





## Preface

This master thesis in ecology was written for the Department of Ecology and Natural Resource Management at the Norwegian University of Life Sciences, and is the completion of my five year education to become a science teacher. This study aims to contribute to the knowledge of evolution of reproduction systems and sexual dimorphism in alpine plant species.

I have gained knowledge of research and of how to work on a long-term project by planning a study, doing field work the summer of 2014, analysing data and writing this thesis.

I would like to thank my superb supervisor, Ørjan Totland (INA), for all the ideas, experience, excellent guidance and support throughout this process. I would also like to thank Johanne, my parents and the buzzard, Laura, for helping me out in the field and entertaining me during the long days at Midtdalsbreen, the people at Finse Alpine Research Center, Knut who helped me with the statistics, my breakfast club and the rest of my friends and roommates for making my time at Ås unforgettable.

Ås, May 2015

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## Abstract

The abundance of pollinators in alpine areas are scarce due to harsh and unstable weather conditions. This scarcity can lead to pollen limitation in unisexual plants and drive evolution towards self-compatible plants. Unisexual plants may have an advantage in favourable years if self-pollinating plants experience inbreeding depressions. Different dependence on pollinator visitation for different sexual morphs of a species can lead to sexual dimorphism in pollinator attracting traits, such as flower size. I did field studies and statistical analyses to investigate the predictions: I) Hermaphrodites will have larger flowers than females, II) visitation frequency will be higher on plants with large flowers and higher flower density, III) female flowers will have higher fruit production, and IV) pollen limitation is stronger in females than in hermaphrodites.

The field studies were done at Finse on Hardangervidda in Norway with three populations of *Silene acaulis* along an environmental gradient from 1300 to 1450 m.a.s.l. during June to August 2014. To study effects of plant and flower size, abundance of plants and sex on flower visitations and fruit production, 20 pairs consisting of one female and one hermaphrodite plant were observed. To quantify pollen limitation I performed a supplemental pollination experiment on 5-6 pairs in each of the three populations.

Hermaphrodites had 25.9 % larger flowers than females, and a higher pollinator visitation frequency. Visitation frequency was also higher in populations with high plant abundance and increased with flower size. Females had 4.2 times higher fruit set than hermaphrodites. Results from the supplemental pollination experiment show that hermaphrodites have a stronger pollen limitation, and increased significantly their mean seed production by 124 % after supplemental pollination, while females did not significantly increase their seed production after supplemental pollination.

These results show that there is a sexual dimorphism in *Silene acaulis* at Finse. I suggest that the difference in flower size and fruit set is because hermaphrodites are functionally males in this population, and have a higher reproductive output, by pollen dispersal, from increased pollinator visitation, and therefore have larger flowers than females. This gynodioecious population may be evolving towards dioecy, but it is also possible that seed production is higher in hermaphrodites, relative to females, in less favourable seasons than the summer of 2014.



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## Introduction

The species richness and abundance of pollinators decrease with altitude (Warren et al. 1988), and is naturally scarce in alpine areas (Arroyo et al. 1985; Larson & Barrett 2000; McCall & Primack 1992; Reid & Lortie 2012; Totland & Sottocornola 2001), due to the harsh conditions in alpine environments (Alatalo & Molau 2001) where the weather is unpredictable, windy and cold (Körner 2003; Molau 1993; Reid et al. 2014). Unstable access to pollinators may drive the evolution of self-compatible species and individuals, as a reproductive assurance (de Vos et al. 2012; Lazaro et al. 2015; Lloyd 1992; Reid et al. 2014; Zhang et al. 2008). Unisexual animal-pollinated plants without the ability to self-pollinate are completely dependent on pollinator visitation for seed production (Richards 1986), and pollinator visitations is positively correlated with fruit and seed production (Barrett 2010; Burd 1994; Lortie & Reid 2012). The presence of individuals of different sexual morphs within a population may be an evolutionary result of an unstable environment, where the ability to self-pollinate is important for reproductive assurance in stressful years and unisexual plant can have higher reproductive success in favourable years (Barrett 2003; Philipp 1980).

Pollinators usually prefer to visit large flowers over small (Campbell 1991; Eckhart 1992; Galen 1989; Galen 2000; Ågren et al. 1986), sometimes causing a higher reproductive success in large-flowered individuals (Galen 2000). Large flowers represent a resource investment, which may reduce resources available for survival, reproduction and growth (Galen 2000). Plants may evolve traits to improve pollinator attraction as an evolutionary response to pollen limitation (Ashman 2000), such as increased flower size (Ashman & Diefenderfer 2001; Lazaro et al. 2015; Wilson et al. 1994). The pollinator selectivity on different floral traits may affect the evolution of sexual dimorphism (Ashman 2000), if the attraction of pollinators is less important for reproductive success in one morph than in the other (Charnov & Bull 1986; Eckhart 1992; Shykoff 1992). According to Bateman's principle; male reproduction is limited by number of matings achieved (pollinator visitations in plants) while female reproduction is limited by resources (Burd 1994; Wilson et al. 1994), and this can drive sexual dimorphism in floral traits in dioecious and gynodioecious populations (Burd 1994).

Plants can be male, female or hermaphrodites, and while male and female are dependent on outcrossed pollination, many hermaphrodites have the possibility of self-pollination (Alatalo & Molau 2001; Keller & Schwaegerle 2006; Richards 1986; Shykoff 1992). Plants that self-pollinate must allocate resources to both male and female functions, while female and male



individuals can allocate these resources to either seeds or pollen, which may give them higher fitness (Delph et al. 1999). Outcrossing is often favoured since it prevents inbreeding depression (Barrett 2003; Dufay & Billard 2012; Lloyd 1975; Maurice et al. 1998; Shykoff 1988; Sun & Ganders 1986) and is an important process for evolution (Barrett 2010). Hermaphrodites with the ability to self-pollinate often have mechanisms promoting outcrossing (Barrett 2002; Barrett 2003; Bernasconi et al. 2009). These mechanisms may lead to different reproductive strategies in plant species (Richards 1986).

Gynodioecy (i.e. the coexistence of female and hermaphrodite individuals) is a common breeding system in the arctic and alpine (Hermanutz & Innes 1994; Molau & Prentice 1992). Females in gynodioecious species often have a significantly higher reproductive output, as estimated by fruit (Delph & Carroll 2001; Lortie & Reid 2012; Maurice et al. 1998; Reid et al. 2014) and seed production (Keller & Schwaegerle 2006; Shykoff 1988) than hermaphrodites. This is important for persistence of female plants in a gynodioecious population (Charlesworth 1981; Dufay & Billard 2012; Lloyd 1975; Sun & Ganders 1986), because the hermaphrodites provide one-half of the genetic material through pollen dispersal (Charnov 1982; Reid et al. 2014).

In this thesis I study the cushion forming plant *Silene acaulis*. This is a common perennial species in arctic and alpine areas of Eurasia and North America (Lid & Lid 2005). *Silene acaulis* populations can have males, females and hermaphrodite individuals and is gynodioecious in my study area (Alatalo & Totland 1997; Lid & Lid 2005). Hermaphrodites have the availability to self-pollinate (Shykoff 1988), and fruit production in *S. acaulis* is partly limited by resource availability (Alatalo & Little 2014) and may be strongly limited by pollen availability (Alatalo & Molau 2001).

I studied the effects flower size and number of flower visitors had on the female reproductive success of hermaphrodites and females in *Silene acaulis*; which factors influence pollinator visitation; and how pollen limitation differ between the two morphs, along an environmental stress gradient from a low alpine site (1300 m elevation), to a high alpine site (1450 m elevation) at Finse in south-alpine Norway.

