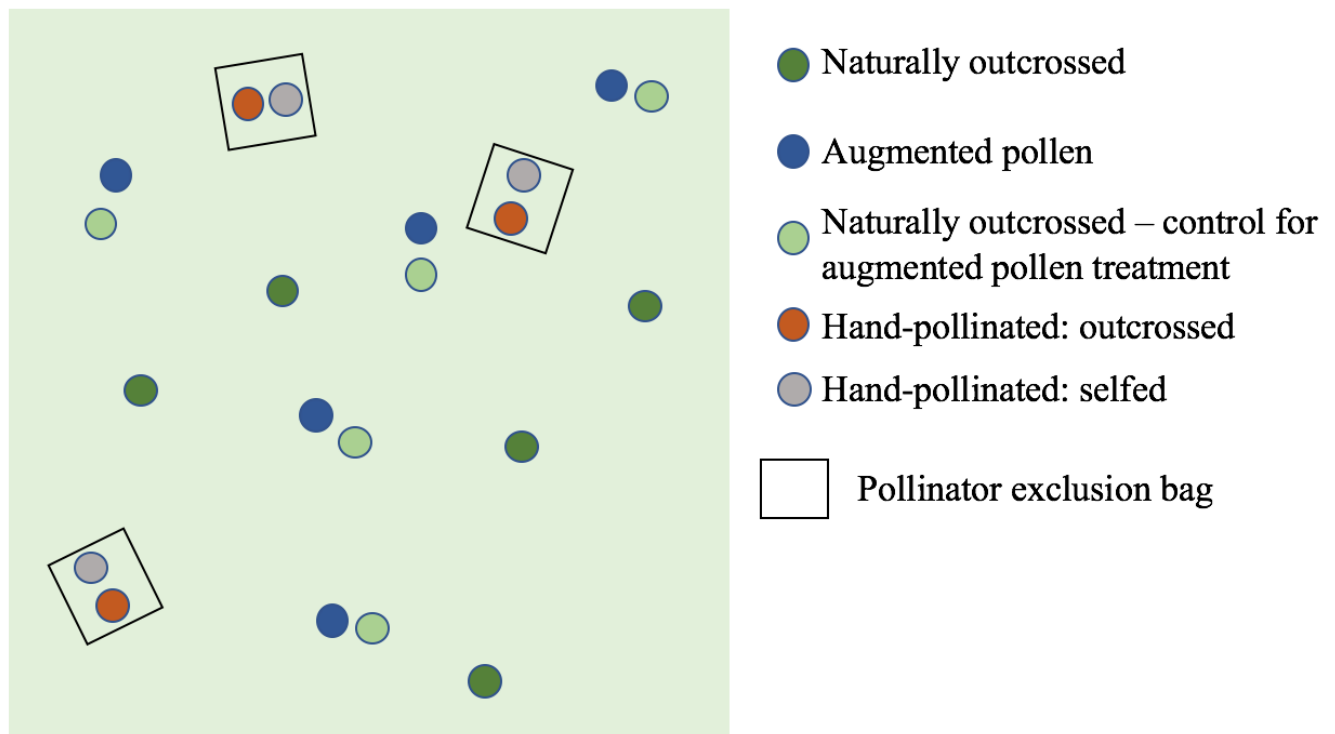


Figure 1: Diagram of pollination treatments in the field.

Fruit and Seed Collection and Analysis

Mature fruits were collected on 4 September, 2016. Unfortunately, the fruits of most inflorescences were already dehiscent and had most likely released some seeds. Nevertheless, all marked inflorescences were clipped and bagged in labeled glassine envelopes. These fruits were

stored at room temperature until they were analyzed starting on 26 September.

For each bagged sample, the number of aborted and mature fruits were counted along with the total number of seeds. An average seed weight was calculated based on the weight of 10 seeds from a single inflorescence.

