

Flowering as a Reproductive Strategy of the Sexes of the
Gynodioecious *Silene acaulis*

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A thesis submitted to the
University of Colorado at Boulder
in partial fulfillment
of the requirements to receive
Honors designation in
Environmental Studies
May 2018

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Abstract

Populations of the alpine cushion plant *Silene acaulis* in Colorado have female and hermaphrodite plants, each with respective flowering and fruiting throughout their growing season. In order to understand the differences between the two sexes, I collected flowering data throughout the season as well as fruit and size data and completed statistical analyses. I found similar quantities of flowers for the sexes, twice the fruit production by females, more flowers but fewer fruits on larger plants, and longer flowering ranges for hermaphrodites. Females are more successful at producing fruits from their flowers, whereas hermaphrodites must produce both pollen and seeds. Both sexes follow decreasing trends of fruit production with increasing size. Flowers by size increases faster for hermaphrodites, though fruits per size increases faster for females. These findings may be used for conservation efforts as *Silene acaulis* plays an important role in ecosystem stability as a nurse plant and will certainly be affected by a changing climate.

Preface

At the University of Colorado at Boulder, the classes I took within the Department of Ecology and Evolutionary Biology introduced me to the world of plants, seemingly mundane from outside but fascinating within. In the spring of 2017, I took a Conservation Biology course with Dr. Daniel Doak. I greatly enjoyed the class and Dr. Doak's field and approached him for more information. He invited me to apply for a research grant within his lab to work as an REU over the summer, and I was soon offered the position. That summer provided me with the research that became this project.

The research for this project began in June 2017. I lived at the Mountain Research Station for ten weeks as part of the Research Experience for Undergraduates (REU) program at the Niwot Ridge land designation in the Arapahoe and Roosevelt National Forests in Colorado. During those ten weeks, I continued an ongoing project of the Doak Lab. Dr. Dan Doak and Dr. Bill Morris have been studying *Silene acaulis* since 1995, and specifically at Niwot Ridge since 2001. For the past two summers (2016 and 2017) there were two REU students living at the Mountain Research Station and collecting flowering data on *Silene acaulis*.

As part of the REU program, we designed and completed a project relating to that research. That REU project eventually morphed into this honors thesis.

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Introduction

The understanding of sexual reproduction begins for most people with the reproductive system of most animals. Animals are typically limited to one of two sex options, and sex is typically an inherited trait, often mediated by presence or absence of sex chromosomes. Plant reproductive systems, in contrast, are far more varied than simple male-female sexual reproduction. Plant species have sexual systems that can include male and female parts in the same flower, different flowers on the same plant, different mating systems, and or even changes in reproductive function throughout a plant's lifespan.

The populations of *Silene acaulis* in North America contain females and hermaphrodites, a system referred to as gynodioecy. The strategies for hermaphrodites must be modified from that of either males or females, and so this species presents an opportunity to expand understanding of the costs and strategies of different forms of sexual reproduction.

My fascination with *Silene acaulis* was centered around the differences between the two sexes, and my project sought to address the reproductive differences between them, and specifically how flowers were employed to further fitness in each of the two sexes.

The main data I collected was the flower numbers on each of approximately 500 plants throughout the flowering season, providing a measure of reproductive effort that could also be tied to plant size and relative seed production data. I developed my project around the flowering data collection, and chose to specifically examine the differences between the two sexes to better understand how the sexes differed in their use of flowers. My project also incorporated the relationship between flower quantity and plant size, as a proxy for age.

Before collecting the data, I formulated four research questions as follows. How does flower number, a measure of reproductive effort, differ by sex? What are the ranges of flowering duration for the sexes, and do these differ? How is flower number related to plant size? How does the ratio of flowers to fruits (mature flowers that have produced seeds) differ by sex and size?

The current available literature section of this thesis focuses on life history of the species, species interactions, elevational gradients, and reproductive successes of the sexes. I seek to advance our knowledge of these topics by discussing flowering as a reproductive strategy that may be employed differently by females and hermaphrodites.

Background

In this background section I will provide information on *Silene acaulis* in order to explain the current state of the research surrounding the species and to educate my readers on the project topic. I will cover SA species biology, SA at Niwot Ridge, gynodioecy, differences between the sexes, differences in sexual systems, and capability as a nurse plant.

Species Biology

Silene acaulis (henceforth, SA) is a decumbent, alpine cushion plant found across the Northern Hemisphere in high elevation regions (Hulten 1958). SA grows throughout the Rocky Mountains as far south as New Mexico, USA and through British Columbia, Canada and Alaska, USA. SA

also grows in arctic and alpine conditions throughout Europe including Sweden, France, Denmark, and Greenland (Gussarova et al. 2015).

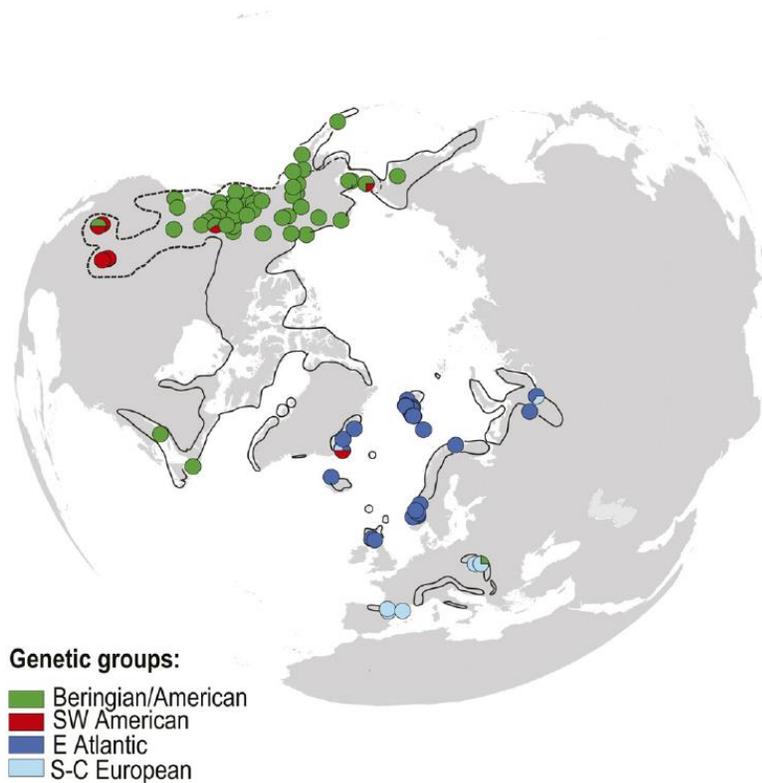


Figure 1. Circumpolar map of SA populations distinguished by genetic groups. Graphic courtesy of Gussarova et al. 2015.

The small, needle-like leaves are clustered on rosettes, where the leaves come from a common small stem. Many rosettes form a cushion. The leaves are replaced at the beginning of each growing season after the snow melts and remain until the end of the summer.



Figure 2. (Left) Plants up to a decade are often only a single rosette. Photo courtesy of Dan Doak. (Right) A flowering *Silene acaulis*.

The plants produce many small pink flowers. The sex of a plant can only be gauged after it begins flowering, as that is the only clear phenotypic expression of sex. Hermaphrodite flowers have anthers, and females do not. Individual flowers typically remain open for several days to a week.

Silene acaulis is very long-lived and regularly exceeds 300 years of age (Morris and Doak 1998). SA grows very slowly. The largest plants may only reach 20 centimeters in diameter and may grow by a single or few rosettes per year, or experience dieback and loss of cushion area (Morris and Doak 1998). High climactic plasticity in the face of environmental conditions may allow them to shrink for several years in poor conditions and then grow for many years after.