I predicted that:

- I. Hermaphrodites will have larger flowers than females, due to an evolutionary process where hermaphrodites can have many more offspring through their male functions than they can through their female functions, whilst females have a limited number of ovules that can mature to seed. Thus, there is a stronger phenotypic selection on flower size in hermaphrodites than in females to attract more pollinators and sire a larger number of seeds.
- II. Flower visitation frequency is higher in populations with a high abundance of plants, and on plants with large flowers, because there are more insect where the flowering plants is easy to locate and large flowers are easier to detect and may produce more nectar.
- III. Female plants have a higher fruit set than hermaphrodites, since female plants must have a reproductive advantage to persist in a gynodioecious population.
- IV. Pollen limitation is stronger in the female plants than in the hermaphrodite plants, since hermaphrodites have the ability to self-pollinate.

### Study area

My study was conducted between Finsevatn and Midtdalsbreen at Finse in southern Norway (60° 34' North and 7° 28' East) (Figure 1), during July and August 2014. Finse is a mid-alpine area at the mountain plateau of Hardangervidda and the three study populations were at altitudes from 1300 – 1450 masl. The mean July temperature at Finse (from 1961-1990) is 7.0°C, and the mean temperature in July 2014 was 12.1°C (Nowegian Meterological Institute 2014). Thus, the period when I preformed the experiments were warmer than usual. The mean annual precipitation is 1030 mm and most of it falls as snow (Nowegian Meterological Institute 2014). The precipitation in July 2014 was 90.0 mm, the July-normal for 1961-1990 is 85.0 mm (Nowegian Meterological Institute 2014). The abundance of *Silene acaulis* was high in all the three populations.

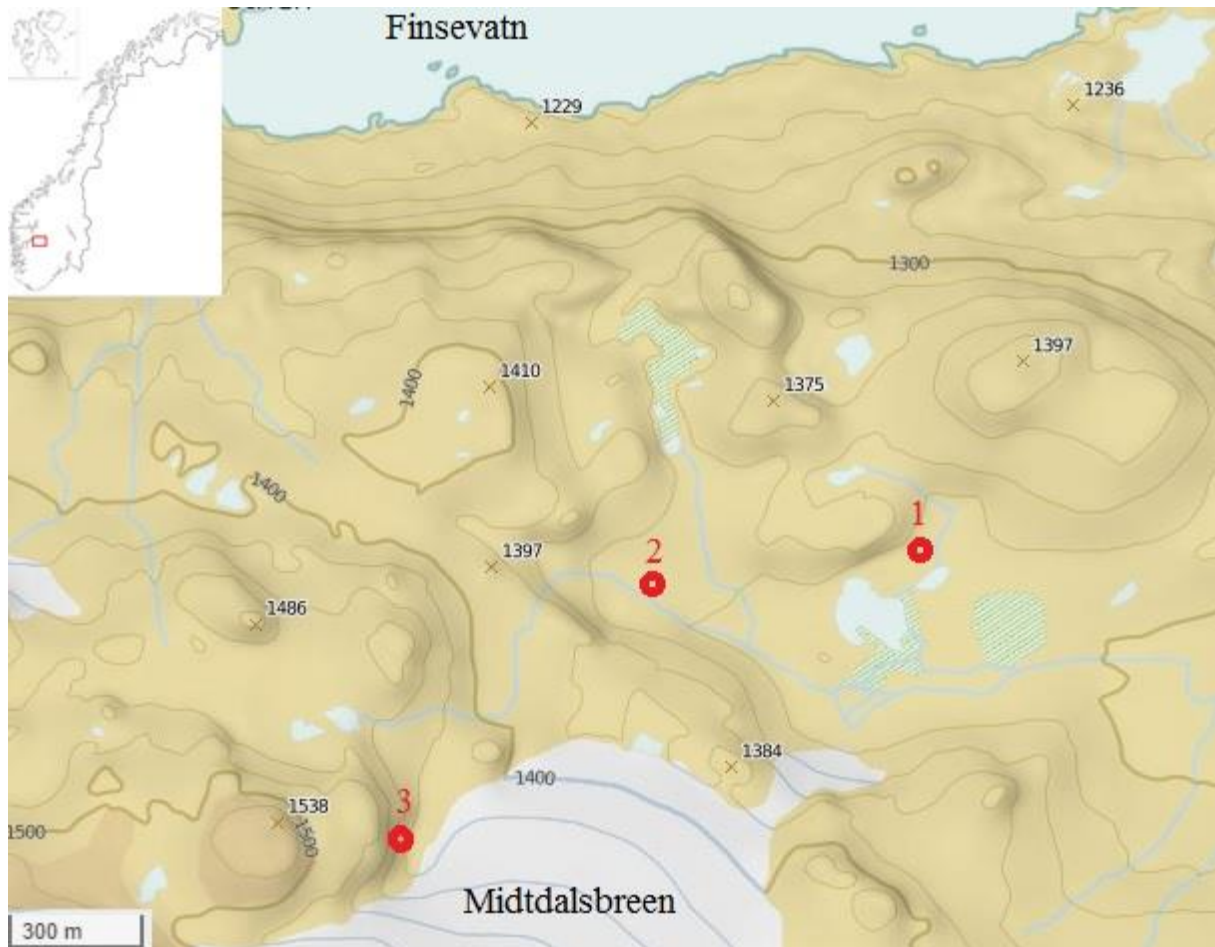


Figure 1) Map of the three populations. The red square in the map of Norway indicates the location of Hardangervidda.

#### Population 1

Population 1 was on a wind exposed, slight east facing slope, 1300 m.a.s.l.. The soil gets its moisture from rain. The area is characterized by large boulders, a thin layer of moraine materials (Geological survey of Norway 2015) (appendix II), and the dominant plant species *Salix lapponum*. This population had 0.29 individuals of *Silene acaulis* per square meter.

#### Population 2

Population 2 was on a slight east-facing slope, 1350 m.a.s.l., near a small glacier-melting river and the ground was covered by eskers (Geological survey of Norway 2015). The soil is moist during most of the season, due to constant melting of snow upslope. The main plant species except *Silene acaulis* was mosses and lichens. This population was more sheltered from wind than population 1, and had the highest density of *Silene acaulis* with 1.32 individuals per square meter.

### Population 3

Population 3 was on a wind-exposed steep north-east facing slope, 1450 m.a.s.l., and is situated ca 150 m east of the glacier outlet Midtdalsbreen. The slope was covered by a thick unstable layer of moraine materials (Geological survey of Norway 2015). The vegetation was scarce with some mosses, lichens and grasses, and this was the driest site of the three populations. This population had the lowest density of *Silene acaulis* with 0.22 individuals per square meter.

