

Factors Driving Flowering Phenology and Reproductive Success in *Silene acaulis* and  
implications for response to climate change

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## Abstract

Along with survival, successful reproduction is one of the key features of individual performance that is necessary for a species to survive, including in the face of environmental alterations. This study examined possible drivers of reproductive success in alpine cushion plant *Silene acaulis*, and the implications of reproductive patterns for long-term stability of populations in the face of climate change. First, I asked whether the timing of flowering in a long-lived alpine plant species influenced whether flowers successfully produced fruit, and whether that timing was correlated with the plant's long-term seed production. I found that the timing of flowering did have an effect on long-term fruit set – plants that flowered earlier tended to produce more fruit per unit area overall. Secondly, I asked whether some individuals in the population consistently produce more fruit than others, and if trends in fruit production varied at the population level. I found that some individuals consistently produce more fruit than their neighbors, which suggests that some variation in fruit set within a population is due to individual differences (possibly genetic or microhabitat differences). I examined possible correlations between climate factors and fruit production. I used summer temperatures and precipitation as climate indicators, and found that both were negatively correlated with fruit set. I also found that variation in population fruit set was lower than expected, suggesting that disparate individual responses to environmental variation buffer population fruit set.

*Key words:* Phenology, reproduction, alpine, *Silene acaulis*, climate change, Colorado

## Introduction

Successful local reproduction in plant species is critical for persistence of the population and species as a whole. Species that have limited windows for flowering and reproduction have additional constraints on reproductive processes. Climate factors and pollinator availability may affect timing of flowering and fruit production, and both of those variables are likely inconsistent year to year. Additionally, as climate patterns shift over time, species may have shorter windows for important life processes, including reproduction. Understanding the abiotic and biotic drivers of successful reproduction may provide insight into how population dynamics may change if patterns of reproduction shift. One key feature of these responses involves variation in individual performance. In particular, asynchrony on the individual level in responses to climate may provide a buffer in the species' response to climate change, while highly correlated responses can lead to population-wide failure of reproduction could put populations at risk.

This study explored possible drivers of reproductive success in *Silene acaulis*, a long-lived, slow-growing alpine cushion plant. *Silene acaulis* individuals only flower for a week or two during the summer season. One component of this study involved tests to understand the effect of flowering time on reproductive success. An individual plant flowering at the same time as the rest of the population may be more successful if the entire site appears more attractive to pollinators. On the other hand, flowering at the same time as the rest of the population means competing for pollinator attention – so perhaps flowering earlier or later is beneficial, though early or late freezes could make this strategy risky. To address this tradeoff, this study explored

the effect of flowering time within a two-year period on long-term reproductive success (measured by fruit production).

A second part of the work used long-term data to explore whether some individuals in the population consistently outperform their peers. If some plants always do better or worse than the population average, perhaps individual variation plays a role in determining success, independently of climate factors. I also examined whether the same individuals flower early or late each season compared to the population, and whether those early or late bloomers produce more fruit. Finally, I looked at whether deviations from typical fruit set showed a trend on a population level – whether, in some years, the individuals of a whole population did better or worse than usual. A trend in deviations from average fruit production across a population may hint that climate factors played a role in certain years.

## Background

### *Study Motivation*

All flowering plants that exist in seasonal climates make a tradeoff when they decide when to open their flowers (Tooke and Battey 2010). For species in temperate and other regions with pronounced cold seasons, plants that flower early in the season benefit from a longer growing season and more chances for pollinator attention, but also risk damage from late spring freezes. A further effect comes from the need to flower when other, nearby members of the same species are also in flower (Gomez 1993). Cues for the timing of flowering can be based on local environmental conditions like precipitation and temperature patterns, as well as day length. In addition, pollen availability can alter the duration of flowering.

In the alpine, the growing season is limited, so timing of flowering is especially critical. Alpine plants are required to adapt to long winter seasons and thus to a limited period with sufficient water, sunlight, and pollinator availability to successfully flower and seed. A plant's decision on when to flower may influence how successfully it reproduces that year.

My work has focused on *Silene acaulis* (Caryophyllaceae), an alpine cushion plant widely distributed around the Northern Hemisphere in alpine and arctic habitats. Cushion plants are common in the alpine. Standing only a few inches tall, their characteristic cushion shape retains nutrients and water, and acts as a temperature regulator (Alatano & Totland 1997), while their low stature shelters them from the winter conditions. As a result, cushion plants often inhabit early successional or extremely harsh sites, making them important dominants in many tundra habitats. *Silene acaulis* (henceforth, *Silene*) has a very tight, elliptical pattern of thick, durable short leaves in clusters called rosettes. Each cushion has a single taproot, which anchors the plant to its surface and supplies water and nutrients to the individual. The tightly-bound

shape and short rosettes makes the size of the entire cushion easy to measure, and also makes individuals easy to distinguish from each other most of the time. Typically, *Silene* grows in dry, rocky areas. The cushion provides a more stable, regulated microenvironment that can create new suitable habitat for other alpine species. It is rarely found below tree line. The plants are incredibly hardy and survive in harsh conditions, growing slowly and reproducing slowly (Morris and Doak, 1988). Thus, their potential for relatively rapid adaptation is small. The ability of alpine plants to extend range limits is poorly understood, but their natural dissected habitat in mountainous regions is likely to impose significant barriers to dispersal.

With *Silene* and other similar plants around the world, we may see substantial effects of changing climate patterns on flowering time and fitness. In particular, warmer temperatures have been shown to encourage flowering earlier in the season (Alatalo and Totland 1997). If flowering begins sooner in the season but pollinators are still unavailable, reproductive success of the population may decrease. Similarly, earlier flowering may subject plants to spring freezing events, even with increases in average temperatures. All these possible effects could have serious consequences for population persistence, especially if migration of populations is impeded by long generation times and difficult dispersal. In my thesis, I have asked several questions about the proximate causes of flowering time in *Silene* populations living on Niwot Ridge, Colorado and about patterns of reproductive success, as well as addressed some of the consequences of plant responses to climate. In particular, I address the following questions:

1. How does timing of flowering influence seed set? Particularly, if an individual has most of its flowers open at the same time as the rest of the local population, does that individual tend to have a higher (or lower) fruit set?



2. Is there consistency among individuals in a population in who flowers early or late? If so, did flowering early or late in 2016-2017 correlate to their long-term seed set?
3. Do some plants consistently have higher fruit set and is inter-annual variation in reproductive success correlated among individuals? If I find that plants vary in success in the long-term, then perhaps differing responses in fruit set on the individual level will buffer effects of changing climate conditions.
4. Does fruit set vary with climate factors? If fruit set is correlated with summer precipitation or temperature, then we might expect population fruit set to change as climate patterns change. If fruit set is not uniformly affected by climate factors (individuals appear to be affected differently by different weather each year), then perhaps fruit set will not be as variable as expected with changing climate.