Niwot Ridge Long Term Study

The flowering data comes from Niwot Ridge in the southern Rocky Mountains in Colorado. I performed data collection at four permanent plots over four slightly different but paired microclimates. The sites were established in 2001 by Daniel Doak and Bill Morris (Doak and

Morris 2010). Each of the four plots has several hundred permanently marked plants, each mapped and also marked with numbered metal tags or colored plastic toothpicks.

In the 16 years since the sites were established, the tagged *Silene acaulis* plants have been censused at the end of each flowering season (late July) to gather demographic data. In addition to sex, each plant has yearly data on survival, size of cushion, and number of fruits produced.

The four sites on Niwot Ridge represent the conditions the general population faces in the region. SN1 and SN4 are on the East Knoll. SN2 and SN3 are on the West Knoll. SN1 and SN2 are on the tops of the knolls, while SN3 and SN4 are on the sides. SN1 and SN2 are considered the dry sites, as their position on the hills means the wind blows snow off throughout the winter, and their snowmelt date is earlier in the summer than for the wet sites. SN2 and SN3, the wet sites, remain covered in snow for the majority of the winter. The snow melts in the early summer and provides them with enough moisture to produce their communities. Additionally, melting snow runoff waters the wet sites.

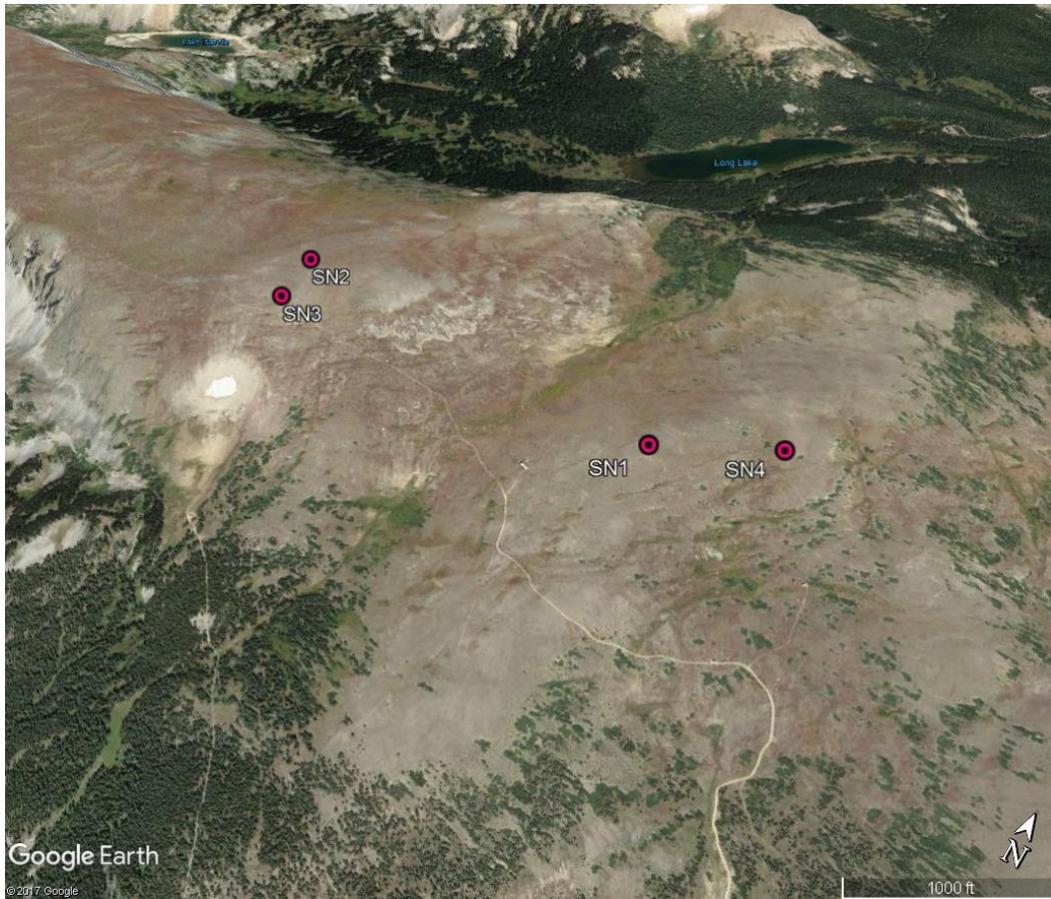


Figure 3. Niwot Ridge as viewed from the southeast. Image courtesy of Lucas Piedrahita.

Niwot Ridge sits at approximately 11,000 feet above sea level, providing the climactic conditions needed for alpine plants.

Gynodioecy

The *Silene acaulis* populations in Colorado are gynodioecious – containing females and hermaphrodites but not males. The individual plants remain the same sex throughout their life, as opposed to plants with a dichogamous sexual system, in which individuals produce flowers of both sexes but at different times. The hermaphrodite flowers are “perfect flowers” and contain

both male and female reproductive parts. They are able to produce both pollen and ovules in the same flower and are capable of self-pollinating with assistance from an insect. The female plants have flowers that produce pistils but not stamen. Females must rely on the hermaphrodites for pollination. Hermaphrodites are self-compatible, though self-pollination results in seeds of lesser quality and reduced survivorship (Shykoff 1988; Keller and Schwaegerle 2006).



Figure 4. (Left) A hermaphrodite flowering. (Right) A female flowering. The hermaphrodite's flowers have noticeable pollen on the anthers.

Gynodioecy is often considered to be an intermediary evolutionary stage between hermaphroditism and dioecy (Charlesworth and Charlesworth 1978). Gynodioecy is found in less than 1% of angiosperm species, including a species of the same genus, *Silene vulgaris*.

Sexual Differences

There are several ways the sex of a gynodioecious plant is determined. Cytoplasmic male sterility genes (CMS) are maternally inherited from females alone, and suppress the pollen production of an otherwise hermaphroditic flower, and thus create something functionally female (Hanson and Conde 1985; Braun et al. 1992; DeHaan et al. 1997; Schnable and Wise 1998; Klaas and Olson 2006). Nuclear restorer genes restore the male function lost by CMS to create hermaphrodites (Manicacci et al. 1996). Nuclear genes can be inherited from either pollen or ovule, though never from a female mother. Two hermaphrodite parents, however, could both pass down nuclear restorer genes to create more hermaphrodites. The seeds, therefore, tend to carry the sex of their mother. Hermaphrodites produce seeds that are hermaphrodites, and females produce seeds that are female.

Due to their different routes for fitness, females and hermaphrodites potentially have a different resource allocation strategy. Females channel all their excess nutrients and water towards making seeds, while hermaphrodites can split those resources between pollen and seeds. This split in hermaphroditic resources suggests a possible reduction in quality or quantity of both pollen and seeds, as compared to males and females respectively.

For SA males and hermaphrodites, there is no difference in ability of well-developed pollen to successfully sire a seed (Philipp et al. 2009). The problem lays in the numbers. Males have more well-developed pollen grains per anther, but are smaller plants overall with fewer flowers and thus fewer anthers, leading to a hermaphroditic advantage (Philipp et al. 2009).

Hermaphrodites have an advantage over females in that they are able to produce offspring via pollen. The resource input required to make pollen is much lower than that needed for seeds,

while the genetic contribution of a hermaphrodite parent is the same for both types of sexual propagules. Thus, with the assumption that sex is genetically inherited based on the sexes of the parents, it follows that the hermaphrodites would produce greater total offspring than females (or even males), and over several generations the population would be reduced to solely hermaphrodites. As this is not the case, there must be some advantage for females that allows them to produce the next generation of females (with CMS inheritance) and not be outcompeted.