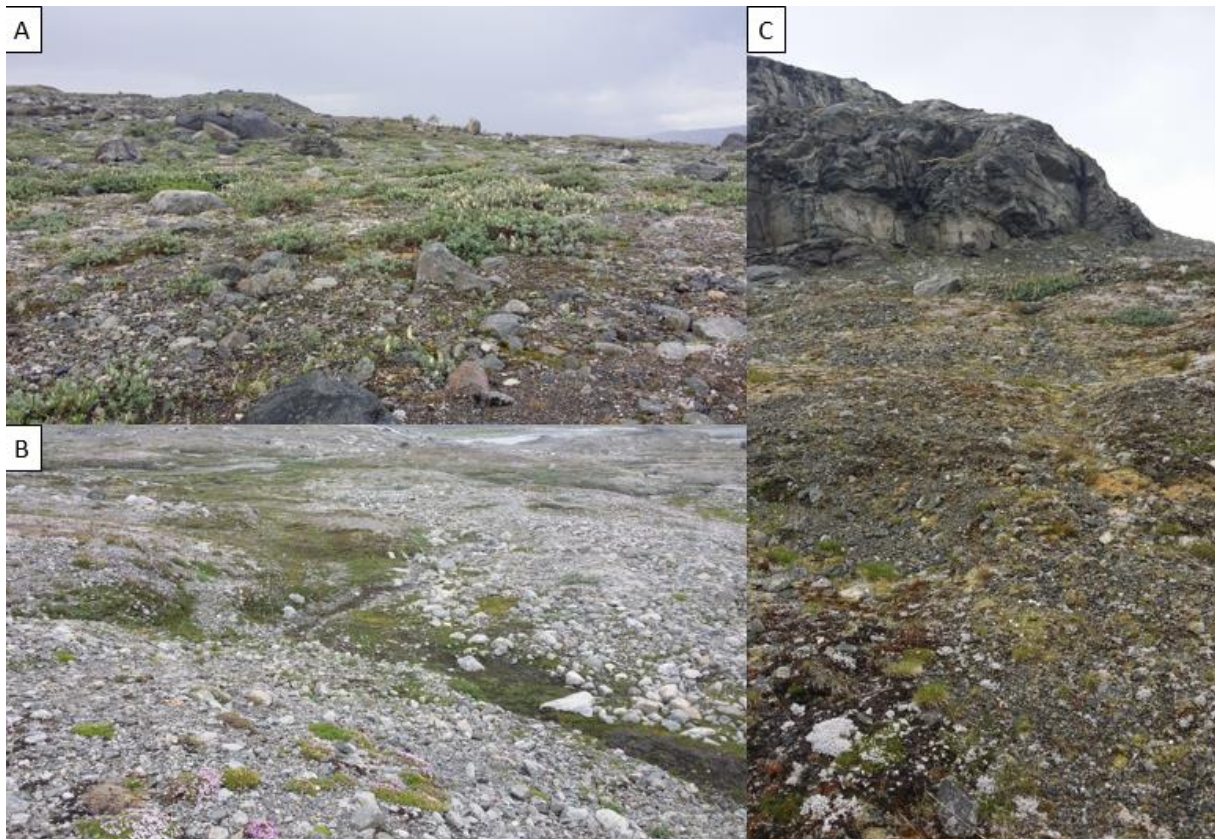


Figure 2) Photographs of A) population 1 facing north, B) population 2 facing east and C) population 3 facing south-west.

### Study species

*Silene acaulis* L. Jacq. (Caryophyllaceae) is a long-lived perennial evergreen cushion plant common in North-America and Europe (Keller & Schwaegerle 2006; Lid & Lid 2005; Reid et al. 2014), and have an almost circumpolar distribution (Chernov 1988). It has narrow leaves with rigid hairs at the tip (Lid & Lid 2005). Each individual has a single and strong taproot (Morris & Doak 1998). The flowers have five petals that are usually pink, but sometimes white (Lid & Lid 2005), and have a yellow nectar band at the petal base (Swales 1979). The flowers are open, with a nectar-tube and nectar producing glands at the stamen base (Jürgens et al. 1996) and have a sweet smell which is attractive to visitors (Jones & Richards 1962).

Plants flowers from early until mid-summer (Jones & Richards 1962; Lid & Lid 2005), each flower blooms for approximately one week, and this does not differ between sexual morphs (Shykoff 1988). The gender expression of *S. acaulis* is highly variable and range from monoecious (Warming 1920), andromonoecious (Warming 1920), dioecious (Desfeux et al. 1996; Gleason & Cronquist 1963; Warming 1920), trioecious (Hermanutz & Innes 1994; Müller 1883) and gynodioecious (Keller & Schwaegerle 2006; Lid & Lid 2005; Shykoff 1988; Såstad 1991). There is no clonal reproduction (Morris & Doak 1998), but hermaphrodites have the availability to self-pollinate (Shykoff 1988; Shykoff 1992).

The styles and papillae in hermaphrodites vary in length, and are often shorter than female styles and papillae (Hermanutz & Innes 1994; Jones & Richards 1962; Shykoff 1992). Style length may affect the seed production (Shykoff 1992). Hermaphrodites starts the male phase first, and enters the female phase after pollen dispersal (Alatalo & Totland 1997; Jones & Richards 1962). The flowers have approximately 30 ovules per ovary in both female and hermaphrodite flowers (Shykoff 1988).

*Silene acaulis* grows in snow beds and tundra (Lid & Lid 2005), and on wind-exposed ridges, open grassland and rocky slopes (Larcher et al. 2010), mainly on alkaline ground (Lid & Lid 2005). The flowers are visited by bumblebees, moths, flies, beetles, butterflies and ants (Reid et al. 2014), and dipteran species are the most important visitor on *S. acaulis* at Finse (Totland 1993).



Figure 3) Photographs of A) *Silene acaulis* with a *Bombus alpinus* visitor and B) close-up of a hermaphrodite with a *Zygaena exulans* visitor.

## Method

In order to examine if flower size, fruit set and flower visitation differed between the two morphs and the three populations I chose 20 pairs of *Silene acaulis* plants in each of the three populations. Each pair consisted of a female and a hermaphrodite plant. The pairs were selected by lining up a 30 meter measuring band and choosing the nearest female and hermaphrodite to every 3 meter along the line. The gender of the plants was identified with females having sterile stamens and hermaphrodites having pistils and stamens with pollen. In some pairs the two plants had a less than three meters to the next pair, and in some pairs the two plants were further apart than three meters, due to the patchy occurrence of *S. acaulis* in the populations. I marked the hermaphrodites with blue, numbered sticks and the females with orange, numbered sticks. I do not think that the different colours affected the pollinator visitations.

## Flower size

I measured flower size at approximately mid-flowering of each plant by measuring the longer diameter across petals (corolla diameter). I measured the size of 10 flowers on each plant, situated along a line from one edge over the centre of the plant to the other edge (following the diameter line of a plant). I measured all the flowers on the plant if the plant had 10 or less open flowers.

## Statistical analyses for flower size

I used the mean flower size of ten flowers for each plant and the mean of all flowers on the plant that had less than 10 flowers. I used a general linear ANOVA model to examine if flower size differed between the two morphs and among the three populations, if the interaction between population and morph explained the difference, and included plant size as a continuous variable. Pairs nested within populations was included in the models to account for any variation in flower size caused by the spatial position of plants.

## Plant size

In order to account for any effect of plant size on flower size, I measured the size of each plant by measuring the longest surface diameter and the diameter perpendicular to this. The area of plants (plant size) was calculated using the formula for an ellipse; as follows

$$Area = \frac{a}{2} \cdot \frac{b}{2} \cdot \pi$$

, where  $a$  is the longest diameter and  $b$  is the perpendicular diameter to  $a$ .

Plant size was used to determine flower abundance on each plant by dividing number of open flowers by plant size at the time of registration for effects on flower visitations, and the total number of flowers on each plant divided by plant size for the effects on fruit production.

### Flower visitation

In order to examine if flower visitation activity differed between morphs and populations, I measured flower visitation activity on the selected plants by observing one pair at the time, for five minute periods and counting the flower visitors that visited one or more of the open flowers during this period. The visitors were classified as flies, butterflies, bumblebees or as other. Each plant in population 1 was observed five times, in population 2 six times, and in population 3 four times. The last observation was done when more than 50 % of the plants had less than two flowers.

I noted the time of day when each observation started. I measured wind speed by holding an anemometer approximately 1 m above ground for the whole period. Light conditions was noted as sun, no sun, or partly sunny during the observation period. I measured temperature in the shadow approximately 5 cm above ground, close to the plant. Finally, I counted the number of open flowers on the plant after each observation period.

### Statistical analyses for flower visitation

I used the link function of square root to get a better distribution of the residuals than the link function of log gave to test which predictors determined visitation frequency (number of visitors in a five minute interval), The number of visitors in a time period is a counting variable and therefore I used Poisson regression analyses to test if visitation frequency was related to the categorical predictors: light, morph and population and the continuous predictors: flower density, number of flowers, number of nearby plants, flower size, time of day, wind and temperature. I used a Tukey post-hoc test to find significant differences between groups.