Analyzing Styles with Fluorescence Microscopy

One style was collected from each hand-pollinated, bagged plant (outcrossed and selfed treatments). These styles were collected with forceps and were placed in a fixative (3:1, ethanol: acetic acid) on 15 July and 19 July. Ten extra styles were collected and placed in fixative from non-study plants in order to practice the methods necessary for preparation for fluorescence microscopy. On 28 July, any styles that appeared to be not too mature were also collected from naturally outcrossed flowers, however the vast majority of these flowers within the study patches were too mature (beginning to dry up and turn brown) for collection. These samples were stored in the fixative at room temperature until they were processed on 5 October at the University of Missouri. The styles were then “cleared” in 8M NaOH for 1 hour and 30 minutes at 60°C. Samples were then washed twice with distilled water before being stored in distilled water inside a refrigerator until they were ready for slide mounting. The samples were mounted on slides in a few drops of 0.01% aniline blue dye in 0.9M KPO₄ buffer. All slides were analyzed and photographed with a fluorescence microscope. The styles were not clear enough to be able to follow or count pollen tubes, however, it was possible in some cases to count the number of pollen grains on the surface of the stigma. Since these pollen grains had remained on the stigmas throughout the fixative and clearing processes, they were used to measure pollen load.

Acoustic Analysis

Hundreds of hours of audio data were collected between 10 and 24 July. In order to sort and analyze this data for recorded bumble bee buzzes, I worked with Dr. Candace Galen (University of Missouri) and Dr. David Heise (Lincoln University). Dr. Heise has been developing an algorithm based on Computational Auditory Scene Analysis that can detect and record buzzes via their fundamental frequencies (Miller-Struttman et al. 2016, manuscript submitted for publication). This software recorded fundamental frequencies from 120-240 hz . Previous research found that *B. Sylvicola*'s wingbeat frequency during flight ranged 195-220 hz while its wingbeat frequency while foraging ranged 300-450 hz (Macior 1968).

Because this software was relatively new and was actively being improved when I performed my data analysis, the output data had to be ground-truthed with the actual audio files. In order to ground-truth the output data, one random 10 min sample was selected and analyzed for each patch. For each patch, a random day of audio collection was selected. Two random 5 min samples were selected from that audio file (one from the morning half, and one from the afternoon half) using the software's output data in order to select samples in moments where buzzes were detected. Each 5 min sample was listened to and annotated twice on Audacity, before the output data was consulted for ground-truthing. True positives, false positives, false negatives, and pollination buzzes were all recorded during the ground-truthing process. Most false positives were caused by airplanes, wind, and birds.

Statistical Analysis:

All statistical tests for this project were run in JMP, version 7.0.2 (SAS Institute, Cary,

NC). For all statistical models, the distribution of residual variation was examined and when residuals were non-normal, trait values were transformed (e.g. log-base 10). One-way ANOVAs were used to examine differences in fruit and seed production across different treatments as well as different patches of *P. groenlandica*. When differences between treatments were found, contrasts (*t*-tests) were used to examine which treatment levels significantly differed in terms of total fruit count, mature/total fruit count, and average seed weights. Simple linear regressions were used to examine the relationships between inflorescence heights and factors such as total seed count and mature/total fruit count. ANCOVAs were used to examine the effect of the number of buzzes recorded within a patch (in 10 minutes) on the average seed weight within that patch. Two factors involving plant visibility were used as covariates in these tests: the number of inflorescences within a patch as well as the size of the patch. I expected that patch visibility covariates would better elucidate the direct relationship between buzz pollination events and plant reproduction.

Results

Pollinator Ecology

In order to test how the amount of pollen deposited on a flower may limit seedset, I compared various measures of fitness across treatments and patches. Once a difference was found between treatments (Table 1), *t*-tests were used to determine which specific treatments differed. There was no significant difference in total seed counts between the augmented pollen (AP) treatment and the control or naturally outcrossed treatments. While there was no significant difference between average seed weights for the AP treatment and the control treatment, the AP treatment had slightly heavier seeds than the naturally outcrossed treatment (*t*-test; DF = 1, F =

3.73, $P < 0.10$) (Figure 2). Significant differences were found across treatments when it came to the total number of fruits counted (Figure 3). However, the AP treatment had significantly more mature fruits (and fewer aborted fruits) per total fruit compared to the naturally outcrossed treatment (t -test; $DF = 1$, $F = 4.93$, $P < 0.05$), but not compared to the control treatment (Figure 4).

Several Analysis of Covariance tests were used to investigate the effects of the number of recorded buzzes on average seed weights. When controlled for factors involving the visibility of the plants, the number of recorded buzzes predicted the average seed weight within that patch. The two covariates used for these tests were the number of inflorescences within a patch ($RSquare = 0.53$, $P = 0.052$) and the size of that patch ($RSquare = 0.71$, $P = 0.061$).