### *Breeding Systems of Angiosperms*

Angiosperms, or flowering plants, have evolved a variety of different sexual systems. One end of the spectrum is gender monomorphism, where individuals of a population are all essentially hermaphrodites – they produce both seeds and pollen. On the other end, gender dimorphism exists when individuals of a population are grouped either as males and females (dioecy), females and hermaphrodites (gynodioecy), or males and hermaphrodites (androdioecy) (Ashman 2006). Many species, including *Silene acaulis*, have breeding systems that may differ around the world. Dioecy exists in about 6% of angiosperms (Ashman 2006), and several different evolutionary pathways to dioecy have been proposed.

In the case of gynodioecy in *Silene*, a mitochondrial mutation exists that sterilizes pollen function and creates females, and a nuclear mutation that restores pollen function creates

hermaphrodites (McCauley and Olson, 2003). So, for example, if an ancestral, purely hermaphroditic population of *Silene* existed, a female could arise if there is a mutation in the mitochondrial genome that causes pollen to be sterile. Mitochondrial alleles are only inherited via seeds, so mitochondrial mutations can essentially increase their own transmission rate by increasing investment in seed production. The check on this feedback loop is that once females increase in frequency, males that can fertilize many females have an advantage. So, any nuclear mutation that can counteract the mitochondrial mutation to restore pollen function is favored, and the fluctuation continues (McCauley and Olson, 2003). Most populations of *Silene* likely have multiple alleles that sterilize pollen and multiple alleles that counteract those to restore pollen function.

### *Silene acaulis*

*Silene acaulis* is a long-lived alpine plant with light pink flowers. The population of *Silene acaulis* here in Colorado is a gynodioecious one, meaning it consists of females and hermaphrodites (Delph 2004). Females produce seeds and males produce both seeds and pollen. The seeds of female plants have been shown to be stronger (measured in terms of quality of the seed, quantity of seeds produced, or both) (Shykoff, 1988; Delph and Mutikainen, 2003), but females rely on hermaphrodites to receive pollen, whereas hermaphrodites can self-pollinate. Females tend to have higher fruit sets and higher quality seeds that produce more successful offspring (Delph, 2004; Delph & Carroll, 2001; Sakai et al., 1997; Shykoff et al., 2003), which has a maintaining effect on gynodioecy. Hermaphrodites tend to produce larger flowers than females (Philipp, Jakobsen, & Nachman, 2009) and can contribute to the next generation via both seed and pollen production.

Large *Silene* cushions are very old, but significant dieback can occur even in one year, so inferring the age of small cushions is difficult. Age estimates for large cushions in southern Alaska are around 75-100 years (Morris and Doak 1998). In more northerly sites, growth rates are slower and plants can be several hundred years old. For reference, the “large” *Silene* individuals are comparable in size to a dinner plate.

Doak and Morris have been monitoring four different *Silene* populations along a longitudinal gradient in order to better understand the life-history of these plants and implications as preferable habitats shift northward (or, in localized areas, higher in elevation). Their populations are located in arctic and in south-central Alaska, on Niwot Ridge, Colorado (the same population used for this study), and in New Mexico. Their work has shown that *Silene* individuals in extremely harsh conditions can live several hundreds of years while persisting at their small size. Doak & Morris show that southern populations (with warmer climates) have higher growth rates, but lower survival and recruitment than northern populations.

Several studies on *Silene acaulis* reproduction and phenology have been done. Kempe (2014) conducted a study on the correlation between synchrony of flowering time and reproductive success and found that synchrony on a spatial scale of about 50 m<sup>2</sup> had a positive effect on fruit set. This is likely because an entire population flowering at once has a better chance of attracting pollinators, so if an individual is in synchrony with its neighbors, its chances of receiving pollinator attention is higher (Kempe 2014). In the alpine, pollinator encounters and activity are limited, so synchrony may be incredibly important for reproductive success.

Reid and Hooper (2014) examined the effects of pollen availability on female *Silene acaulis* viability. Because females cannot produce pollen as a means of reproductive fitness, they must produce twice as many seeds as their self-compatible hermaphrodite counterparts to persist

(Reid and Hooper 2014). This strategy maintains gynodioecy as long as sufficient pollinator activity is available; however, reduced pollination rates in the alpine, frequently attributed to fewer pollinators and mismatched timing of flowering and pollinator availability, may disrupt this system (Reid and Hooper 2014). Thus, females will likely be much more sensitive in the face of declining pollination rates in alpine plants, and reproduction dynamics may be altered.

A study on Niwot Ridge, Colorado by Delph and Carroll examined the sex-ratio variation of 10 different *Silene* populations (i.e. the proportion of females compared with hermaphrodite individuals). Sex ratio, here, is the ratio of females to hermaphrodites at each site. Sex ratio can be influenced by factors such as pollinator activity or site quality, and is subject to fluctuations over time. Delph and Carroll found that the frequency of females was not correlated the quality of the site, though significant variation in sex ratio was found between sites. Delph also showed that seed fitness was positively correlated with site quality (using ecological measures) and female frequency in the area. She also showed that hermaphrodites had more variable and lower fruit set compared to females. Her results suggest that climatic factors could play a role in female frequency, and thus impact the breeding system of *Silene* (Delph and Carroll 2001).

A study on a subdioecious population (one containing males, females, and hermaphrodites) of *Silene acaulis* in Greenland compared pollen viability and capacity between males and hermaphrodites and found that per anther, males produced more pollen, but that because hermaphrodites are more abundant, they were more likely to sire seeds than their male counterparts (Philipp et al 2009). Thus, excluding a dramatic change in environmental conditions, the population is likely slowing becoming a gynodioecious system like we have here in Colorado (Philip et al 2009).

Date of snowmelt has long been regarded as a major factor determining when cushion plants begin to flower (Thorhallsdottir 1998; Kempe 2014; Alatalo & Totland 1997). A study in Iceland found that onset of flowering varies greatly between years and that initial flowering of 75 species in the area was strongly correlated with ambient air temperature, and thus that phenology and flowering patterns will likely adapt rapidly to earlier springs and warmer temperatures (Thorhallsdottir 1998). This will likely have implications for pollinator competition, seed bank dynamics, and species richness in the area (Thorhallsdottir 1998).

### *The Portfolio Effect*

The basis of the idea of a portfolio effect is in the financial investment world; diversification between assets tends to reduce risk, as many components are variable but aggregate to a more stable level (Schindler et al 2015). In biological systems, components at a finer scale tend to be more variable due to stochastic events and inherent complexities - but on larger scale, we observe that the system as a whole tends to be more stable (Schindler et al 2015).