Delph et al. (1999) measured seed quality as a function of seed mass, endosperm mass, nitrogen and phosphorus content, and energy content. They determined that seeds produced by females and hermaphrodites did not vary significantly in any of those variables, yet female-produced seeds had higher survivorship than their hermaphroditic-produced counterparts. A later study found that smaller seeds actually germinated more successfully (Keller and Schwaegerle 2006).

What advantages do females have over hermaphrodites to allow them to persist?

First, females flower more because they can redirect resources hermaphrodites would use for pollen. Though smaller, their flowers were more abundant (Shykoff et al. 2003).

Second, female flowers contain more ovules per flower (Delph and Carroll 2001) and thus have a higher chance of seeds finding a suitable place for germination and successfully germinating.

Third, females have higher fruit set (Shykoff 1988; Morris and Doak 1998; Delph et al. 1999; Delph and Carroll 2001; Shykoff et al. 2003).

Finally, females produce 4.4 times the seed offspring as hermaphrodites (Morris and Doak 1998).

Additionally, as females cannot self-pollinate, their seeds have an inherent genetic advantage to hermaphrodite seeds, which are often produced via self-pollination. Inbred, self-pollinated seeds had a reduced survival of more than 30% when compared to outcrossed, female produced seeds (Keller and Schwaegerle 2006).

Sexual Variety

Not all populations of *Silene acaulis* have the same sex ratios – or even sexes. *Silene acaulis* is polymorphic and has a range of sex ratios across populations, as well as non-gynodioecious sexual systems in some populations.

Populations of SA have been recorded as monoecious, having solely hermaphroditic plants; andromonoecious, having both hermaphroditic and male plants; trioecious, having male, female, and hermaphroditic plants; dioecious, having male and female plants; and gynodioecious (Warming, Eugenius 1920; Shykoff 1988; Alatalo and Molau 2001; Ashman 2002; Casimiro-Soriguer et al. 2015). For example, the Greenland population is trioecious and contains hermaphrodites, females, and males (Philipp et al. 2009).

Sex ratios change within much smaller geographical regions as well. While a general population may have a certain sex ratio, the elevational difference of subpopulations can alter the frequency of the sexes. This is because females use resources more efficiently and thus outperform hermaphrodites in nutrient-low conditions (Asikainen and Mutikainen 2005; Petry et al. 2016). Females exist in higher proportions of the total population at higher altitudes (Alatalo and Molau 1995; Delph and Carroll 2001; Asikainen and Mutikainen 2005; Petry et al. 2016). Higher

female frequency has been noted in over a dozen species, and recorded as early as Darwin (1877).

The elevation increases mentioned above are associated with decrease in growing season length and temperature, increase in precipitations, and later snowmelt (Petty et al. 2016) as well as increased winds (Arroyo et al. 2003). Females tend to do better in these conditions than hermaphrodites, and thus constitute a greater portion of the population.

Other factors may contribute to this imbalance as well. Ashman (2002) noted limited resources may force hermaphrodites to abandon seed production in favor of the less costly pollen production, which would limit hermaphrodite seed production and thus the next generation of hermaphrodites (Harder and Barrett 2006).

In a similar gynodioecious species, *Eurya japonica*, hand pollination to reduce pollen limitation led to a higher fruit set among females (Wang et al. 2015). SA females are also more sensitive to reduced pollination and needed to produce twice the seeds as hermaphrodites to persist (Reid et al. 2014). This may explain why females collect more pollen over longer time (Shykoff 1992).

Nurse Plant

SA is considered an “ecosystem engineer” or “nurse plant” due to its important ecological function (Antonsson et al. 2009). The cushion formed by SA is well-adapted to the harsh alpine conditions, and the space within the cushion provides an environment for other plant species to grow. The cushion provides nutrient accumulation, shade, herbivory protection (Nunez et al. 1999; Callaway et al. 2002) and protection from strong winds and temperatures (Callaway and

Walker 1997; Arroyo et al. 2003; Badano et al. 2006). These conditions create a suitable environment for other plants, mostly graminoids (Antonsson et al. 2009).

The nurse plant effect of cushion plants is found to be more effective at higher elevations, where the abiotic stresses are more prominent (Callaway et al. 2002; Antonsson et al. 2009).

Silene acaulis and other vascular plants can control leaf temperatures, keeping the cushion much warmer than the surrounding environment (Gauslaa 1984). Warmer temperatures within the cushion reduce the abiotic stress exerted on smaller plants growing in the cushion.

The same benefits are received by other species outside the producer trophic level. Though less studied, arthropod richness and diversity were significantly higher within a cushion than in the surrounding environment, especially in cushions with tall plant growth (Molenda et al. 2012).

This nurse plant effect has been argued to be important for ecosystem health. Many of the smaller species rely on cushions, especially in higher elevations, and it is possible that these the populations would die out if cushion plants were to disappear (Badano et al. 2006; Antonsson et al. 2009).

Silene acaulis is an interesting species to study for climate change effects for these reasons, as their fate is intertwined with the fates of dozens of other species.



Figure 5. A *Silene acaulis* mixed with *Geom rossi*, *Minuartia obtusiloba*, *Antennaria media*, and grasses.

Further Review of the Literature

Other challenges exist for these cushion plants. The changing climate poses threats to a climate-specific plant. *Silene acaulis* in Sweden responded quickly to artificial climate manipulation by lengthening stems and leaves, with an overall greater biomass short-term. Long-term, *Silene* cover decreased. Thus, SA can react with plasticity to unusual weather in a given year, but cannot adapt to new, harsh conditions (Alatalo and Little 2014). With extremely long generation times, evolution of the species to adapt to new conditions is likely extremely slow (Morris and Doak 1998).

Anther smut is an infection that sterilizes flowers of hermaphrodites, but high female frequency correlated with low infection rates (Hermanutz and Innes 1994). There is no data on anther smut on Niwot Ridge.

Methods

I followed two methods, one for field data collection and one for data analysis using the R program. The Methods section is split into two subsections to address these properly.

Field Methods

I collected flowering data during the summer of 2017 at the Niwot Ridge Biosphere Reserve, a part of the Arapahoe and Roosevelt National Forests, on approximately 500 individuals in four subpopulations on Niwot Ridge. The flowers on each cushion were counted thrice weekly over the entire flowering period. Flowers with petals spread at an angle greater than 45 degrees and with intact stamen and pistils were considered open. Buds, unfurled flowers, and broken or old flowers were not counted. At the end of the flowering season, I counted the total number of flowers on each cushion by noting all broken, fruiting, withered, or still-open flowers. This total provides a relative measure of total floral production.

A week later, a census team and I returned to Niwot Ridge for the annual demographic census. That week, we counted the number of fruits on each plant. This value gives the quantity of seed-containing fruits, as well as the flowers successfully pollinated. Seed quantity within fruits was not determined.



Figure 6: Collecting data at SN1.

During the same July census, the plants were measured to determine growth. The cushions were measured for diameter at the widest length in millimeters, and then diameter of the widest diameter perpendicular to the first. Then the measurer estimated a value of percent missing (PM) from the oblong shape created by the two diameters. Cushion area was later calculated from those three values and is in units of square millimeters. Plants with fewer than 20 rosettes were not measured for area, but rather rosettes were counted and recorded. These data are archived at ([www.duke.edu/~wfmorris/Silene Polygonum Data](http://www.duke.edu/~wfmorris/Silene_Polygonum_Data)).

Analysis Methods

The flowering data from throughout the flowering season and demographic data from the census were compiled into spreadsheets by Dr. Megan Peterson. These data were collected in part by

past researchers including Dr. Dan Doak, Dr. Megan Peterson, Dr. Bill Morris, Elijah Hall, Ellen Waddle, and Lucas Piedrahita. The spreadsheet includes variables such as plant location, flowers open per day, first and last flowering day, and plant area. There are a total of 78 variables in the spreadsheet after removing several I did not need, though all 78 were not employed in this project. Many were reproductive and growth data from the past 16 years and thus of little use for my comparison of the sexes. I used the statistical analysis programming language and software R to complete my thesis project.