Source	Total Fruit Count	Mature/Total Fruit Count	Average Seed Weight (mg)
Treatment	15.77***	49.65***	2.014
Patch	1.47	1.78+	2.24*
Patch*Treatment	0.48	1.41	0.82

Table 1: F values from one-way ANOVA tests. P values are indicated as follows: + $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

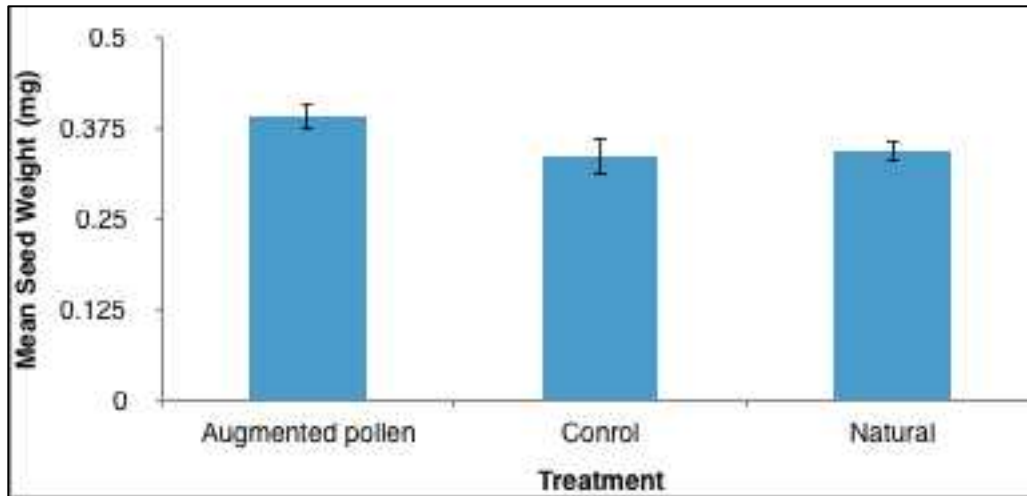


Figure 2: Mean seed weight (mg) across the three treatments with standard error bars.

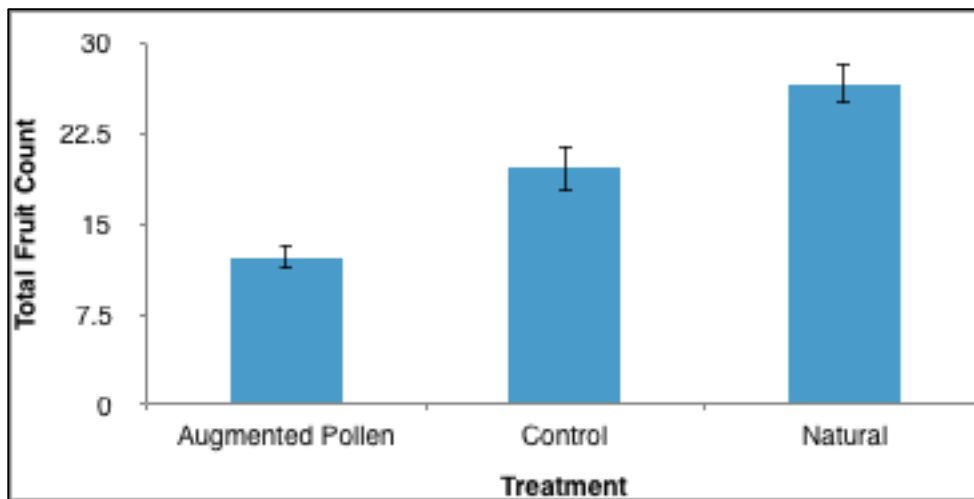


Figure 3: Mean total fruit count for each of the three treatments. Standard error bars are shown.