## Fruit production

In order to examine if fruit set is determined by morph, population, flower abundance on plant size I counted the number of fruits on the plant two weeks after the majority of flowers on individual plants had withered. I express reproductive success as the total number of fruits produced by the plant divided by its total number of flowers. I estimated the total number of flowers by counting the number of buds, open flowers and withered flowers at peak flowering of each plant.

I registered the abundance of the two morphs in all three populations, to account for effects on visitation frequency. To quantify the abundance of *Silene acaulis* plants in the populations I counted all plants in a 2 meter radius around each of the plants. These were registered as female, hermaphrodites or, in case of plants without flowers or with only buds, as unknown.

## Statistical analyses for fruit production

I calculated the values for fruit set by dividing number of fruits by number of flowers. I transformed the values of 0 and 1, to get a better normal distribution of the residuals for the analysis of fruit set, by using the formulas:

$$0 = \frac{1}{4n} \text{ and } 1 = 1 - \frac{1}{4n}$$

, where  $n$  is the number of observations. This was done to prevent the numbers 0 and 1 when I transformed all values for fruit set with the formula:

$$\text{asin}(\sqrt{x})$$

, where  $x$  is the value of fruit set.

I used a general linear ANOVA model to analyse the fruit set on the plants. This was done with morph and population as fixed factors, pair nested within population as a random factor, and flower density on each plant, mean visit and flower size as continuous covariates. I used a Tukey post-hoc test to find significant differences between groups.

## Supplemental pollination experiment

To examine if seed and fruit production were limited by pollen availability, and if pollen limitation differed between morphs and populations, I conducted a supplemental pollination experiment. I chose 5-6 pairs, consisting of one females and one hermaphrodite plant, as close as possible, in each of the three populations I used for the other studies. On each of the plants



I selected 3 pairs of flowers to receive only natural pollination (control) or natural and additional pollen (supplemental pollination), where the flowers in each pair were as similar as possible with regard to distance from the edge, size and sun conditions. On plants with few flowers (less than 25 flowers), these three pairs were marked and hand pollinated the same days. On plants with more than 25 flowers the three pairs were marked and supplementally pollinated on different days. This was done approximately mid-season, and most of the flowers were in bloom at the same time, due to the short blooming in the warm weather.

The control flowers were marked with green pieces of plastic drinking straw around the peduncle because I assumed that this would have the least effect on pollinators, whereas the supplementally pollinated flowers were marked with blue straw because it would not matter if this affected the pollinators, since the supplemental pollinating would saturate the pollen need for the flower. I collected pollen-laden anthers from plants situated 2-10 meters away from the plant in the experiment, and applied this pollen to the three stigmatic lobes of the flowers registered to receive supplemental pollen on the same day, by directly daubing the anthers onto the mature stigmas of the flowers. Mature pollen and stigmas was determined by using a hand-held lens. The mature pollen was light yellow with visible grains and the mature stigmas had tiny papillas. The supplemental pollination on each flower was done twice with one day between, except for two plants where rainy weather caused me to conduct the second pollination two days after the first pollination. I counted the total number of buds, open and withered flowers when I pollinated the plants the first time. I collected fruits approximately two weeks after the supplemental pollination, when they were close to mature but not yet dehiscent, and placed them in individual envelopes and counted the total number of fruits on the plant. The collected fruits were opened under a dissecting microscope and the mature seeds were counted.



*Figure 4) Photograph of a fruit capsule and mature seeds from Silene acaulis.*

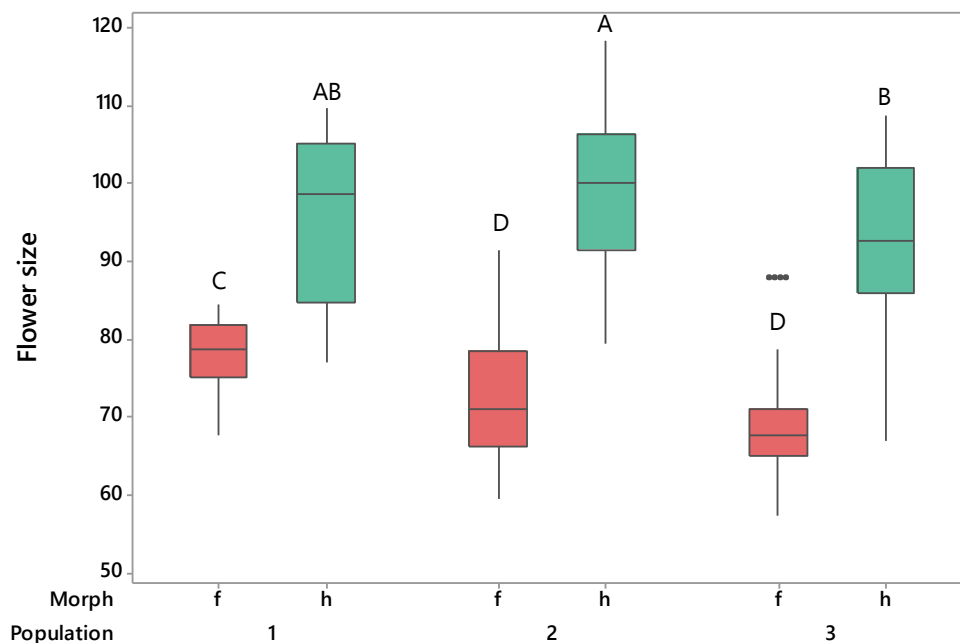
## Statistical analysis for the supplemental pollination experiment

I used a log transformation to get a better normal distribution of the residuals for the analysis of seed production in the supplemental pollination experiment. I used a general linear ANOVA model to investigate the factors and covariates affecting the seed production on in this experiment. This was done with the fixed factors: morph, population, experiment and the interaction between experiment and morph, and the covariates: flower density, fruit set and mean flower size.

## Results

### Flower size

ANOVA showed that hermaphrodites had significantly larger flowers than females (Table 1). The mean flower size (as measured in corolla diameter) for hermaphrodites in population 1, 2 and 3 were respectively  $97.14 \pm 2.3$  mm,  $99.32 \pm 1.9$  mm and  $92.56 \pm 3.9$  mm, and for female plants the mean in the three populations were  $77.84 \pm 1.1$  mm,  $72.7 \pm 1.3$  and  $70.07 \pm 2.8$  mm (mean  $\pm$  95 % confidence interval). Plants in population 3 had the smallest flower size, and the largest female flowers were in population 1 and hermaphrodites in population 2 (Figure 5).



*Figure 5) Boxplot of flower size of Silene acaulis (corolla diameter in mm) of the different morphs, female (f) and hermaphrodites (h), in the different populations (1, 2 and 3). Where the boxes which share a letter are not significantly different on a 95 % confidence level. The stars above boxes are outliers.*

Plant size, population, pairs nested in population and the interaction between morph and population was also significant factors explaining the difference in flower size. The sources tested in this model have an adjusted R<sup>2</sup>-value of 0.87, and explains much of the variation of flower size. Residuals are shown in appendix I (Figure 11).