In this thesis, one of the questions I will address is whether there is a similar portfolio effect that stabilizes *Silene* populations. If reproductive success is variable on the individual level, and fluctuations occur inconsistently throughout the population, do aggregate fruit yields have less variation, and have a stabilizing effect on populations over time? The mechanisms that stabilize small or at-risk populations in the face of inherent variability are poorly understood and incredibly vital to conservation efforts. While *Silene* is not currently an at-risk species, a better understanding of the functioning of this alpine plant's population dynamics could provide insight about other species.

### *Other Comparable Studies*

A study conducted in the Himalayas examined the effects of facilitative effects of cushion plants (Chen et al 2015). They found that soil nutrient availability determined facilitative effects of cushion plants at high elevations. The presence of two cushion plants studied (*Potentilla articulata* and *Arenaria polytrichoids*) increased species richness and diversity compared with areas they were absent in. They also found that nitrogen and potassium levels were higher within the cushion than right outside (Chen et al 2015), which may aid colonization by other species.

Luisa et al (2010) found that for *Silene acutifolia*, a relative of *S. acaulis*, flowering time in northwest Spain varied quite a bit year-to-year on both an individual and population level. The data taken in their study were very similar to this one; they collected information on initial flowering time, total flowering time, and peak flowering time. They did note, however, that the order in which individuals flowered was largely maintained each year they did their study. They also found that the variation in flowering time did not affect reproductive success at an individual level (Luisa et al 2010).

## Methods

### *Population/Site Details*

We studied populations of *Silene acaulis* at four sites on Niwot Ridge, Colorado. Two of the sites were on the western side of the ridge (West Knoll sites) and the other two on the eastern side (East Knoll). Two to three transects (~20 x 1m each) were created at each of the four sites, and every *Silene acaulis* individual along each transect was identified – most individuals are given a numbered tag, though younger individuals were labeled with colored toothpicks for convenience instead, since survival is lower for new recruits. Each plant has a specific X-Y coordinate location along the transect, with the X coordinate the location along the transect tape, and the Y coordinate length away from the tape. A total of 629 individuals were included amongst the four sites.

### *Data Collection*

Each day during the summer season of 2016, we visited two of the four sites. Thus, the flowers on each individual plant were counted every other day. We recorded the date and number of flowers on the individual cushion, as well as the sex once the flowers were open widely enough. The dates were converted into day of the year, and I began data collection the first day that flowers were open at any of our study populations. The number of flowers on each cushion was counted at each visit, giving information about total flowering time (total number of days that flowers were open on an individual), total flower count, peak flowering time (day that most numbers of flowers were recorded) and initial flowering time (day that first flower was recorded). Sex was easy to identify once flowers opened; the hermaphrodites have visible stamen and anthers with pollen, while females do not.

At the end of the field season, the size of each cushion was estimated from the length of the minor and major axes (the shape of most cushions are approximately ellipses), which are then used to compute area. Many cushions have significant dead area, or dieback, so a percent estimate of area missing/dead on the cushion was also recorded so that the actual live area could be estimated accordingly.

Once the flowering season had ended on Niwot Ridge, we counted the total number of flowers produced by each cushion, as well as the total number of flowers that produced fruit. The fruits that contained a seed are distinguishable with a naked eye, as they are much fuller at the base. We also recognize that actual number of seeds may vary slightly between fruits, but approximately twenty seeds are in each fruit, with relatively low variability, so fruit number is seen as a reasonably good estimate of reproductive output. This allowed us to calculate fruit set (a measure of reproductive success) as the proportion of flowers that successfully produced fruit.

Dan Doak and Bill Morris have collected data on the size and number of fruits produced for each of these individuals since 2002. I also used climate data from the PRISM Climate Group. I accessed Prism data to obtain precipitation estimates for June, July, and August from 2002 onward, as well as mean July temperature for that same time period. Additionally, in 2017, two other undergraduate students working with the Doak lab collected flowering time data using the same methods described above (and on the same individual plants), so detailed data sets for the flowering season in the summer of 2016 and 2017 are used in this study.

### *Data Analysis*

For several of my analyses, I used mixed linear models, with multiple fixed effects and individual plant identity as a random effect. Some models used fruit number as the response



variable and were conducted as generalized linear models with Poisson-distributed error. I also used an information-theoretic framework to determine support for alternative models for the same dependent variables, using AIC values to judge support for alternative model forms. All data analysis was conducted in R version 3.2.3. Packages used include ggplot2, lme4, and Hmisc.

### *Climate Data*

The climate estimates used were borrowed from PRISM Climate Group at the University of Oregon. The group uses complex models that extrapolate weather data from different hotspots to estimate weather everywhere. We used total precipitation from June to August as a measure of summer precipitation, and mean July temperature as a temperature indicator.



**Fig 1.** Location of Niwot Ridge, Colorado. (*Google Maps*)

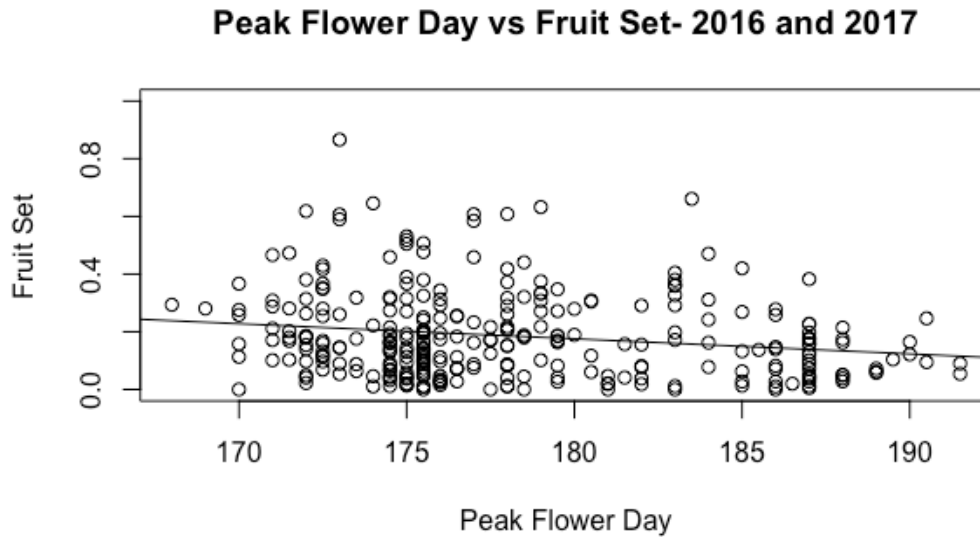


**Fig 2.** Location of *Silene acaulis* population sites marked by a star. Two sites on West Knoll, two sites on East Knoll on Niwot Ridge, Front Range, Colorado. (Google Maps)