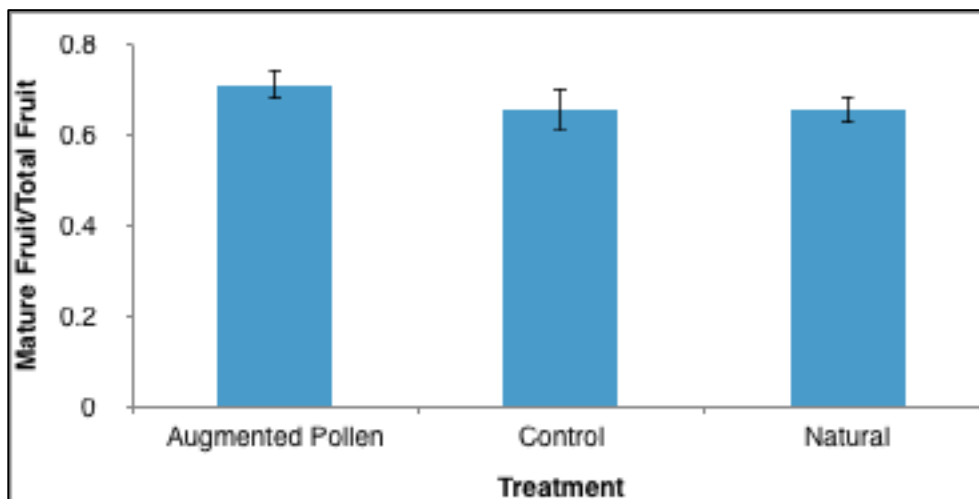


Figure 4: Mean ratio of mature fruits to total fruits for each of the three treatments. Standard error bars are shown.

Reproductive Allocation

A series of regressions were run to test how reproductive efforts were related to each other. Taller plants had more flowers which led to more seeds (Table 2). There was also a positive correlation between inflorescence height and the ratio of mature fruit to total fruit (Table 2; Figure 5).

	Inflorescence Height (cm)
Seed Count	0.38; 17.53***
Mature fruit count/Total fruit count	0.14; 0.016***

Table 2: Regressions involving the impact of inflorescence height. Results shown: RSquare value; slope of linear line of it; *** P < 0.001.

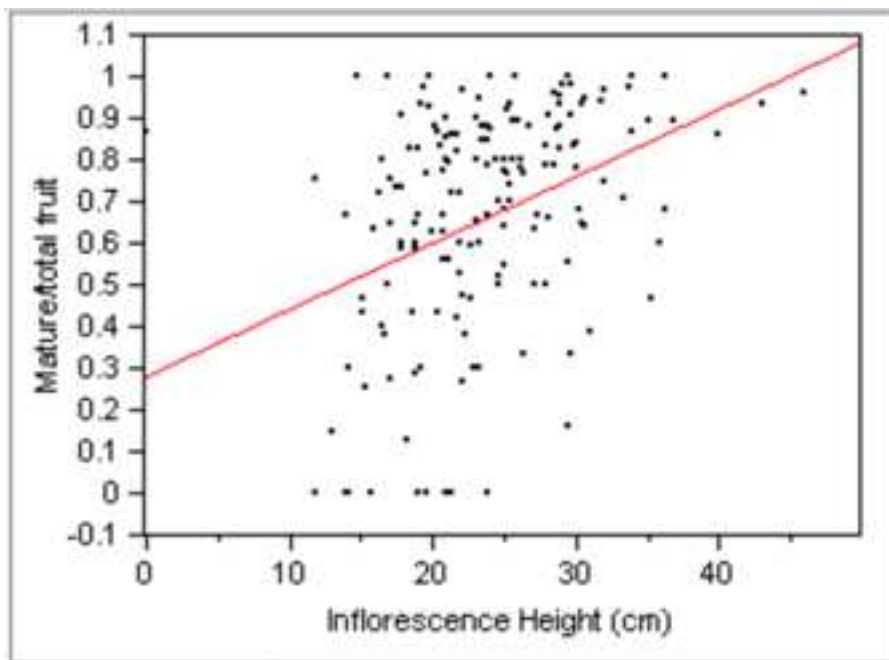


Figure 5: Relationship between inflorescence height (cm) and ratio of mature fruits to total fruits. The linear line of fit is shown: Rsquare = 0.143, P < 0.01.

Visibility/ Plant ecology/ Competition

While the positive correlation between inflorescence height and the ratio of mature fruit to total fruit (Table 2) may indicate the allocation of resources in taller plants, it may also be an indicator of the visibility of taller inflorescences to pollinators. There was no relationship found between the percentage of *Salix* sp. cover and the number of mature fruits or seeds. There was also no relationship between the presence of other interspecific flowers and the number of mature fruits or seeds.