*Table 1) Analysis of variance of flower size of Silene acaulis of a general linear model tested on morph, population, plant size and the interaction between morph and population. Numbers in bold are significant on a 95 % confidence interval.*

<b>Source</b>	<b>df</b>	<b>Adj SS</b>	<b>Adj MS</b>	<b>F-value</b>	<b>P-value</b>
Morph	1	38711	38710.5	1299.88	<< <b>0.01</b>
Population	2	3653	1826.6	6.02	< <b>0.01</b> *
Par(population)	52	23657	454.9	15.28	<< <b>0.01</b>
Morph*population	2	1513	756.5	25.40	<< <b>0.01</b>
Plant size	1	845	84 <sup>o</sup>	28.38	<< <b>0.01</b>
<b>Error</b>	438	13044	34.2		
Lack-of-fit	37	13044	377.2		
Pure error	401	0	0.0		
Total	496	111074			

R<sup>2</sup> = 88.26 % (R<sup>2</sup>adjusted = 86.70 %)

\* Not an exact F-test

*Table 2) Coefficients of flower size of Silene acaulis of a general linear model tested on morph, population, plant size and the interaction between morph and population.*

<b>Term</b>	<b>Coef</b>	<b>SE Coef</b>	<b>T-value</b>	<b>VIF</b>	<b>P-value</b>
Constant	81.343	0.687	118.36		<<0.01
Morph					
f	-11.205	0.311	-36.05	1.61	<<0.01
Population					
1	4.270	0.424	10.07	1.49	<<0.01
2	-0.027	0.432	-0.06	1.81	0.95
Morph*population					
1 f	1.771	0.412	4.30	1.46	<<0.01
2 f	-2.304	0.375	-6.15	1.56	<<0.01
Plant size	0.015	0.003	5.33	5.03	<<0.01

## Morph abundance

There were highest abundance of *Silene acaulis* individuals, in a 2 meter radius around the plants, in population 2 (Table 3). The highest percentage of females were in population 3 and lowest in population 1 and the highest percentage of hermaphrodites were in population 1 and lowest in population 2 (Table 3).

Table 3) the percentage of female, hermaphrodites and unknown plants in the three populations. The total number of plants in a 2 meter radius around each of the 120 plants in the field work.

Population	Total number of plants	Female	Hermaphrodites	Unknown
1	187	37 %	52 %	11 %
2	1005	44 %	40 %	16 %
3	139	50 %	46 %	4 %

### Flower visitation

The visitation frequency increased with number of flowers on the plant in both hermaphrodites and females (Figure 6) with p-values  $\ll 0.01$  and  $R^2$ -adjusted at 0.26 for hermaphrodites and 0.20 for females.

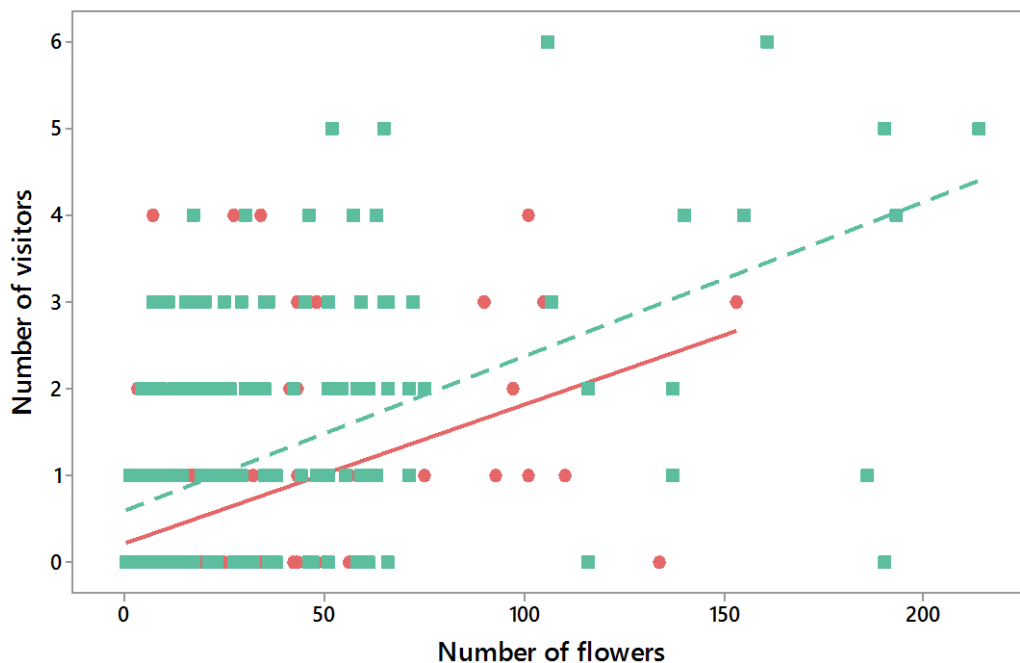


Figure 6) Scatterplot of insect visitors on *Silene acaulis* to number of flowers on the plant where green squares are hermaphrodites and red circles are female plants. Both with regression line.

Temperature, flower density, total number of flowers on plants, population and morph significantly explained variation in visitation density (Table 4). The abundance of nearby plants, wind, time of day, flower size and light conditions were not significant predictors for visitation density in the observation period. Nearby plants and population are correlated (Table 5). The adjusted  $R^2$  value for this analysis was 0.32. The residuals are shown in appendix I (Figure 13).

Table 4) Deviance table of a Poisson regression analysis on the number of visitors on *Silene acaulis* tested on flower density on the plant, nearby plants in a one meter radius, wind, temperature, number of flowers, mean flower size, morph and the three populations. Numbers in bold are significant on a 95 % confidence interval.

Source	df	Adj dev	Adj mean	$\chi^2$	P-value
Regression	12	245.40	245.40	245.40	<<0.01
Morph	1	17.25	17.25	17.25	<<0.01
Population	2	3.85	1.92	3.85	0.15
Flower density	1	2.44	2.43	2.43	0.12
Nearby plants	1	0.29	0.29	0.29	0.59
Wind	1	3.42	3.42	3.42	0.06
Temperature	1	8.59	8.59	8.59	<0.01
Number of flowers	1	124.49	124.49	124.49	<<0.01
Time of day	1	2.34	2.34	2.34	0.13
Flower size	1	2.39	2.39	2.39	0.12
Light	2	5.73	2.86	5.73	0.06
<b>Error</b>	459	495.75	1.08		
<b>Total</b>	471	741.16			

R<sup>2</sup> = 33.11 % (R<sup>2</sup>adjusted = 31.49 %)

Table 5) Coefficients of a Poisson regression analysis on the number of visitors on *Silene acaulis* tested on flower density on the plant, nearby plants in a one meter radius, wind, temperature, number of flowers, mean flower size, morph and the three populations.

Term	Coef	SE Coef	VIF
Constant	0.57	0.29	
Morph			
h	0.33	0.08	3.07
Population			
2	-0.21	0.11	5.16
3	-0.02	0.09	1.83
Flower density	0.25	0.16	1.16
Nearby plants	0.00	0.00	4.41
Wind	-0.04	0.02	1.28
Temperature	0.03	0.01	2.03
Number of flowers	0.01	0.00	1.38
Time of day	0.42	0.28	1.25
Flower size	0.00	0.00	3.17
Light			
yes	0.16	0.07	1.77
no	0.07	0.07	2.10

The most common visitor in all three populations was flies with 77-95 % of the visitations (Table 6). Population 2 had the highest percentage of bumblebees where this group amounted to 20 % of the visitors (Table 6) and population 3 had the highest percentage of butterflies