The plants too young to be sexed were removed from the data. Any plants that never flowered were also removed from the dataset. Plants with a rosette count rather than area are too young to flower and were not considered in this project, so the measurement of size is not skewed by rosette counts. The resulting dataset was reduced to 272 plants – those with a clear sex, flower and fruit count, and size. Of those, there were 95 females and 177 hermaphrodites. I began with testing the relationships between a variety of variables, as I was trying to gauge the connections between them.

The variables I will refer to include sex, flower number, fruit number, size (as a measure of square millimeters), population (SN1-SN4), fruits per flower (fraction of total flowers that became fruits), peak flowering day (day with highest number of flowers), first flowering day, last flowering day, and flowering range (number of days between first and last flowering days)

I primarily used linear models (denoted as `lm` in R) to test relationships between different variables. For the first research question, I used linear models to test the effects of sex on flower number and fruit number, as well as interactions with population on flower and fruit number.

For the second research question, I used linear models to test the effect of sex and size on several different dependent variables: peak flowering day, flowering range, first flowering day, and last flowering day.

For the third research question, I used linear models to test the effects of sex, size, and population on dependent variables of flower number and fruit number.

For the fourth, I tested independent variables of sex, size, and population against the dependent variable of fruits per flower.

The population variable was included to see the difference in the four different microclimates.

The tests were run both with and without population, using the function ANOVA to test the relationship between population and the other variables rather than each population against each variable.

Linear models with statistically significant p-values were noted for further analysis.

Results

In order to organize my findings and adequately answer the four research questions, the Results section is split into four subsections. For all colored figures, purple refers to females and green refers to hermaphrodites.

Research Question One

The mean total flower count for females was 29.34, and the mean total flower count for hermaphrodites was 30.7. There is no statistically significant difference between these values, as the p-value is 0.75. This is visible in Figure 8a.

	Median	Mean	Max
Female Flowers	12	29.56	217
Female Fruits	4	12.43	92
Hermaphrodite Flowers	17	30.75	202
Hermaphrodite Fruits	3	6.74	67

Figure 7. Summary table of flower and fruit data.

The mean fruit count for females was 12.43, and the mean fruit count for hermaphrodites was 6.74. These values are statistically significant (p-value of $<2e-4$) (see Figure 8b).

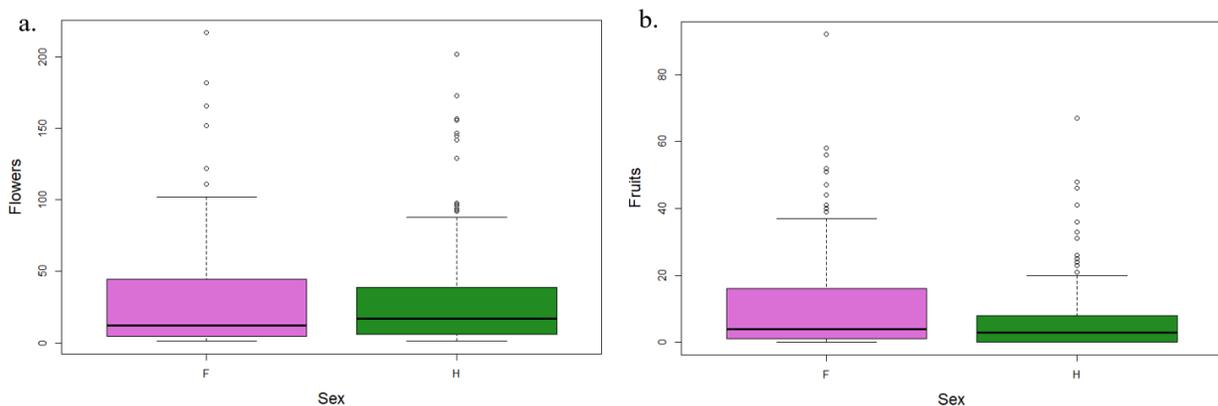


Figure 8. Flower number is similar for females and hermaphrodites, though fruit number is not.

To understand other potentially confounding variables, I used population as the independent variable for flowers and fruits. I found that SN4 had the highest flower count, of statistical significance ($p\text{-value} = 6e-10$), but that there was no significance in fruit count by population.

Figure 9 is plots of these values.

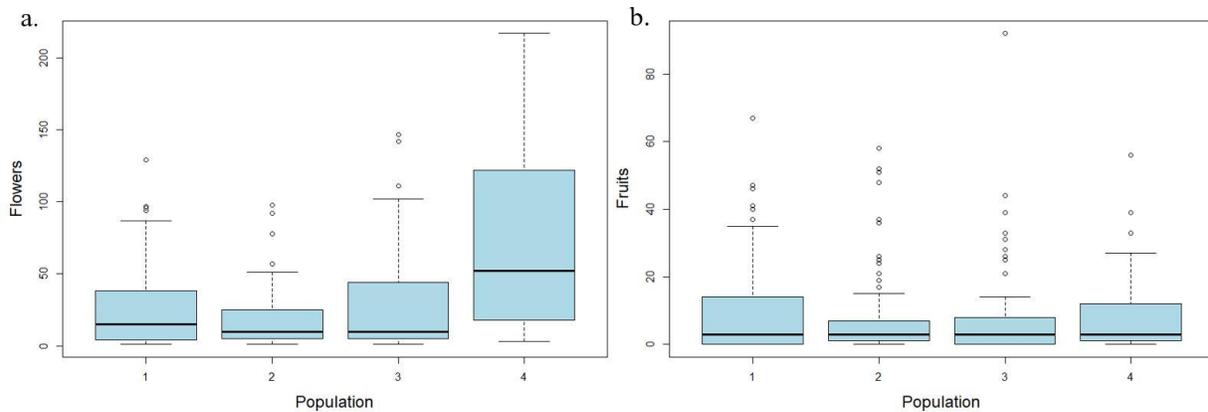


Figure 9. Flower number was similar in SN1-SN3 but higher in SN4. Fruits were similar in all four populations.

Research Question Two

In relation to the second research question, hermaphrodites had a longer flowering range of an additional two days, which is statistically significant ($p\text{-value} = 0.000896$). Females had an average range of 7.2 days, while hermaphrodites had a range two days longer of 9.2 days. Figure 10 shows not only the range but also the first, last, and peak flowering day for flowering plants.