Evolution and Self-compatibility

The vast majority of the hand-pollinated plants that were kept underneath pollinator exclusion bags resulted in aborted fruits. When comparing the few inflorescences that had mature fruits, on average those that were outcrossed had a higher number of seeds per mature fruit compared to those that had been selfed (Figure 6). Florescence microscopy found that the stigmas from selfed flowers had significantly more pollen grains on their surfaces than outcrossed plants (Figure 7).

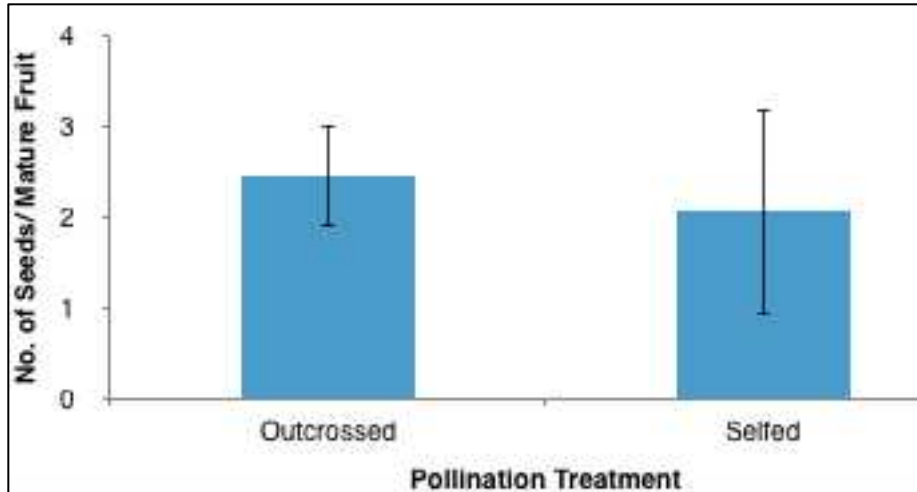


Figure 6: Average number of seeds per mature fruit for both pollination treatments. t -test: 2.708; F ratio: 7.3551; $P = 0.0204$; DF for treatment = 1; DF for t -test = 11

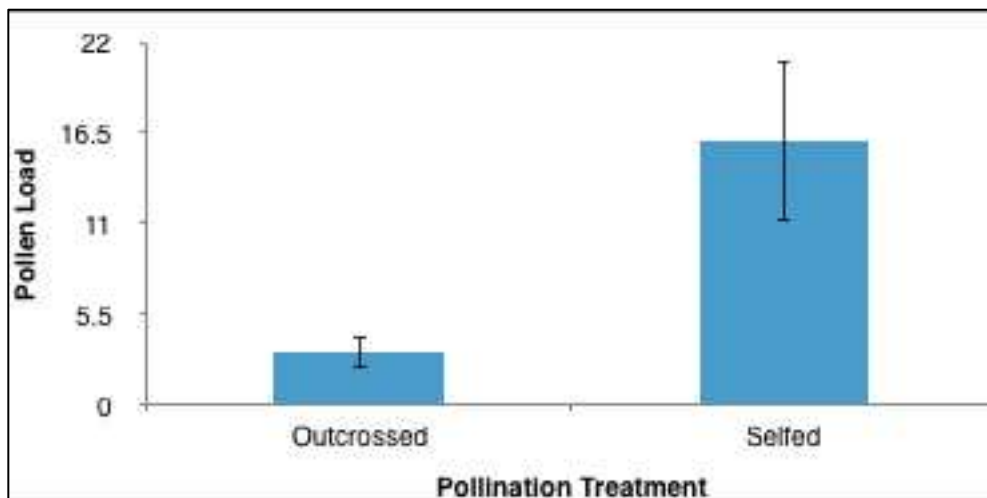


Figure 7: Average pollen load for both pollination treatments. t -test: -2.557; DF=30; F ratio: 6.5407; $P = 0.0158$

Discussion

With the current changes in our global climates, it is important to study the plant-pollinator interactions that may be impacted. Because alpine ecosystems are particularly susceptible to shifts in climates (Settele et al. 2014), they contain species interactions that can be useful for such studies. This study focused on the interactions between various bumble bee species and the wildflower *Pedicularis groelandica*. By studying the adaptations and behaviors that form the

bases of these interactions, one may better hypothesize how predicted climate trends may impact alpine species. *P. groenlandica* and its pollinators offer an interesting model system for research because of the evolved buzz pollination interactions that they depend on.