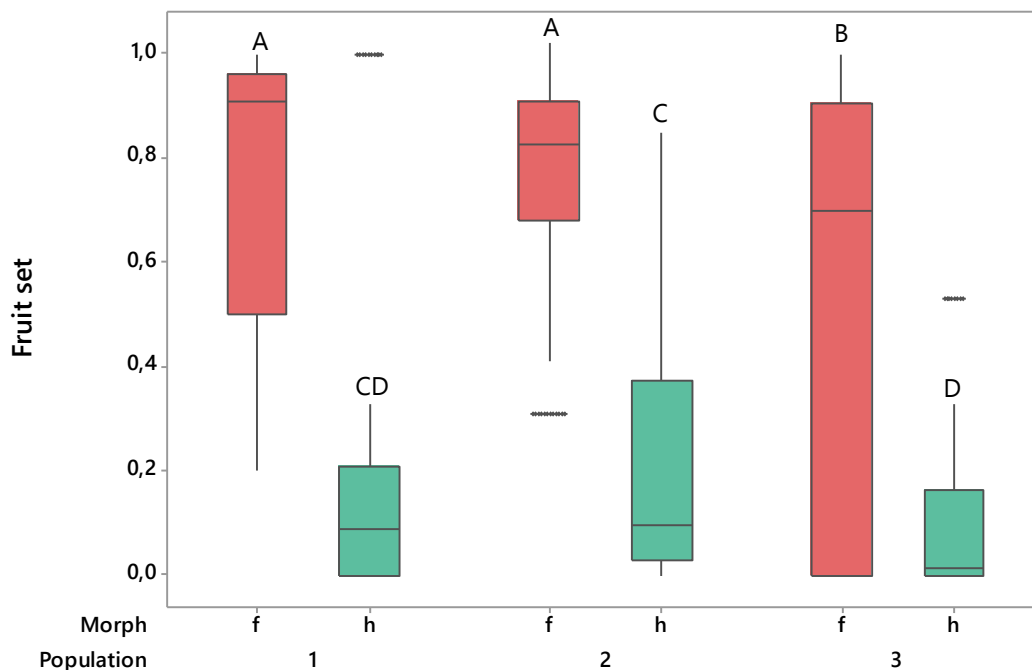
where 21 % of the visitors were butterflies (Table 6). The total number of visitors was highest in population 2 and lowest in population 3 (Table 6).

*Table 6) Visitors on Silene acaulis in the three populations. Total number of visitors for all observations, mean number of visitors per observation round and percentage of flies, butterflies and bumblebee visitations.*

Population	Total number of visitors	Mean number of visitors	Flies	Butterflies	Bumblebees
1	134	27	95 %	4 %	1 %
2	192	32	78 %	1 %	21 %
3	44	11	77 %	20 %	2 %

### Fruit production

Female plants set significantly more fruit than hermaphrodites (Figure 7, Table 7). The mean fruit set for females in population 1, 2 and 3 were respectively  $0.77 \pm 0.05$ ,  $0.77 \pm 0.03$  and  $0.55 \pm 0.09$  and for hermaphrodites in the three populations mean fruit set were  $0.17 \pm 0.05$ ,  $0.23 \pm 0.05$  and  $0.10 \pm 0.03$  (mean  $\pm$  95 % confidence interval). Population 3 had the lowest fruit set of the populations (Figure 7).



*Figure 7) Boxplot of fruit set in Silene acaulis (total number of fruits/total number of flowers) of female (f) and hermaphrodites (h) in population 1, 2 and 3. Boxes that share a letter are not significantly different at a 95 % confidence level. The stars over and under the boxes are outliers.*

Fruit set increased significantly with flower size (Table 7) in both female and hermaphrodites (Figure 8) with P-values  $\ll 0.01$  for both morphs, and  $R^2$ -adjusted of 0.07 for hermaphrodites and 0.06 for females.

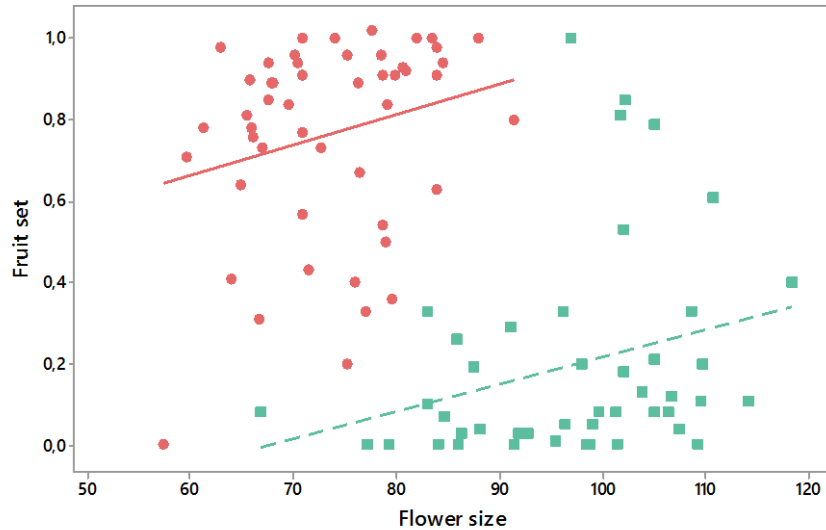


Figure 8) Scatterplot of the fruit set of *Silene acaulis* (total number of fruits/total number of flowers) on flower size where red dots are female and green squares are hermaphrodites. Both with regression line.

The fruit set increased with visitation frequency for both hermaphrodites and females (Figure 9) with p-values at  $< 0.01$  for hermaphrodites and  $\ll 0.01$  for females and adjusted  $R^2$ -value of 0.08 for hermaphrodites and 0.12 for females.

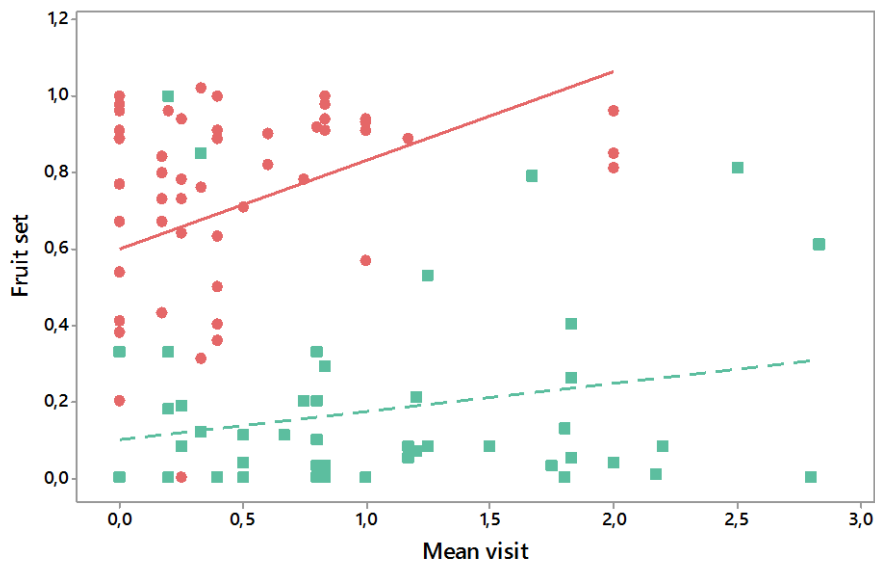


Figure 9) Scatterplot for fruit set of *Silene acaulis* (total number of fruits/total number of flowers) to mean number of visitors for the observation periods, where red dots are female and green squares are hermaphrodites. Both with regression line.

Morph, pairs nested in populations, flower density on the plant, mean visitation rate and flower size significantly explained variation in fruit set among plants (Table 7). Population did not significantly explain the difference in fruit set. Morph and flower size is correlated with VIFs of 5.97 and 7.89, respectively (Table 8). The sources tested in this model has an adjusted R<sup>2</sup>-value of 0.85 and explains much of the variation of fruit set. The residuals are shown in appendix I (Figure 12).