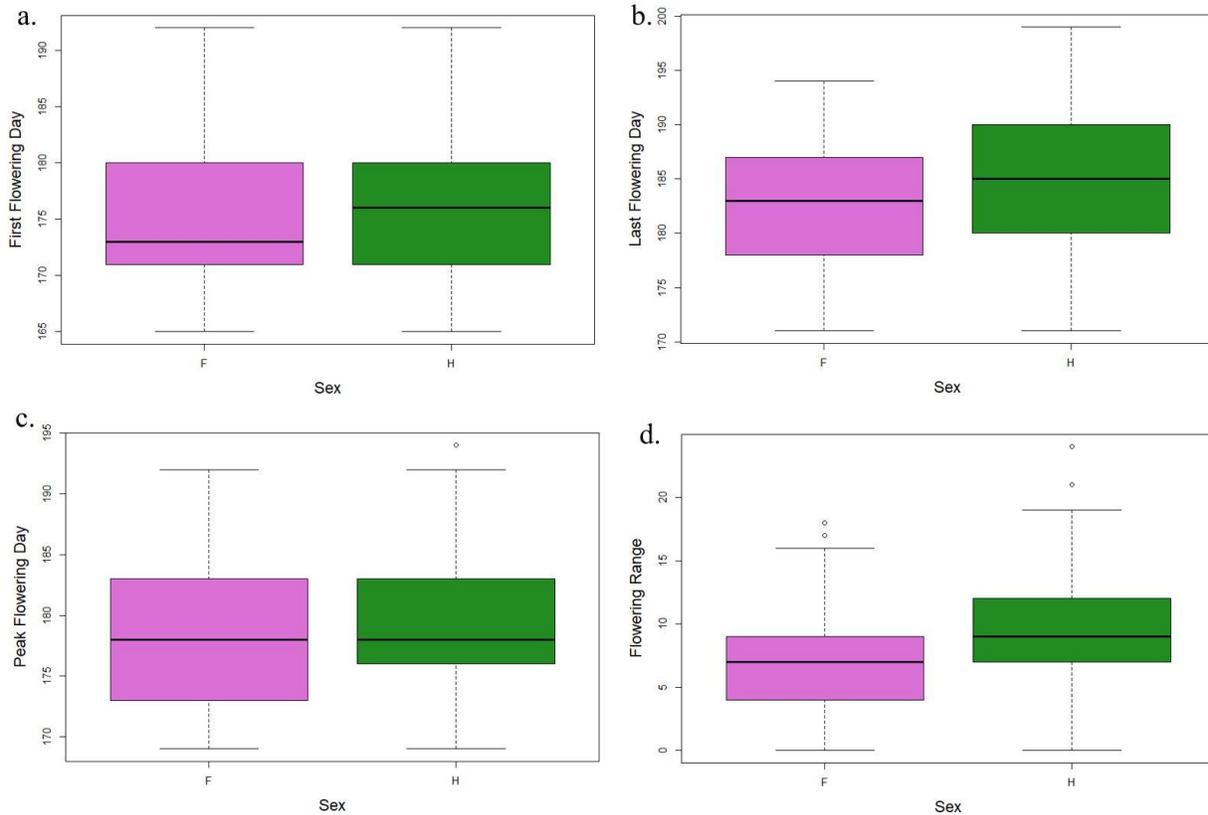


Figure 10. Plots of the (a) first flowering day, (b) last flowering day, (c) peak flowering day, and (d) flowering range with independent variable of sex.

Figure 10 displays the similarity in first flowering day, which was not statistically significant. The last flowering day was longer by just under a day for hermaphrodites (female last day was day 181.6, hermaphrodite last day was day 182.4). This is not statistically significant, as the p-value is 0.33. Peak flowering day was slightly later by a fifth of a day for hermaphrodites, which is not statistically significant (p-value =0.7). Range of flowering period is the only significant difference, as discussed above.

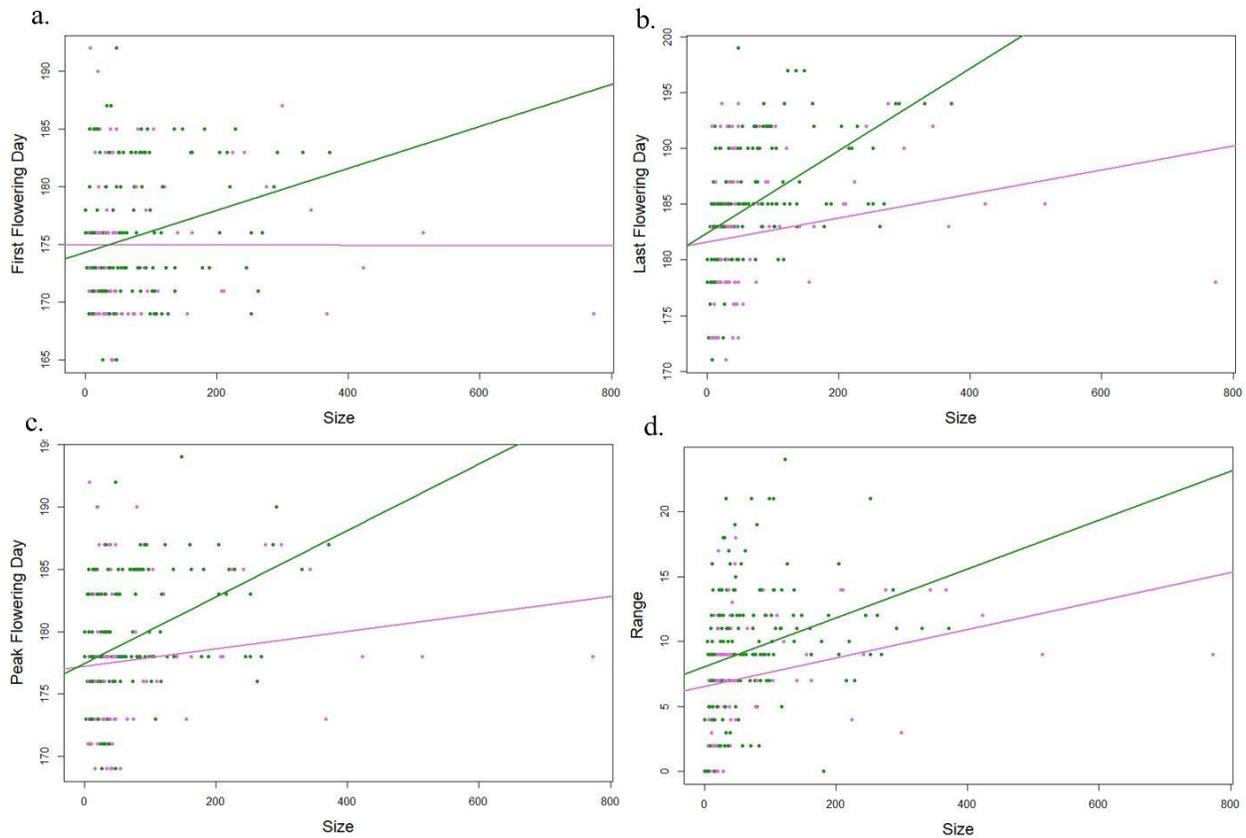


Figure 11. A composite of (a) first flowering day, (b) last flowering day, (c) peak flowering day, and (d) flowering range as dependent variables to the independent variable size and colored by sex.

Figure 11 above shows the flowering time (distinguished by first, last, and peak flowering days as well as range) each as a function of the plant size with interaction of sex. The first flowering day is later for hermaphrodites than females, overall, and had more variance than females, which tended to begin flowering at the same time regardless of size, as shown in Figure 11a. First flowering day differences are statistically significant with the interaction of sex and size (p -value = 0.0159). Last flowering day follows a similar trend, with hermaphrodites flowering later than females for their given size. This is again significant for the interaction (p -value = 0.000241). Hermaphrodites do have a longer flowering range than females (Figure 11d) and so the later ceasing of flowering is logical. Both sexes end flowering later in larger plants. The peak

flowering day is later for hermaphrodites, which is significant only with combined interaction of sex and size (p-value =0.00364). The range is longer for hermaphrodites for any given size, which is significant for sex and size separately but not for the interaction (p-values=0.0454, 0.00584, and 0.2, respectively).

Research Question Three

An ANOVA test of flowers as a function of sex and size shows a non-statistically significant relationship between flowers and sex, but a significant relationship between flowers and size as well as flowers and sex and size (p-values=.2245, 1.43e-11, and .00211, respectively). This suggests a switch in fitness reliance by hermaphrodites from seeds to pollen with increased size.

Flowers increased with size for both sexes, though at a faster rate for hermaphrodites (0.3255) than for females (0.1925) as shown in Figure 12a. Fruits increased at a slightly faster rate for females (0.04568) than for hermaphrodites (0.03238) as shown in Figure 12b.