## Results

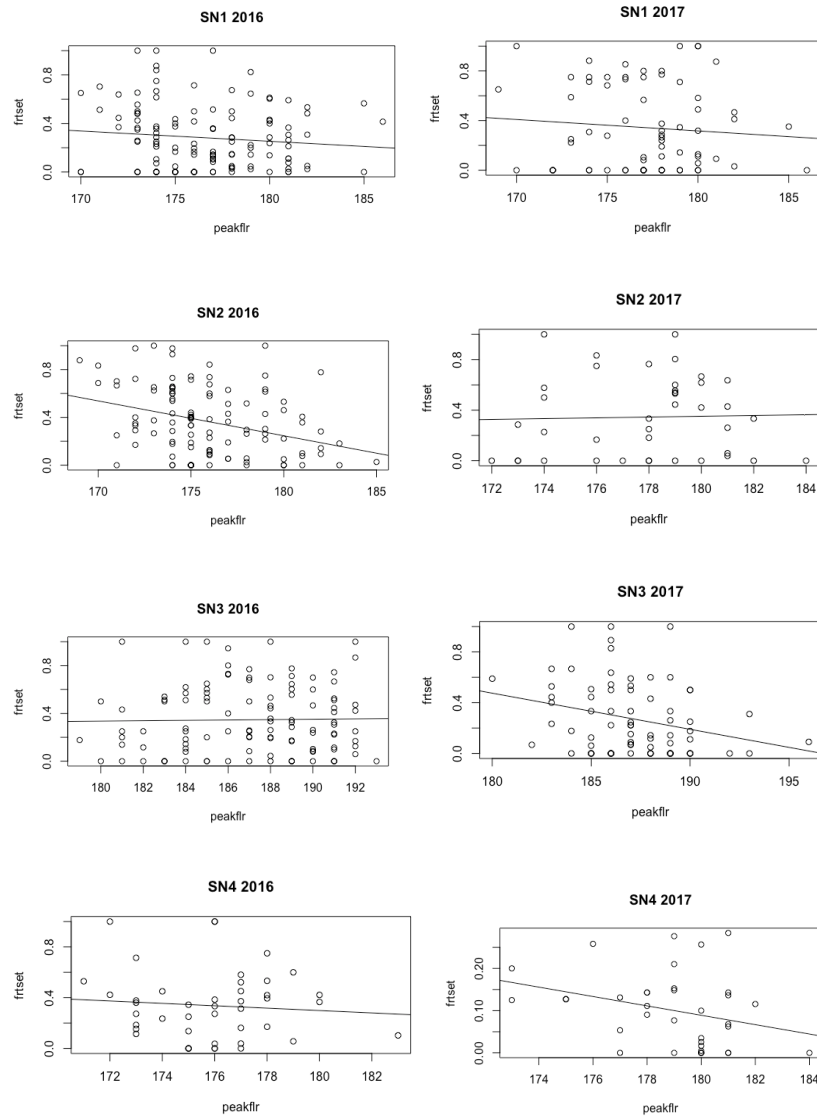
### *Fruit Set and Flowering Time*

We used a linear regression model with fruit set as the response variable and peak flowering time for both years and all four populations combined (see Table 1). In this model, fruit set was negatively correlated with peak flowering time; earlier flowering was associated with slightly higher fruit set (Fig. 3). But, if site is included in the model, there is no significant effect of peak flowering day on fruit set. If a regression is done on each site separately though, we see a significant relationship at SN2 in 2016 and SN3 and SN4 in 2017, in all cases showing that earlier flowering is beneficial (Figure 4). But, there were much weaker relationships for the remainder of sites.



**Figure 3.** Peak flowering day and fruit set for all individuals for both years that flowering data exists (2016 and 2017).  $p=0.001225$ ,  $r^2 = 0.0316$ .

Presumably, these variable results were because sites differ quite substantially in their peak flowering time. The correlation between flowering time and fruit set was difficult to tease apart without isolating site and year. Overall, earlier flowering tended to yield slightly higher fruit set, but, as suspected, differences at the population level and between years obscured the pattern; in some years, at some populations, the correlation is much stronger. Figure 4 shows peak flowering day and fruit set for each individual per year, separated by site, where the difference is more obvious between site and year.

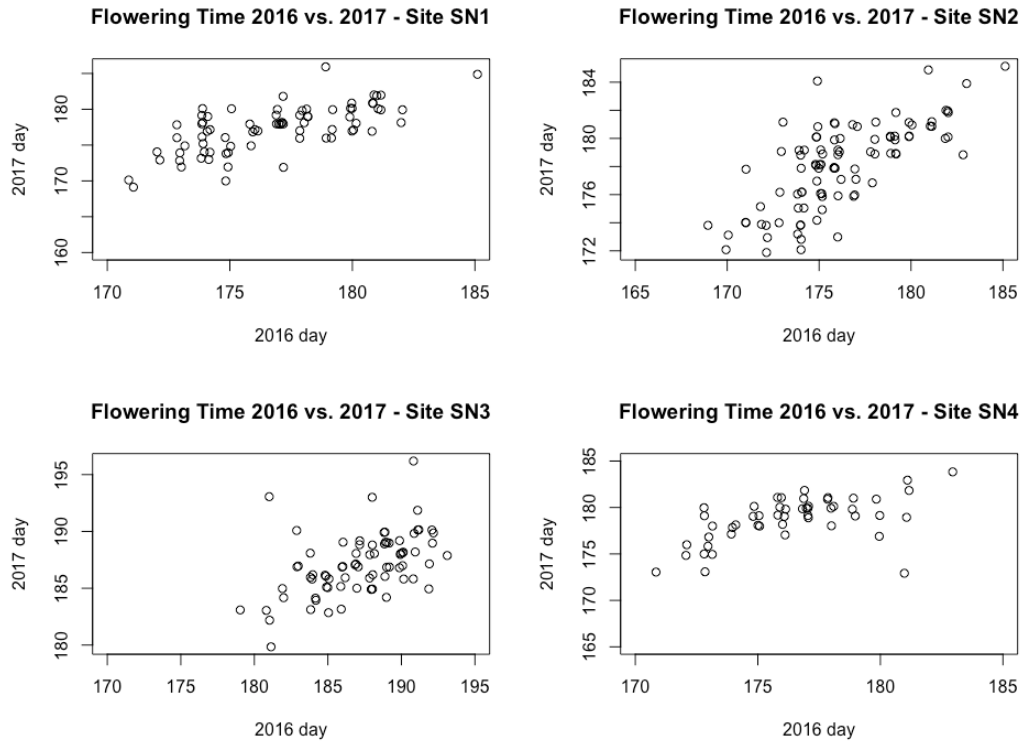


**Figure 4.** Fruit set and peak flowering time regressions separated by site and year. p-values, ordered by site then year:  $p = .2258, .004163^{***}, .8446, .5773, .4595, .8582, .019^{***}, .0688$ ;  $r^2$  values:  $.01191, .1075, .003445, .007457, .007956, .0008504, .07506, .09411$ .