Several findings from my research fill in some of the gaps of knowledge concerning the ecology and reproductive strategies of *P. groenlandica*. First, the reproductive success of *P. groenlandica* appears limited by the amount of pollen that is deposited on each flower. This indicates that continued drops in bumblebee populations due to global climate change may have consequences for the successful reproduction of *P. groenlandica*. Second, my results provide evidence that pollinator visitations and effects may be monitored and measured by acoustic recordings of their flight buzzes. When controlled for by certain factors concerning the visibility of the flowers, a positive correlation was found between the number of recorded flight buzzes and the average seed weight within a patch. These results provide more evidence that acoustic monitoring of bumblebees may be a useful, viable means of studying their behaviors and populations. Third, I found that taller plants tend to have more mature fruits per total fruits compared to shorter plants. This finding points to one of the impacts that encroaching *Salix* sp. may have on neighboring plants. While these were the significant trends that were found in this project, there were several other findings that may help to inform future research with *P. groenlandica*.

Pollinator Ecology and Reproductive Allocation

Part of the purpose of this project was to explore the interactions between *P. groenlandica* and its pollinators. One interesting element of this mutualistic relationship was determining whether or not the amount of pollen being deposited by a pollinator was limiting to the

reproductive success of an individual plant. By adding extra outcrossed pollen to certain inflorescences, I found that fewer fruits were likely to be aborted and that the seeds tended to weigh more. This indicated that while other environmental factors may be limiting the reproductive output of these study plants, pollen may specifically be limiting the number of fruits that mature and the weight of the consequent seeds. These trends in pollen limitation differ from the findings of Yang et al. (2004) who found that while pollen was not limiting, inbreeding depression and pollen interference had an impact on the abortion rates of two different species of *Pedicularis*. On the other hand, Karrenberg and Jensen (2000) found that the size of a population may influence whether or not it is at risk to pollen limitation.

The findings from the pollen augmentation portion of the research complement my findings from the audio analysis of recorded *Bombus* buzzes. I found that the number of recorded buzzes within a patch (from a random ten minute sample) was positively correlated with the average seed weight of the naturally outcrossed plants in that patch. This correlation was only found when the test was controlled for the effects of the number of inflorescences within the patch or the size of that patch. The correlation between pollinator visits and seed weight would be expected considering the fact that pollen deposits may potentially limit the viability of seeds (via their weight). However the notion that pollinator flight buzzes may be recorded and utilized as a measure of pollinator visitations or abundance is a new technique for the field.

As work continues on Dr. David Heise's computer algorithm, there are several ways that it can be improved and made more useful for studies such as this one. Firstly, it has now been established that ideally, audio recordings in the field will be taken on calm days with no rain and little wind. This will leave the airplanes as the major source of detected false positives. Work must be done to selectively remove intervals of time where airplanes were flying above the

recorded area. Also, the algorithm must be altered in order to make it possible to detect buzzes at higher frequencies such as pollination buzzes.

The data suggests that reproductive efforts, when pollen is not limiting, may be allocated towards making more mature fruits and towards making more heavy seeds. Rather than using the excess pollen grains to simply produce more seeds, the plants seem to be allocating resources to more flowers, creating fewer aborted fruits, which in turn will produce heavier seeds. Heavier seeds have been found to be an indicator of viability (Heschel and Paige 1995).

Visibility and Competition/plant ecology

Neighboring, flowering plants (both intra- and interspecific) can impact the successful pollination of one another (Hegland 2014; Fang and Huang 2016). In terms of pollinator visits, neighboring flowers can either aid in the attraction of pollinators to a dense flower patch, or they may act as competitors for the attention of said pollinators. Pollen competition can also occur between interspecific or intraspecific flowers. Heterospecific pollen transfer (HPT) has highly variable effects on the plant receiving the HP which may depend on certain traits of both the receiving plant and donor plant (Ashman and Arceo-Gómez 2013). Macior (1970) found that many of the different species of bumblebee that visit *P. groenlandica* often have heterospecific pollen on them. In my field experiment, I found that the number of neighboring, interspecific flowers within a patch had no effect on the various measures of the reproductive success of the *P. groenlandica*.