*Table 7) Analysis of variance of fruit set of Silene acaulis in a general linear model, tested on flower density on the plant, mean visits of insects, mean flower size, morph and pair in the three populations. Numbers in bold are significant on a 95 % confidence interval.*

<b>Source</b>	<b>df</b>	<b>Adj SS</b>	<b>Adj MS</b>	<b>F-value</b>	<b>P-value</b>
Morph	1	17.68	17.68	505.19	<< <b>0.01</b>
Population	2	0.12	0.06	0.11	0.89 *
Pair(population)	52	33.77	0.65	18.56	<< <b>0.01</b>
Flower density	1	0.17	0.17	4.78	<b>0.03</b>
Mean visit	1	0.47	0.47	13.29	<< <b>0.01</b>
Flower size	1	0.82	0.82	23.57	<< <b>0.01</b>
<b>Error</b>	438	15.32	0.04		
Lack-of-fit	37	15.32	0.41		
Pure error	401	0.00	0.00		
Total	496	118.45			

R<sup>2</sup> = 87.06 % (R<sup>2</sup>adjusted = 85.35 %)

\* Not an exact F-test

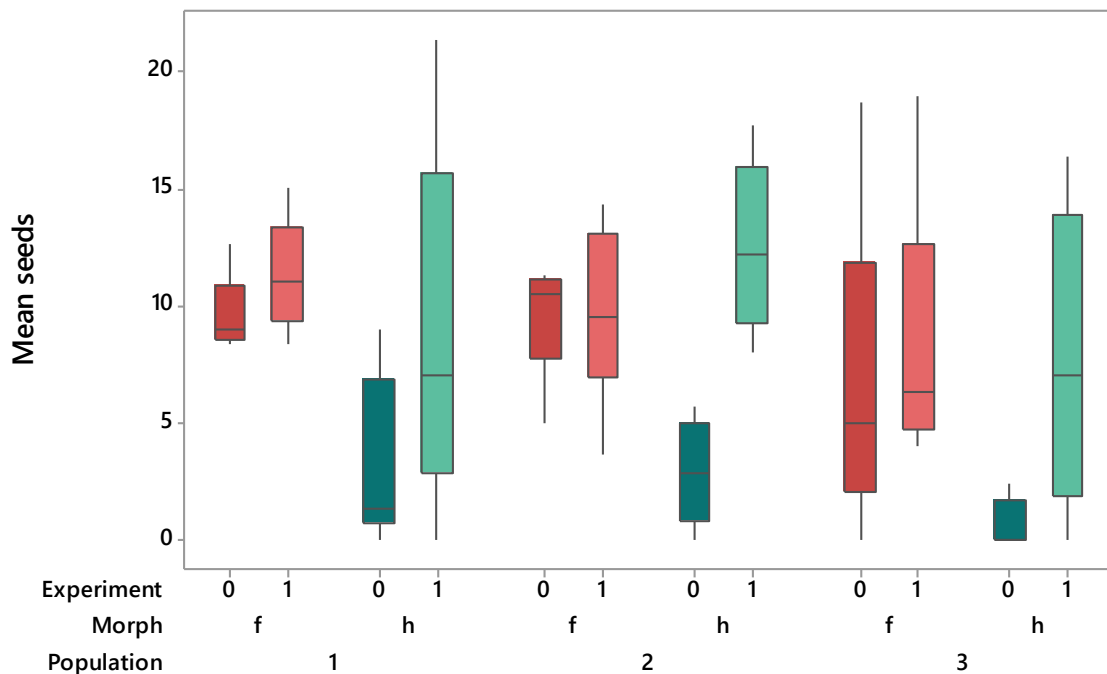
*Table 8) Coefficients of fruit set of Silene acaulis in a general linear model, tested on flower density on the plant, mean visits of insects, mean flower size, morph and pair in the three populations.*

<b>Term</b>	<b>Coef</b>	<b>SE coef</b>	<b>T-value</b>	<b>VIF</b>	<b>P-value</b>
Constant	0.072	0.133	0.54		0.59
Morph					
f	0.46	0.021	22.48	5.97	<<0.01
Population					
1	0.017	0.014	1.22	1.37	0.22
2	0.014	0.012	1.19	1.19	0.23
Flower density	-0.173	0.079	-2.19	2.17	0.03
Mean visit	0.077	0.021	3.65	3.19	<<0.01
Flower size	0.008	0.002	4.85	7.89	<<0.01



## Supplemental pollination experiment

All female plants produced seed on at least one flower, whilst three of the hermaphrodites produced no seeds or fruit. Seed number per fruit differed significantly between control and supplementary pollinated flowers, but not between morphs or populations (Table 9). The difference in seed number per fruit in control and supplemental pollinated flowers was significantly higher in hermaphrodites than in females (figure 10) where mean seeds in female increased insignificantly with 12 % (from  $8.6 \pm 2.2$  to  $9.7 \pm 2.2$ ) and mean seeds of hermaphrodites increased significantly with 124 % (from  $2.3 \pm 1.4$  to  $9.8 \pm 2.3$ ), and the interaction between the pollination treatment and morph was significant.



*Figure 10) Boxplot of mean seed production of Silene acaulis in the three populations. Separated by morph (greens are hermaphrodites and red are females) and by treatment (where dark is control and light are supplementally pollinated).*

Morph, population, flower density and flower size was not significant in explaining the difference in seed production (Table 9). And there is some correlation between morph and flower size with VIFs of 4.76 and 4.46, respectively (Table 10). This analysis have an adjusted  $R^2$ -value of 0.52, and the residuals are shown in appendix I (Figure 14).

Table 9) Analysis of variance in a general linear model of seed production in *Silene acaulis*, tested on fruit set, experiment treatment, morph and the interaction of morph\*experiment. Numbers in bold are significant on a 95 % confidence interval.

Source	df	Adj SS	Adj MS	F-value	P-value
Morph	1	0.08	0.08	1.12	0.19
Population	2	0.40	0.20	2.70	0.08
Experiment	1	1.45	1.45	19.76	<< <b>0.01</b>
Experiment*Morph	1	0.81	0.81	11.07	< <b>0.01</b>
Flower density	1	0.14	0.14	1.90	0.17
Fruit set	1	0.75	0.75	10.15	< <b>0.01</b>
Flower size	1	0.01	0.01	0.10	0.75
<b>Error</b>	55	4.05	0.07		
<b>Total</b>	63	9.60			

R<sup>2</sup> = 57.84 % (R<sup>2</sup>adjusted = 51.71 %)

Table 10) Coefficients of a general linear model of seed production in *Silene acaulis*, tested on fruit set, experiment treatment, morph and the interaction of morph\*experiment.

Term	Coef	SE Coef	T-value	VIF	P-value
Constant	0.25	0.40	0.63		0.53
Morph					
f	0.08	0.07	1.06	4.76	0.29
Population					
1	-0.01	0.05	-0.24	1.63	0.81
2	0.11	0.05	2.11	1.55	0.04
Experiment					
0	-0.15	0.03	-4.45	1.00	<<0.01
Experiment*Morph					
0 f	0.11	0.03	3.33	1.01	<0.01
Flower density	0.79	0.58	1.38	1.67	0.17
Fruit set	0.56	0.18	3.19	2.47	<0.01
Flower size	0.00	0.00	0.32	4.46	0.75

## Discussion

This study has shown that there is a sexual dimorphism in the two morphs of *Silene acaulis* at Finse. I found a significant difference between female and hermaphrodites in flower size, visitation frequency, fruit production and pollen limitation. Hermaphrodites had larger flowers and a higher number of pollinator visitors and female plants had higher fruit set and were less limited by pollen in seed production. This shows that hermaphrodites allocates more resources to pollination attraction by having larger flowers (Wilson et al. 1994), which is in line with Bateman's principle (Burd 1994) and theories of pollinator visitations being more important for male reproduction than for female reproduction (Charnov 1982). The results are also in accordance with theories of female maintenance in gynodioecious populations (Charlesworth & Charlesworth 1978; Charlesworth 1981), by having female advantage in fruit production (Dufay & Billard 2012; Lloyd 1975).

### Flower morphology

My first prediction; that hermaphrodites have larger flowers than female, was supported by my results. Indeed, hermaphrodites had on average 25.9 % larger flowers than females in the three populations at Finse. Other studies on *Silene acaulis* have also found significantly larger flowers in hermaphrodites than in females (Alatalo & Molau 1995; Hermanutz & Innes 1994; Lortie & Reid 2012; S astad 1991). Pairs and populations also significantly explained variation in flower size among individuals, suggesting that micro climate, soil conditions, nutrient availability and other abiotic factors also contribute to determining flower size in *S. acaulis*. Population 3 had the smallest flowers and this population is located at the highest elevation, close to the glacier Midtdalsbreen. This indicates that flower size is resource limited in high altitudes and adverse habitats (Alatalo & Molau 1995).