### *Correlation in flowering time inter-annually*

One of the questions posed by this study was whether the same individual plants showed consistent performance from year to year. A component of this question is whether individuals flowered early or late in both seasons. At each site, individuals showed a strong correlation in flowering in the same order between years – the same individuals that flowered early in 2016

also flowered early in 2017, and same pattern goes for individuals that flowered during the peak time or later. Figure 5 shows the correlation between peak flowering time in 2016 and 2017, separated by site.



**Figure 5.** We used date of peak flower (measured as the day of the year that the majority of the flowers were open on the plant) and ran a Pearson’s product-moment correlation test in R to determine whether day of peak flower in 2016 was correlated with day of peak flower in 2017, with separate tests for each site.  $r^2$ , Degrees of freedom and p-values, in order of site, are:  $df=77$ ,  $p=5.164 \times 10^{-6}$ ,  $r^2=.2279$ ;  $df=93$ ,  $p=1.4 \times 10^{-11}$ ,  $r^2=.383$ ;  $df=74$ ,  $p=2.62 \times 10^{-6}$ ,  $r^2=.2494$ ;  $df=51$ ,  $p=.161 \times 10^{-2}$ ,  $r^2=.01928$ .

*Does flowering time influence long-term reproductive success?*

Since order of flowering is largely fixed for individuals, at least as far as we can determine with two years of data, we then asked whether mean flowering day between those two years was correlated with mean long-term (16 years) seed set. We used a linear model and found that mean peak flower day for the two years was correlated with average fruit set over the long-

term. Plants that flowered early tended to have more fruit per area over the long-term. However, this trend is somewhat confused by differences in each population's flowering time. SN3, for example, began flowering later and peak flower was ten days later than the any of the other sites, which may affect fruit set and be driving the pattern we're seeing (that plants who flower early tend to do better because of location). Presumably, SN3 flowers late nearly every year, so plant location may impact long-term reproductive success if late flowering is less beneficial.

Intercept	Area	Year	Peak Flower	Precipitation	Precipitation <sup>2</sup>	Mean July Temp	Peak Flower: Precipitation	Peak Flower: Mean July Temp	$\chi^2_{\text{residual}}$	$\chi^2_{\text{conditional}}$	AIC	Delta AIC
7.494 <sup>a</sup> -1	2.355 <sup>a</sup> ***	(categorical)***							.3210345	.5547145	63287.8	0.000
6.669	4.47 <sup>a</sup> ***			-1.002 <sup>a</sup> ***		-2.841 <sup>a</sup> ***			.1537231	.3650395	87621	24333
1.666	3.728 <sup>a</sup> ***			6.716 <sup>a</sup> ***	-3.904 <sup>a</sup> ***				.1256472	.3360300	89984.83	2363
2.866	3.988 <sup>a</sup> ***			-7.367 <sup>a</sup> ***					.1230728	.3325050	90287.94	303
2.6341	2.418 <sup>a</sup> ***					-8.627 <sup>a</sup> ***			.1046797	.3127408	94026.67	3739
1.022 <sup>a</sup>	3.628 <sup>a</sup> ***			-5.253 <sup>a</sup> ***	-9.925 <sup>a</sup> ***	-7.541***					122515.6	28488
1.022 <sup>a</sup>	3.628 <sup>a</sup> ***			-5.253 <sup>a</sup> ***	-9.925***	-7.541***	4.985 <sup>a</sup> ***	4.005 <sup>a</sup> ***			122516	400
6.391 <sup>a</sup>	3.642 <sup>a</sup> ***			3.484 <sup>a</sup> ***	-8.807 <sup>a</sup>	-2.770 <sup>a</sup> ***					124040	1524

**Table 1. Predictive Models of Fruit Set.** Linear mixed models (Poisson regressions) of fruit set were run on combinations of climate variables and fruit set. All models include individual plant as a random effect. Significance codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘.’

*Do some individuals consistently have higher fruit set?*

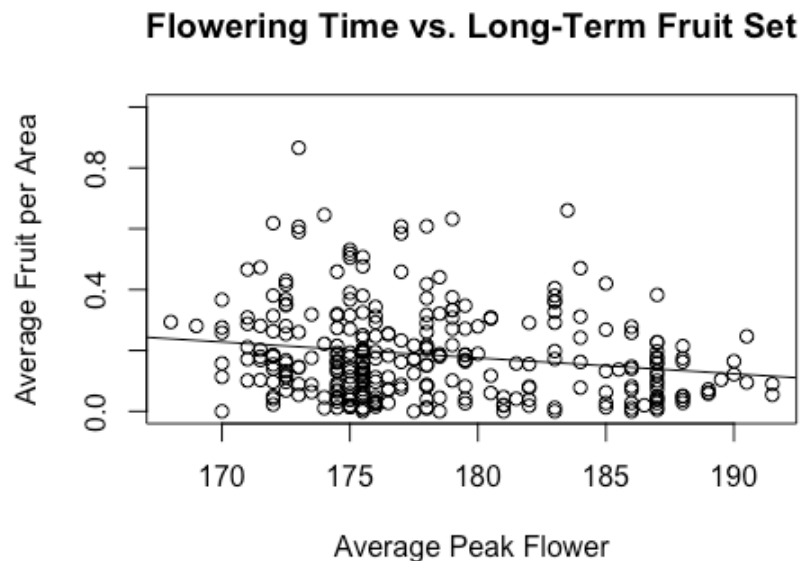
To address this question, we constructed mixed Poisson regression models in R that include individual performance as a random effect on fruit number. In these models the dependent variable is total fruit number, with plant area a fixed effect in all of the models. I ran several different models with individual plant as a random effect and fruit number as a response variable with combinations of year, precipitation, temperature, and peak flower day included. When peak flower day was included, I excluded plants that never flowered from the model. Additionally, when peak flower day was included in the model, I averaged peak day for the two years of available data, and used the mean peak flower day as an estimate for the peak flower day for that individual for all the sixteen years of long-term data. The justification here is that we found peak day 2016 and 2017 to be highly correlated amongst all individuals, as discussed in the previous section. All of the models in the table were generalized linear models with Poisson distributed error. Only females were used for these analyses, since fruit set is a complete measure of female reproductive success. Because hermaphrodites can also gain fitness through pollen dispersal, fruit set does not completely capture reproductive success year-to-year.

I constructed models that also included interaction terms for peak flower and climate variables. I found that the interaction of peak flower and mean July temperature as well as peak flower and precipitation were both highly significant, suggesting that earlier flowering is likely more beneficial for in years with certain weather, and less so for others.