Previous studies found that the visibility of plants to pollinators may be affected by both the height of the individual inflorescences as well as the density and height of the surrounding vegetation. For example, Schlinkert et al. (2016) found that across different species of flowering

plants, the size and height of an individual plant impacted how conspicuous it was to various pollinators (and herbivores) (Schlinkert et al 2016). This ecological trend seems to hold true for *P. groenlandica*, as taller inflorescences were found to have more mature fruits per total fruit. However, the percent *Salix* cover over various inflorescences was not found to have an impact on the various measures of reproductive success of the *P. groenlandica*.

Evolution and Self-compatibility

An important life history trait of *P. groenlandica* that has not been fully studied is its self-compatibility. Eriksen et al. (1993) hypothesized that self-compatibility may be a more prevalent and useful strategy for flowering plants in harsh ecosystems, such as alpine and arctic systems, where pollinators may be more scarce. Therefore, it may be expected that *P. groenlandica* could have some level of self-compatibility. Also, I observed that when a bumblebee would visit an inflorescence, she would often visit multiple flowers on that inflorescence before flying to the next. This would allow for a potential for geitonogamy where a single flower on an inflorescence could be pollinated with pollen from other flowers from the same inflorescence.

My research failed to definitively find evidence for self-compatibility or self-incompatibility within *P. groenlandica*. This was because almost all of the plants within the pollinator exclusion bags aborted their fruits. Several hypotheses may account for the high rates of abortion including the possibility that when these flowers were hand-pollinated, the stigmas were not yet receptive for pollen. It is also possible that the pollinator exclusion bags that were used may have caused for an inhospitable environment for the plants by trapping moisture and by making the air within the bag too warm.

Conclusions

In the face of global warming, it is important to study the plant-pollinator interactions that may be at risk. With bumblebee populations in decline, buzz-pollinated plants such as *P. groenlandica* may be at a particular disadvantage as they rely on a specific, evolved behavior within various species of bumblebees. Through evidence of pollen limitation and the importance of flower visibility, this research has demonstrated some of the dependency of *P. groenlandica* on these pollinators. While this species of wildflower has a fairly wide elevational range, many other subalpine and alpine wildflowers have more limited ranges. Therefore, changes in pollinator behaviors, distributions, or populations may be more impactful to the population dynamics of alpine flowers. Similarly, climate-induced shifts in plant populations within these ecosystems, such as the encroachment of *Salix* shrubs, may have impacts on entire plant communities. *P. groenlandica* is a useful model plant for these studies due to the possibility of recording and quantifying pollinator visitations via the acoustics of pollination buzzes and flight buzzes.

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Sources

Ashman, T. and G. Arceo-Gomez. 2013. Toward a predictive understanding of the fitness costs of heterospecific pollen receipt and its importance in co-flowering communities. *American Journal of Botany* 100:1061-1070.

Buchmann, S. L. 1983. Buzz pollination in angiosperms. In: Jones CE, Little RJ (eds) *Handbook of Experimental Pollination Biology*. Scientific and Academic Editions, NY, pp 73–113.

Cameron, S., J. Lozier, J. Strange, J. Koch, N. Cordes, L. Solter, and T. Griswold. 2011. Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences* 108:662-667.

De Luca, P. and M. Vallejo-Marín. 2013. What's the 'buzz' about? The ecology and evolutionary significance of buzz-pollination. *Current Opinion in Plant Biology* 16:429-435.

De Luca, P., L. Bussière, D. Souto-Vilaros, D. Goulson, A. Mason, and M. Vallejo-Marín. 2012. Variability in bumblebee pollination buzzes affects the quantity of pollen released from flowers. *Oecologia* 172:805-816.

Eriksen, B., U. Molau, and M. Svensson. 1993. Reproductive strategies in two arctic *Pedicularis* species (Scrophulariaceae). *Ecography* 16:154-166.

Fang, Q. and S. Huang. 2016. A paradoxical mismatch between interspecific pollinator moves and heterospecific pollen receipt in a natural community. *Ecology* 97:1970-1978.

Geib, J. and C. Galen. 2012. Tracing impacts of partner abundance in facultative pollination mutualisms: from individuals to populations. *Ecology* 93:1581-1592.

Geib, J., J. Strange, and C. Galen. 2015. Bumble bee nest abundance, foraging distance, and host-plant reproduction: implications for management and conservation. *Ecological Applications* 25:768-778.