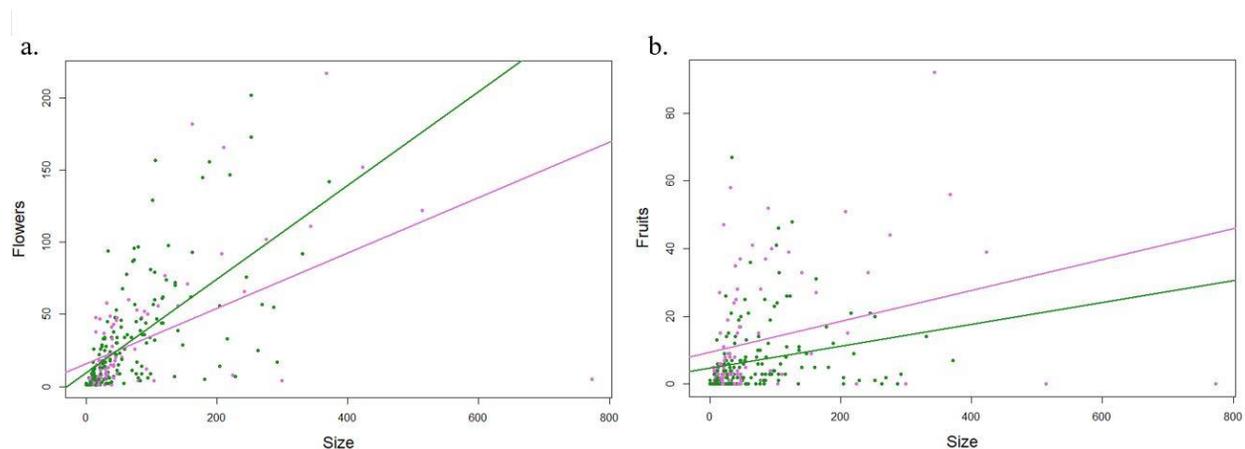


Figure 12. The total flower count increases at a faster rate for hermaphrodites with size increase (a), though fruits do not (b).

The decreasing fraction of fruits per flower at large sizes suggests senescence. As *Silene acaulis* is so long-lived, the plants slowly dieback and produce fewer flowers. There is insufficient data to prove a nonlinear curve, however, as there are so few plants in the larger sizes.

Research Question Four

Females have a higher proportion of flowers becoming seed-producing fruits as 43.6% of their flowers became fruits, and hermaphrodites fruited only 23.6% of their flowers. Seeding ratio as a function of sex is statistically significant ($p\text{-value} = 4.799e-07$). Figure 12 displays fruits per flower as a function of size and sex to highlight the differences between young females and old hermaphrodites.

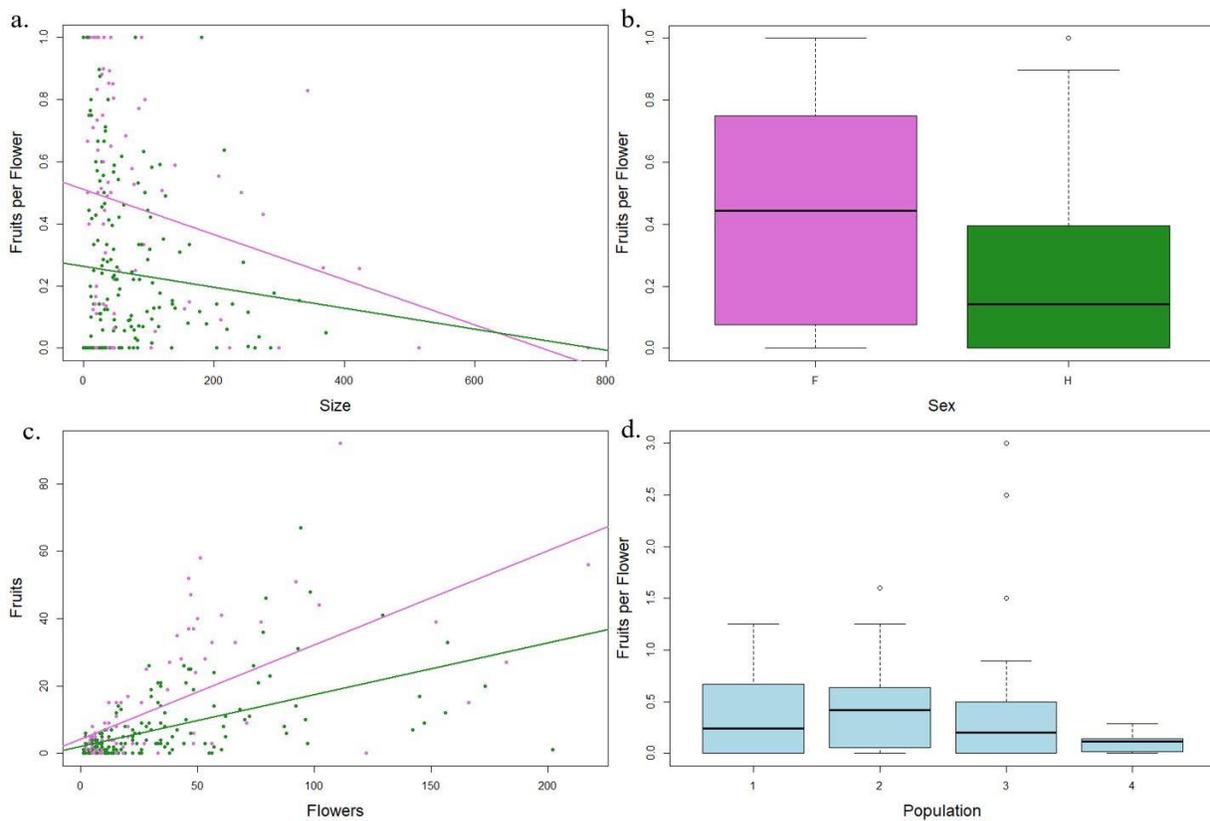


Figure 13. Fruits per flower differed by both size (a) and sex (b). Fruits per flower, or the seeding ratio, is higher for females (p -value = $4.8e-7$) and smaller plants. Females have a higher fruits-to-flower ratio (c) and fruits per flower is lowest at SN4 (d).

Figure 13c is a plot of fruits as a function of flowers to highlight the differences between the sexes. The purple line representing females is consistently higher than the green line, showing a consistently higher seeding ratio for female flowers (p -values = $4.8e-7$ and $7.67e-7$ with interaction of size). Figure 13d refers back to Figure 9. As fruits per flower is a fraction, the higher flower quantity at SN4 with normal fruit quantity led to a much lower fruits per flower fraction, which may influence the other results (p -value of SN4 fruits per flower = 0.000281). For all other analyses and results, population effect was ignored and Niwot Ridge was considered as a whole.

Discussion

Based on Delph et al 1999, female seed quality is no greater than hermaphrodite seed quality. Assuming this is true for the Niwot Ridge population, then quantity of fruits can be used as a proxy for likelihood of seed offspring carrying on the genetic material. Females produced higher quantities of both fruits and fruits per flower, suggesting higher fitness through seeds. This explains how females continue to compete with pollen-producing hermaphrodites.

Previous studies found that females seed more of their flowers than herms (Shykoff 1988; Delph et al. 1999; Wang et al. 2015). My data supported that as well, as females had an average fruit:flower ratio of .436 and hermaphrodites had a ratio of .236, which is statistically significant.

Females produced fruits from 43.6% of their flowers, while hermaphrodites fruited only 23.6%.

The mean number of fruits for females is 12.43 to their mean 29.55 flowers, while

hermaphrodites produced an average of 6.74 fruits to their average of 30.7 flowers. This near-doubling of fruits by females illuminates the ability of non-pollen producing plants to focus on seeds and agrees with conclusions found by Reid et al. (2014) and Shykoff (1988). However, every fruit represents a successful transfer of pollen from a hermaphrodite, and thus the hermaphrodites can still pass along genetic material while the female exerts the resources to create seeds. The offspring will carry on the female-creating cytoplasmic male sterility, however, furthering the sex ratio.