I found that the best model (judged by AIC values) included summer precipitation and mean July temperature as fixed effects for each year. Both summer precipitation and mean July temperature were negatively correlated with fruit set, as shown in boxplots visualizing how fruit set varies with temperature and precipitation (Figures 8 and 9). In addition, individual random



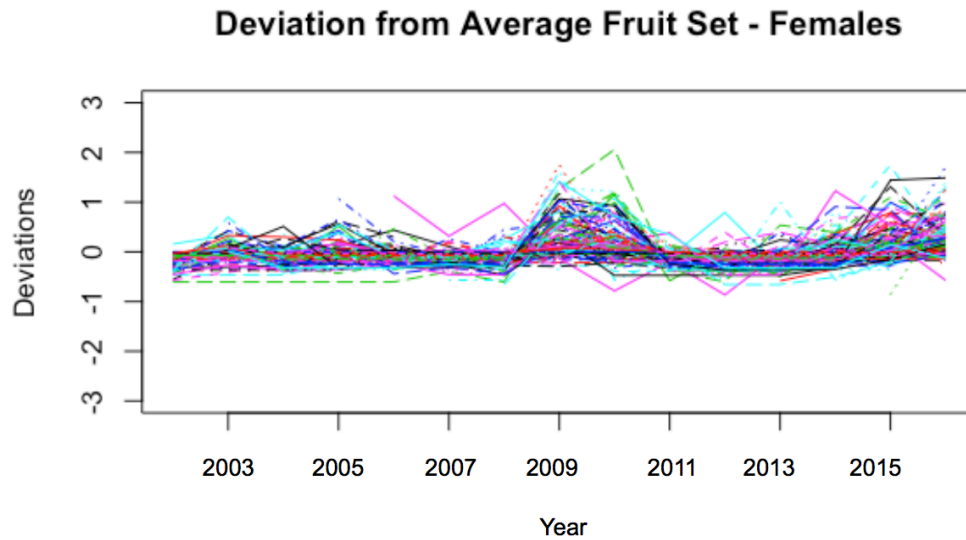
effects were significant in each model. The mean variance for the random effects was .16561 (see Table 1). The levels of significance can also be found in Table 1.



**Figure 6.** Average peak flower was calculated as the mean peak flowering day between 2016 and 2017 for each individual. We compared this mean peak flower day to mean fruit set for the last sixteen years.  $p=.001225$ ,  $df=295$ ,  $r^2=0.0377$ .

*Is inter-annual variation in reproduction correlated among individuals?*

I also wanted to investigate whether patterns in average fruit set were visible at the population level – in other words, were some years better or worse for the entire population as a whole? I calculated deviation from average fruit set from 2002-2017 for each individual (Fig. 7) and looked for patterns in these deviations across years.

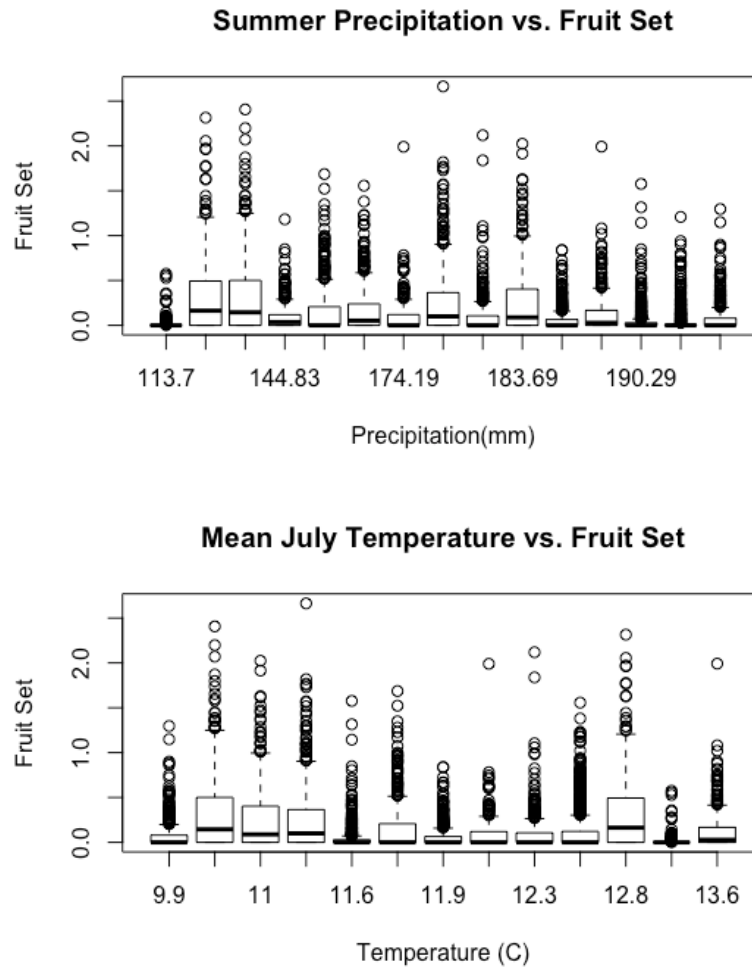


**Figure 7.** Fruit set was calculated as the number of fruit produced per area. This figure shows individual deviations from the average fruit set. Since we are using fruit set as a measure of reproductive success, only female cushions were included, since males can have significant reproductive fitness gain through pollen dispersal, which was not measured. We can see that some years, many of the plants did better than they usually do, but correlation is not exact.

From Figure 7, we see that deviations from average fruit set do occur on the population level. The deviation from average fruit set was calculated as a measure against each individual's average. In some years, many individuals do better than they usually do (years 2008-2010, for example). This is likely due to climate drivers, plentiful pollinator availability, or another external factor that wholly affects the population. Despite evidence that some years are "better" for many individuals, the correlation is not entirely consistent – there is still quite a bit of individual variation. The question of individual variability is addressed in the next section.

Additionally, this calculation does not provide insight into whether some individuals consistently outperform their population's average, since the deviations are weighed against each individual's average performance. The question of individual performance in relation to the population average was addressed in the prior section, where individual plants were included in the model as random effects.

Does climate affect fruit set?



**Figure 8 & 9.** Boxplots of Precipitation and Temperature from each year and the population fruit set. Both mean July temperature and summer precipitation were negatively correlated with fruit set ( $p_{Temp}=0.00068$ ,  $p_{Precip}=0.0012$ ). Boxplots were chosen because for each year, I had single estimates for precipitation and temperature for the entire population – only 16 values total for each, which was difficult to tease apart and visualize in a scatterplot.

### Portfolio Effect

Climate patterns such as precipitation and temperature have been shown to influence population fruit set. Individual variation in fruit set is also loosely correlated amongst individuals (there are some years in which many individuals do better or worse than they usually do).