Goulson, D., G. C. Lye, and B. Darvill. 2008. Decline and conservation of bumble bees. *Annual Review of Entomology* 53:191-208.

Hegland, S. 2014. Floral neighbourhood effects on pollination success in red clover are scale-dependent. *Functional Ecology* 28:561-568.

Heschel, M. and K. Paige. 1995. Inbreeding Depression, Environmental Stress, and Population Size Variation in Scarlet Gilia (*Ipomopsis aggregata*). *Conservation Biology* 9:126-133.

Inouye, D. 2008. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology* 89:353-362.

Karrenberg, S., and K. Jensen. 2000. Effects of pollination and pollen source on the seed set of *Pedicularis palustris*. *Folia Geobotanica* 35:191-202.

Knight, T., J. Steets, J. Vamosi, S. Mazer, M. Burd, D. Campbell, M. Dudash, M. Johnson, R. Mitchell, and T. Ashman. 2005. Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics* 36:467-497.

Konzmann, S. and K. Lunau. 2014. Divergent rules for pollen and nectar foraging bumblebees – a laboratory study with artificial flowers offering diluted nectar substitute and pollen surrogate. *PLoS ONE* 9: e91900.

Kopp, C. and E. Cleland. 2013. Shifts in plant species elevational range limits and abundances observed over nearly five decades in a western North America mountain range. *Journal of Vegetation Science* 25:135-146.

Macior, L. 1964. An Experimental Study of the Floral Ecology of *Dodecatheon meadia*. *American Journal of Botany* 51:96.

Macior, L. 1968. Pollination Adaptation in *Pedicularis groenlandica*. *American Journal of Botany* 55:927.

Macior, L. 1970. The Pollination Ecology of *Pedicularis* in Colorado. *American Journal of Botany* 57:716.

McGuire, C., C. Nufio, M. Bowers, and R. Guralnick. 2012. Elevation-Dependent Temperature Trends in the Rocky Mountain Front Range: Changes over a 56- and 20-Year Record. *PLoS ONE* 7:e44370.

Miller-Struttman, N., J. Geib, J. Franklin, P. Kevan, R. Holdo, D. Ebert-May, A. Lynn, J. Kettenbach, E. Hedrick, and C. Galen. 2015. Functional mismatch in a bumble bee pollination mutualism under climate change. *Science* 349:1541-1544.

Miller-Struttman, N., D. Heise, J. Schul, J. Geib, and C. Galen. 2016. Hacking acoustic signals to monitor pollination services. *PLoS ONE*. Manuscript submitted for publication.

Morales, C., M. Arbetman, S. Cameron, and M. Aizen. 2013. Rapid ecological replacement of a native bumble bee by invasive species. *Frontiers in Ecology and the Environment* 11:529-534.

Potts, S., J. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution* 25:345-353.

Schlinkert, H., C. Westphal, Y. Clough, I. Grass, J. Helmerichs, and T. Tschardtke. 2016. Plant size affects mutualistic and antagonistic interactions and reproductive success across 21 Brassicaceae species. *Ecosphere* 7:e01529.

Scott, R. 1996. *The Alpine flora of the Rocky Mountains*. 1st edition. Univ. of Utah Press, Salt

Lake City, Utah.

Settele, J., R. Scholes, R. Betts, S. Bunn, P. Leadley, D. Nepstad, J.T. Overpeck, and M.A. Taboada, 2014: Terrestrial and inland water systems. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Field, C.B., V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L. White (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 271-359.271

Wagner, J., M. Lechleitner, and D. Hosp. 2016. Pollen limitation is not the rule in nival plants: A study from the European Central Alps. *American Journal of Botany* 103:375-387.

Yang, C., S. Sun, and Y. Guo. 2005. Resource limitation and pollen source (self and outcross) affecting seed production in two louseworts, *Pedicularis siphonantha* and *P. longiflora* (Orobanchaceae). *Botanical Journal of the Linnean Society* 147:83-89.

Yu, W., M. Liu, H. Wang, R. Mill, R. Ree, J. Yang, and D. Li. 2015. Towards a comprehensive phylogeny of the large temperate genus *Pedicularis* (Orobanchaceae), with an emphasis on species from the Himalaya-Hengduan Mountains. *BMC Plant Biology* 15:179.