Reid et al. 2014 discussed a “reproductive assurance hypothesis” stating that females must produce twice the seeds as hermaphrodites to persist, which the study supported. The conclusions of this thesis support that hypothesis as well.

Higher elevations tend to have higher proportions of females, due not to seed quality but resource allocation and resilience through low water availability (Alatalo and Molau 1995; Petry et al. 2016). I would argue this is also due to the higher ratio of seeds produced by females, influencing the sex ratio for the following generation before the survivorship differences can influence the population. The Niwot Ridge population in this project is approximately two-thirds hermaphrodites, with 95 of 272 in this project female and 177 hermaphroditic. The female seed production keeps this population gynodioecious. If hermaphrodites were to outcompete females, the population would still be able to sustain itself, although as an androgynous or monoecious population (Warming, Eugenius 1920).

Flowers and fruits both increase with plant size, showing an increased ability of older and larger plants to attempt more reproduction. This is a logical conclusion as larger plants would have access to more resources via a larger root system and higher photosynthesis rate from a larger plant area. However, fruits per flower decreases with increasing size at a rate of -0.0007269 for

females and -0.0003367 for hermaphrodites (also shown in Figure 13a). This decrease in fitness for larger plants may reduce the impact of older plants' influence on the evolution of the species, as larger plants are older and thus have traits that were selected for many years before the pollination of the seeds that became the smaller and more fecund plants. Simply speaking, as older plants reproduce, they pass on traits that may not be as beneficial as the traits younger plants pass down. If older plants have a lower fecundity rate, the gene pool for the upcoming generation is less irrelevant than if fruits per flower increased with size.

Aging hermaphrodites have a shifting fitness reliance. Hermaphrodites act like females as young plants and like males as old plants. While small (young) they produce more fruits as a ratio of their flower quantity, showing a reliance on seeds to carry on genetic material. As the hermaphrodites age, their fruits per flower decreases at a slower rate than that of females, showing a switch to a fitness reliance on pollen rather than seeds. This is also emphasized by the reduced fruits per flower of females (see Figure 11a).

The longer flowering range for hermaphrodites may be due to an interest to send and receive pollen (as females need only receive) and also to reduce the likelihood of self-pollination. Self-pollination, while possible, produces offspring of reduced quality (Dufay and Billard 2012). Offspring produced via self-pollination has reduced survival by more than 30%, reduced germination rates, and grows slower than female-produced offspring (Shykoff 1988; Keller and Schwaegerle 2006). This may explain the longer range of hermaphrodites in Figures 10 and 11.

The delay in both beginning flowering and ceasing flowering as well as peak flowering day for large hermaphrodites (Figure 11) suggests that the larger plants may take longer to produce their many rosettes again after the winter dormancy, and perhaps that hermaphroditic production of pollen delays flowering. Small females would be more able to respond to the warming summer

and create rosettes and produce flowers quicker. However, this is simply a theory and further research could address this concept.

The significant difference in flowers at SN4 yet lack of corresponding increase in fruits may have some environmental effect that could be investigated. SN4 is the wet site on the East Knoll facing south and at a slightly lower elevation. These factors or the proximity to a wet region and related pollinator abundance may influence seeding success.

Although not addressed in this thesis, flowering synchrony can have a large effect on fruit number. Flowering synchrony occurs when plants in an area to flower at the same time, thus potentially attracting more pollinators but also dealing with increased pollination competition. Differing synchrony across individuals and the four subpopulations may influence fruit quantity and thus fruit per flower quantity.

Conclusion

My first research question focused on the flower and fruit quantities. I found the sexes produced similar flower quantities, though females produced twice the number of fruits. Fruit number was consistent across the four microclimates, though flower number was higher at SN4 and thus fruits per flower was lower.

My second question was for flowering ranges, which was answered with longer ranges for hermaphrodites, presumably to have time to both send and receive pollen and reduce chances of self-pollination.

For the third question, flower quantity did increase with size. Female flowers increased at a rate of 0.1925 per square millimeter size increase and hermaphrodite flowers increased at a rate of

0.3255 per square millimeter size increase. Fruit quantity increased with size as well, at a rate of 0.04568 for females and 0.03238 for hermaphrodites per square millimeter size increase. Thus, flowers are more common on large hermaphrodites and fruits are more common on large females.

The fourth research question on the fruiting ratio found a higher fraction of fruits per flower on female plants as well as smaller plants of both sexes. Females must produce more fruits in order to compete with the disadvantage of not producing pollen. Their higher fruiting ratio explained the continued existence of females able to compete with the “perfect flower” of a hermaphrodite. The high fruits per flower of younger plants may be explained by the senescence of plants in the dieback phase.

The implications of this research extend beyond this project. The difference in the reliance on flowers by the two sexes illuminates the evolutionary progress of the species. Hermaphrodites, despite being the perfect flower, are unable to outcompete females. Females produce offspring of higher quality yet exist in smaller numbers.

The role of SA in the alpine ecosystem as a nurse plant makes this species integral for conservation work. As the climate changes and warmer summer become the new norm, alpine plants limited by elevation have nowhere to move, and may face extirpation. The extirpation or extinction of *Silene acaulis* would have effects throughout the trophic system as the plants and insects that rely on the cushion for shelter are ousted. Additionally, earlier springs could lead to changes in the pollinator abundance and thus limit the seed production of SA, or even favor less successful offspring of self-pollinated hermaphrodites.

Beyond climate mitigation efforts, preemptive conservation measures could include seed collection and storage to preserve the genetic variety, or relocation of the seeds of more durable populations to areas with expected intense warming.

Research on *Silene acaulis* could easily continue. Further research could seek to address resource allocation and the root system to identify methods of nutrient distribution, which could relate back to flower production as there may be a limiting agent in flower or fruit production.

Additionally, research could address patterns of senescence to identify an optimal size for maximum flower and fruit production. The comparative rate of senescence for the two sexes and their rate of dieback coupled with ceasing of flower production could help to create a deeper understanding of the genetic material passed along to the following generations.

Further research could also address flowering synchrony as a factor in fruits per flower seeding success and overall fruit production. Consideration for patches of related plants could add additional complications, as seeds may not scatter far and may lead to inbreeding.