Considering these factors, I wondered whether there was sufficient variation in response to these

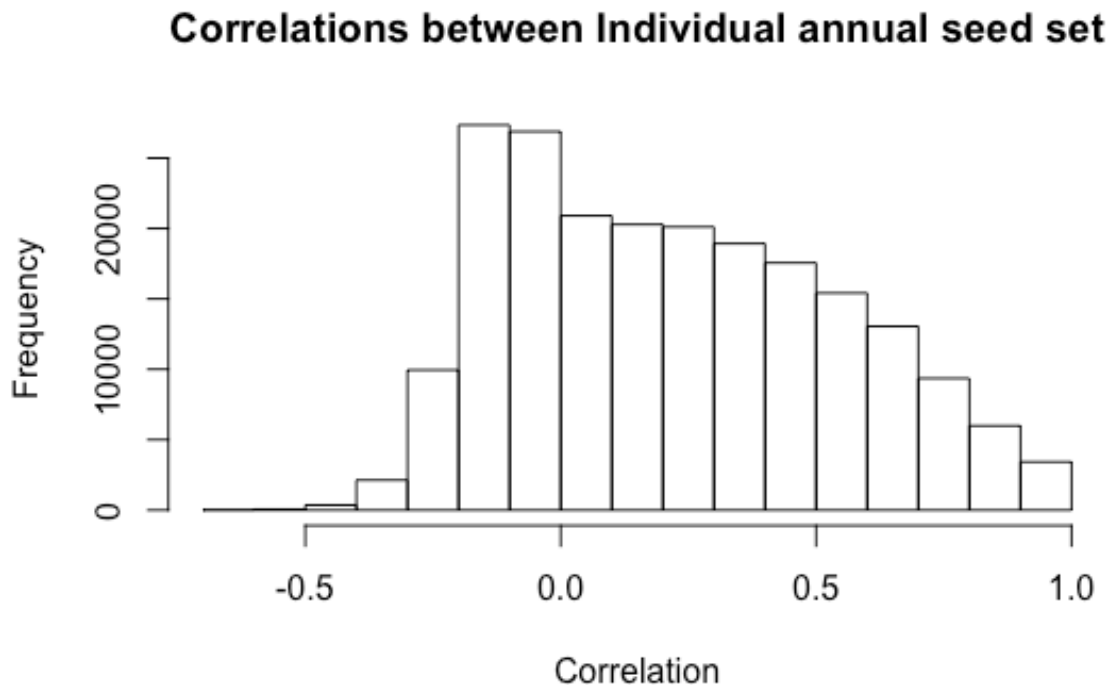
factors to “buffer” population fruit set fluctuation. A null model that estimates annual variation in fruit production if all plants were perfectly correlated in their annual variations was used to predict variation in fruit set at the population level for each site (Abbott et al. 2017), and we compared those values to actual variation seen.

<b>Site</b>	<b>Observed Standard Deviation/Predicted Standard Deviation</b>
SN1	0.5396398
SN2	0.6654962
SN3	0.7176194
SN4	0.8452818

**Table 2.** Observed SD / Predicted SD by site.

The observed variation in fruit set ranged from 54-85% of what would be expected with perfect correlation in variation between individuals. Thus, there is a substantial amount of variation in the way individuals respond to environmental variation that buffers annual population performance in response to current and presumably future climate fluctuation. I created a correlation matrix that generated correlation coefficients (for annual fruit set) for every pair of individuals. A correlation coefficient of zero would mean that the pair has no relation, a coefficient of one means that they are perfectly correlated, and a negative coefficient means that the two individuals are negatively correlated. I created a histogram of each coefficient to get an

idea of how tightly correlated individuals tended to be on average.



**Figure 10.** Histogram of correlation coefficients for each combination of individuals in the study.  $r=.41554$ .

Individual fruit set was weakly correlated across the population. Most individuals were weakly or barely correlated, and some were even negatively correlated. This means that individuals amongst a population differ in their response to external variables and are less correlated than we might suspect. This explains why the observed variation in fruit set is significantly less than we'd expect - correlation between individuals is not tightly bound. A favorable year for fruit production for one individual is an average or even poor year for another individual on the other end of the spectrum.

## Discussion

One motivating question for this study was whether and how flowering time phenology in *Silene acaulis* impacts reproductive success. We found that earlier peak flowering was correlated with higher fruit set for the entire population, but that this trend may be driven by the substantial differences in flowering time between the four sites. When flowering time and fruit set was compared for each site and separated by year, we found that earlier flowering was correlated with higher fruit set only in a few sites and years; in other sites, the trend was much weaker or non-existent. Because some sites flower later (presumably because they are under snow for longer), their “early” flowering time is late by comparison, but fruit set is not negatively affected. Overall, it’s still unclear from this data exactly how date of peak flower compared to an individual’s neighborhood or population affects their fruit set.

Since flowering time may influence seed set, I also investigated whether the same individuals of a population tend to flower sooner or later than their peers, and whether those individuals tend to perform better or worse as a result, using 16 years of fruiting data. One finding from this study was that the individuals within the population flowered in the same order both years, regardless of whether the peak day of the entire site shifted – the order of flowering in 2016 and 2017 was nearly perfectly correlated. Preservation of order of flowering, even if fruit set does not depend on peak flowering day at fine scales, is noteworthy. Potentially, individual microclimates are driving flowering time; one might imagine that the order in which snow melts off each plant is consistent year to year, and this drives flowering time. Additionally, there could be genetic factors that consistently drive a plant to flower earlier or later than average. I found that those earlier-flowering individuals in 2016 and 2017 had a higher average fruit set from 2002-2017, but again, that this may be driven by site differences.

Annual fruit set also showed strong response to climate factors; both July temperature and summer precipitation were negatively correlated with fruit set. PRISM Climate Data estimated precipitation and temperature across the entire range of our sites; however, in reality, each site has different localized weather, despite being so close in proximity. All above tree line, our sites on Niwot Ridge experience high winds throughout the year, and the populations near the top of the ridge consistently have snow blown off and into the valley onto other *Silene* populations. Appendix A1 shows the difference in number of days spent under snow between the four populations. SN3, in 2016, was buried under snow for nearly twice as many days as any other site. SN3 also began flowering later and peak flower was ten days later than the other sites, which may affect fruit set and be driving the pattern we're seeing (that plants who flower early do better). Regardless, the population as a whole produced more fruit during dry, cooler summers and responded negatively to hotter or wetter years. If climate patterns change on Niwot Ridge, reproductive success will presumably shift in response.

I found that peak flowering time in the 2016 and 2017 season was significantly correlated to long-term average seed set on an individual level. Earlier peak flowering time tended to be correlated with higher fruit sets in certain years. The implications of this are slightly unclear; perhaps plants that flower early attract pollinators who are already available without competition from the rest of the population. Additionally, earlier flowering is considered risky for many species, though in the case of *Silene*, which only flowers for less than two weeks, the risk of freezing may not be substantial enough to discourage flowering a week or so early. However, I did find an interaction between success in early flowering and climate variables, suggesting that in some years, risk of earlier flowering pays off, but in other years, it may not.

One question that this study did not have the data to address was whether date of flowering on the ridge is driven mostly by climate. Presumably, climate affects when *Silene* can begin flowering for the season. And, if earlier melting of snow occurs, perhaps *Silene* will respond and flower sooner, regardless of whether pollinators respond in the same way. Having detailed flowering time data for this population in the long-term would provide useful insight into how flowering time is affected by climate, and how that contributes to fruit production.

I also asked whether there was an observable portfolio effect buffering the variation in fruit set year to year on a population level. If this was true, I would have seen that variance in fruit set was less than I'd predict based on aggregate individual variances. I found that the actual variation in fruit set ranged from 54-85% of what I'd expect based on models estimating population behavior with perfectly correlated responses of all individuals; thus, there is a reasonable amount of buffering going on due to individual variation. Individuals tended to be weakly correlated with each other, with some pairs of individuals highly correlated and some not or even negatively correlated. In light of the fact that significant individual effects on fruit set were found, this finding makes sense. If individuals respond differentially, or even have individual genetics that influence seed set uniquely, some of the effects of variation in external factors that contribute to fruit set will be dampened on the population level.

A limitation with my study is the lack of direct observations or experiments about factors limiting reproduction. In particular I did not observe any pollinators; any changes in pollinator availability would likely have a remarkable effect on fruit set going forward. The cues that prompt flowering must be aligned with pollinator cues; if timing becomes mismatched, or if the number of pollinators declines, fruit set will likely decline regardless of favorable or unfavorable weather that year. Additionally, changes in factors that affect reproductive success are also



likely to affect survival, especially amongst young plants. Even though we see a buffering in fruit set, if seedlings do not respond differentially to changes in climate, we still would expect that this buffer would not aid in longer-term population resilience.

## Conclusion

Climate patterns are changing in unpredictable ways and the implications of these changes for alpine and arctic plants are unclear. For these populations to persist, they must continue to produce enough viable seeds. This study has shown that fruit set output on a population level is influenced by climate variables such as temperature and precipitation, as well as individual flowering time and just individual variation.

This study shows that *Silene acaulis* favors cool, dry years for reproduction, which may change in frequency as climate patterns change. I also found that earlier peak flowering influenced seed set for the years we recorded flowering phenology, but also in the long-term seed set of that individual. However, even if the entire neighborhood flowers early, I found that the order of flowering is still largely fixed. It's still unclear why earlier flowering would be linked to improved seed set, or whether the explanation is simply that individuals at sites that melt sooner do better (maybe the site is just better overall), and also just flower earlier because they can.

Though climate and flowering time affect population fruit set, I also showed that there is an observable portfolio effect that buffers the variation in fruit set. Overall fruit set is less variable than expected, which means that some of the variation in individual fruit set dampens the aggregate variation as individual response to driving factors varies. This is good news, as the changes in climate are still somewhat unpredictable at finer scales; if climate becomes more unfavorable to fruit production on Niwot Ridge, this buffer will help to stabilize population reproductive output.

The drivers of reproductive success are interconnected and difficult to tease apart. Further study on *Silene acaulis* and other alpine plant reproduction and population dynamics is needed to better predict how populations will respond to changes in climate and, potentially, pollinator

availability. Because we also found that individual effects contributed to fruit set, there may be a genetic component to reproductive success. Individuals that have consistently produced more fruit in our dataset in the past, however, may not necessarily be most fit to deal with changes in climate. Many factors contributing to reproductive success are still poorly understood, and the dynamics of these processes are subject to change in the future.

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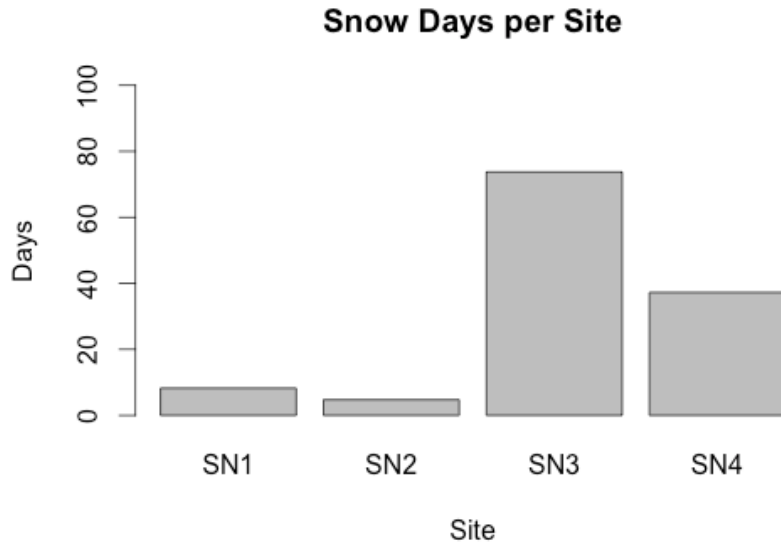
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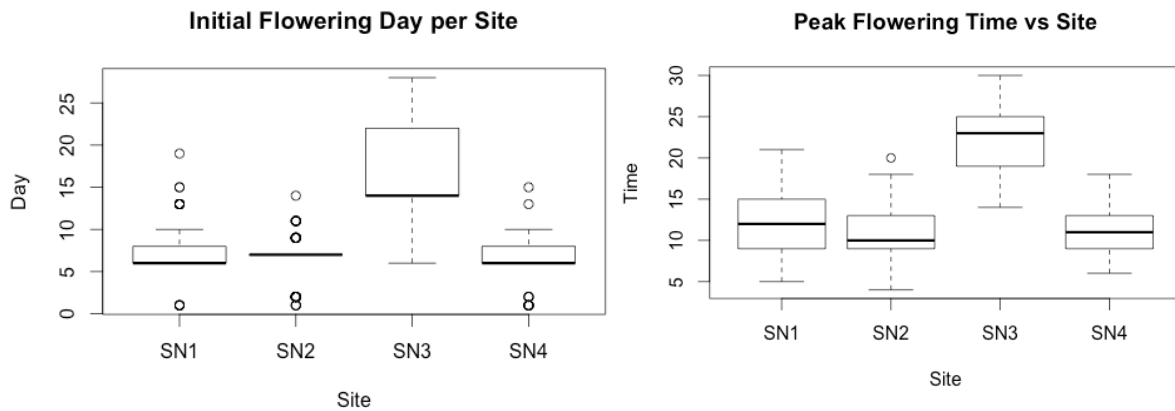
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## Appendix



**Figure A1.**

This figure shows snow days per site (the number of days that the site was buried in snow during the year 2016) across Niwot Ridge. We can see that ecological factors varied even across our four populations.



**Figure A2.** SN3 flowered, on average, nearly 8 days later than any of the other three sites. In the figure below, we can see that SN3 was buried under snow for much longer than any other site.

**Figure A3:** DF: 449; p-value <.0001. There was a significant relationship between peak flowering times and site. Peak flowering time was over a week later at SN3 than any other site.