Bibliography

- Alatalo JM, Little CJ. 2014. Simulated global change: contrasting short and medium term growth and reproductive responses of a common alpine/Arctic cushion plant to experimental warming and nutrient enhancement. *Springerplus* 3:157. doi:10.1186/2193-1801-3-157.
- Alatalo JM, Molau U. 1995. Effect of altitude on the sex ratio in populations of *Silene acaulis* (Caryophyllaceae). *Nord. J. Bot.* 15:251–256. doi:10.1111/j.1756-1051.1995.tb00150.x.
- Alatalo JM, Molau U. 2001. Pollen viability and limitation of seed production in a population of the circumpolar cushion plant, *Silene acaulis* (Caryophyllaceae). *Nord. J. Bot.* 21:365–372. doi:10.1111/j.1756-1051.2001.tb00780.x.
- Antonsson H, Bjork RG, Molau U. 2009. Nurse plant effect of the cushion plant *Silene acaulis* (L.) Jacq. in an alpine environment in the subarctic Scandes, Sweden. *Plant Ecol. Divers.* 2:17–25. doi:10.1080/17550870902926504.
- Arroyo MTK, Cavieres LA, Penaloza A, Arroyo-Kalin MA. 2003. Positive associations between the cushion plant *Azorella monantha* (Apiaceae) and alpine plant species in the Chilean Patagonian Andes. *Plant Ecol.* 169:121–129. doi:10.1023/A:1026281405115.
- Ashman T-LR. 2002. The evolution of separate sexes: a focus on the ecological context (PDF Download Available). [accessed 2018 Mar 16]. https://www.researchgate.net/publication/237105538_The_evolution_of_separate_sexes_a_focus_on_the_ecological_context.
- Asikainen E, Mutikainen P. 2005. Pollen and resource limitation in a gynodioecious species. *Am. J. Bot.* 92:487–494. doi:10.3732/ajb.92.3.487.
- Badano EI, Jones CG, Cavieres LA, Wright JP. 2006. Assessing impacts of ecosystem engineers on community organization: a general approach illustrated by effects of a high-Andean cushion plant. *Oikos* 115:369–385.
- Braun CJ, Brown GG, Levings CS. 1992. Cytoplasmic Male Sterility. In: *Cell Organelles*. Springer, Vienna. (Plant Gene Research). p. 219–245. [accessed 2018 Mar 16]. https://link.springer.com/chapter/10.1007/978-3-7091-9138-5_7.
- Callaway RM, Brooker RW, Choler P, Kikvidze Z, Lortie CJ, Michalet R, Paolini L, Pugnaire FI, Newingham B, Aschehoug ET, et al. 2002. Positive interactions among alpine plants increase with stress. *Nature* 417:844–848. doi:10.1038/nature00812.
- Callaway RM, Walker LR. 1997. Competition and facilitation: A synthetic approach to interactions in plant communities. *Ecology* 78:1958–1965. doi:10.2307/2265936.
- Casimiro-Soriguer I, Buide ML, Narbona E. 2015. Diversity of sexual systems within different lineages of the genus *Silene*. *Aob Plants* 7:plv037. doi:10.1093/aobpla/plv037.

- Charlesworth B, Charlesworth D. 1978. Model for Evolution of Dioecy and Gynodioecy. *Am. Nat.* 112:975–997. doi:10.1086/283342.
- DeHaan AA, Luyten R, BakxSchotman T, VanDamme JMM. 1997. The dynamics of gynodioecy in *Plantago lanceolata* L. .1. Frequencies of male-steriles and their cytoplasmic male sterility types. *Heredity* 79:453–462. doi:10.1038/hdy.1997.184.
- Delph LF, Bailey MF, Marr DL. 1999. Seed provisioning in gynodioecious *Silene acaulis* (Caryophyllaceae). *Am. J. Bot.* 86:140–144.
- Delph LF, Carroll SB. 2001. Factors affecting relative seed fitness and female frequency in a gynodioecious species, *Silene acaulis*. *Evol. Ecol. Res.* 3:465–476.
- Doak DF, Morris WF. 2010. Demographic compensation and tipping points in climate-induced range shifts. *Nature* 467:959–962. doi:10.1038/nature09439.
- Dufay M, Billard E. 2012. How much better are females? The occurrence of female advantage, its proximal causes and its variation within and among gynodioecious species. *Ann. Bot.* 109:505–519. doi:10.1093/aob/mcr062.
- Gauslaa Y. 1984. Heat resistance and energy budget in different Scandinavian plants. *Ecography* 7:5–6. doi:10.1111/j.1600-0587.1984.tb01098.x.
- Gussarova, Allen, Geraldine A., Mikhaylova, Yulia, McCormick, Laurie J., Mirré, Virginia, Marr, Kendrick L., Hebda, Richard J., Brochmann, Christian. 2015. Vicariance, long-distance dispersal, and regional extinction–recolonization dynamics explain the disjunct circumpolar distribution of the arctic-alpine plant *Silene acaulis*. *Am. J. Bot.* 102:1703–1720. doi:10.3732/ajb.1500072.
- Hanson MR, Conde MF. 1985. Functioning and Variation of Cytoplasmic Genomes: Lessons from Cytoplasmic–Nuclear Interactions Affecting Male Fertility in Plants. In: Bourne GH, Danielli JF, Jeon KW, editors. *International Review of Cytology*. Vol. 94. Academic Press. p. 213–267.
- Harder LD, Barrett SCH. 2006. *Ecology and Evolution of Flowers*. OUP Oxford.
- Hermanutz L, Innes D. 1994. Gender Variation in *Silene-Acaulis* (caryophyllaceae). *Plant Syst. Evol.* 191:69–81. doi:10.1007/BF00985343.
- Hulten E. 1958. The amphi-Atlantic plants and their phytogeographical connections. *K Sven. Vetenskapsakad Handl* 7:1–340.
- Keller SR, Schwaegerle KE. 2006. Maternal sex and mate relatedness affect offspring quality in the gynodioecious *Silene acaulis*. *J. Evol. Biol.* 19:1128–1138. doi:10.1111/j.1420-9101.2006.01101.x.
- Klaas AL, Olson MS. 2006. Spatial Distributions of Cytoplasmic Types and Sex Expression in Alaskan Populations of *Silene acaulis*. *Int. J. Plant Sci.* 167:179–189. doi:10.1086/498965.
- Manicacci D, Couvet D, Belhassen E, Gouyon PH, Atlan A. 1996. Founder effects and sex ratio in the gynodioecious *Thymus vulgaris* L. *Mol. Ecol.* 5:63–72.

- Molenda O, Reid A, Lortie CJ. 2012. The Alpine Cushion Plant *Silene acaulis* as Foundation Species: A Bug's-Eye View to Facilitation and Microclimate. *Plos One* 7:e37223. doi:10.1371/journal.pone.0037223.
- Morris W, Doak D. 1998. Life history of the long-lived gynodioecious cushion plant *Silene acaulis* (Caryophyllaceae), inferred from size-based population projection matrices. *Am. J. Bot.* 85:784–784.
- Nunez CI, Aizen MA, Ezcurra C. 1999. Species associations and nurse plant effects in patches of high-Andean vegetation. *J. Veg. Sci.* 10:357–364. doi:10.2307/3237064.
- Petry WK, Soule JD, Iler AM, Chicas-Mosier A, Inouye DW, Miller TEX, Mooney KA. 2016. Sex-specific responses to climate change in plants alter population sex ratio and performance. *Science* 353:69–71. doi:10.1126/science.aaf2588.
- Philipp M, Jakobsen RB, Nachman G. 2009. A comparison of pollen-siring ability and life history between males and hermaphrodites of subdioecious *Silene acaulis*. *Evol. Ecol. Res.* 11:787–801.
- Reid A, Hooper R, Molenda O, Lortie CJ. 2014. Ecological implications of reduced pollen deposition in alpine plants: a case study using a dominant cushion plant species. *F1000Research* 3:130. doi:10.12688/f1000research.4382.1.
- Schnable PS, Wise RP. 1998. The molecular basis of cytoplasmic male sterility and fertility restoration. *Trends Plant Sci.* 3:175–180. doi:10.1016/S1360-1385(98)01235-7.
- Shykoff J. 1988. Maintenance of Gynodioecy in *Silene Acaulis* (caryophyllaceae) - Stage-Specific Fecundity and Viability Selection. *Am. J. Bot.* 75:844–850. doi:10.2307/2444003.
- Shykoff JA, Kolokotronis S-O, Collin CL, López-Villavicencio M. 2003. Effects of male sterility on reproductive traits in gynodioecious plants: a meta-analysis. *Oecologia* 135:1–9. doi:10.1007/s00442-002-1133-z.
- Wang H, Matsushita M, Tomaru N, Nakagawa M. 2015. Differences in female reproductive success between female and hermaphrodite individuals in the subdioecious shrub *Eurya japonica* (Theaceae). *Plant Biol.* 17:194–200. doi:10.1111/plb.12189.
- Warming, Eugenius. 1920. The structure and biology of arctic flowering plants. Kjøbenhavn.