I found no significant difference in flower density, flower number or plant size between the two morphs, while others have found that females have more flowers and higher flower density than hermaphrodites (Lortie & Reid 2012). The reason for this sexual dimorphism can be that female plants may have higher fitness gains by increased flower number than by increased flower size (Ashman 2005; Lortie & Reid 2012). It may also be less important for the reproductive success of females to attract pollinators than for hermaphrodites who also gain fitness through male function, and this can drive this sexual dimorphism (Charnov & Bull 1986; Eckhart 1992). Large flowers is a resource expenditure (Galen 2000), and female

allocation to flowers cause a higher number of ovule number, while the expenditure on flower size may increase male reproductive success in hermaphrodites.

### Flower visitation

The results supports my prediction that populations with a high plant abundance and plants with a high flower density had the highest pollinator visitation. Moreover, there was a positive relationship between the number of flowers on plants and their mean number of visits.

Hermaphrodites had more visitors than females and hermaphrodites had significantly larger flowers than females, indicating that flower size attracts pollinators and are important for the male reproductive success in hermaphrodites. Lortie and Reid (2012) found similar results in their study, where insect pollinators spent more time on hermaphrodites than on female plants. Pollinator foraging theory predicts that pollinators will visit flowers that give the lowest energy cost and highest energy reward (Richards 1986). Herein is distance between flowers and amount of nectar per flower important factors (Richards 1986).

The selective pressure on flower size may be greater in hermaphrodites if the number of pollinator visits are more important for male than for female reproductive success (Charnov & Bull 1986; Eckhart 1992). Hermaphrodites can have countless numbers off offspring as males when there is sufficient pollinator visitations carrying pollen to other flowers, while females have a limited number of ovules, and thus a limited number of offspring.

Female flowers are dependent on pollinators carrying adequate pollen quality and quantity to set fruit (García-Camacho & Totland 2009). It is therefore important that pollinators transport enough pollen between conspecific flowers, to prevent pollen limitation (Wilcock & Neiland 2002). The most common visitor to flowers of *Silene acaulis* in all three populations was flies, which is in line with previous studies at Finse (Alatalo & Totland 1997; Totland 1993). The high abundance of individuals from the Diptera order compensate for its inefficiency as pollinators (Larson et al. 2001; Ssymank et al. 2008), compared to bumblebees and butterflies.

The lowest number of visitors were in population 3, which also had the lowest density of plants and the smallest flowers, while population 2 had the highest number of visitors and highest density of plants. This can indicate that there is a positive relationship between pollinators and the availability of their food source, which is line with the pollinator foraging theory (Richards 1986). This will also apply to plants with high flower density, if the flowers

on high density plants produce a similar amount of nectar as flowers on plants with low flower density.

Flower visitation was positively related to temperature. This suggests that there will be a higher pollination rate with increasing temperature. The summer of 2014 was especially warm, where the mean July temperature was 5.1°C warmer than average (mean from 1961-1990) (Nowegian Meterological Institute 2014). This may have positively influenced the pollinator visitations and fruit set at Finse when I conducted this studies. On the other hand, the high temperature may also have speeded up the flowering period, giving the pollinators a shorter period to conduct the pollination, or create a tropic mismatch between flowering plants and their pollinators (Reid et al. 2014), which may have reduced pollinator visitation and fruit set.

### Fruit production

Females had a 4.2 times higher mean fruit set than hermaphrodites. This supports my third prediction, which stated that female plants have a higher fruit set than hermaphrodites to persist in a gynodioecious population.

This results in sexual difference on fruit set is also found in previous studies on *Silene acaulis* where hermaphrodites had a lower fruit set than females (Delph et al. 1999; Hermanutz & Innes 1994; Lortie & Reid 2012; Maurice et al. 1998; Shykoff 1988). This difference in female reproductive success is crucial for the maintenance of females in gynodioecious populations (Charlesworth & Charlesworth 1978; Charlesworth 1981), since females must have twice as high fruit set (Reid et al. 2014) or a significantly higher seed and seedling survival through better seed provisioning than hermaphrodites to persist in a gynodioecious population (Alatalo & Molau 2001; Dufay & Billard 2012; Shykoff 1988).

Population 3, which had the lowest pollinator visitation, also had significantly lower fruit set than the other two populations, with 33 % lower fruit set in females and 67 % lower fruit set in hermaphrodites than the mean of population 1 and 2. Pollinator visitations significantly explained variation in fruit set, and had a stronger positive relationship with fruit set in females than in hermaphrodites. This also occurred in Lortie and Reid's (2012) study where the frequency of pollinators had no relationship on fruit set in hermaphrodites, but had a positive relationship with fruit set in females. The reason for this may be that hermaphrodites gain increased amount of pollen dispersed to a greater number of plants with increasing visitor frequency, and is not completely dependent on their own fruit production to have

offspring (Charnov & Bull 1986). This is in line with Bateman's principle (Bateman 1948), stating that pollinator attraction through increased corolla size should have a greater effect on male than on female reproductive success (Burd 1994; Galen 2000), which may drive the evolution of sexual dimorphism on flower size (Charnov & Bull 1986; Eckhart 1992).

### Supplemental pollination experiment

My forth prediction; stating that female plants are more pollen limited than hermaphrodites, because hermaphrodites have the possibility of reproductive assurance through self-pollination, was not supported. On the contrary, the results suggest that hermaphrodites were more limited by pollen availability than females, since females did not increase their seed production after supplemental pollination, whereas hermaphrodites had significantly higher seed production after supplemental pollination. This result, combined with the very low fruit and seed production under ambient pollination in hermaphrodites, suggests that hermaphrodites function primarily as males in this area, that they may produce fruits and seeds only under high pollen availability.

Hermaphrodites enter the male phase first during anthesis (Alatalo & Totland 1997), and the female phase is entered when the petals are starting to wither. This may cause a higher visitation frequency while the flowers are in the male stage, and thus cause insufficient pollen deposition for seed set during the female reproductive phase. It is also hypothesised that late arriving pollen have less effect on fertilization than early arriving pollen (Shykoff 1992), and since stigmas of hermaphrodite flowers becomes receptive to pollination later than stigmas of females, hermaphrodites may recruit a lower pollen tube number (Shykoff 1992) for ovule fertilization.

There was an overall low fruit set in hermaphrodites, with a mean of 10-23% in the three populations. This may indicate that self-pollination is not common in these populations of *Silene acaulis*. The mismatch in timing of mature pollen release and stigma receptivity (dichogamy), as occur in *S. acaulis* may prevent selfing (Barrett 2003). Plant species with high levels of self-pollination also use less resources on corolla size and nectar production (Lloyd 1987). This is in contrast to resources spent on flower size in hermaphrodites in the Finse population of *S. acaulis*, which further suggests that there are low levels of self-pollination in hermaphrodites in this area.

Female seed production was not pollen limited, and this supports the reproductive assurance hypothesis where hermaphrodites have a low fruit set in favourable years where reproductive

output is maximised by out-crossing and unisexual plants have an advantage (Reid et al. 2014; Shykoff 1992). Female plants may be more affected by the absence of pollinators as measured in fruit set, while hermaphrodites have a significantly lower decrease in fruit production in the absence of pollinators (Reid et al. 2014). This also support the reproductive assurance hypothesis of hermaphrodites being a poorer fruit producer in favourable years, but have an advantage in years of low pollen availability (Alatalo & Molau 2001).

Three of 16 hermaphrodites did not produce fruit or seeds, even when they were supplementally pollinated, and therefore functioned solely as males. Similar results was detected by Shykoff (1988), where four of 31 supplementary pollinated hermaphrodites in her study on *Silene acaulis* failed to produce fruits. Hermaphrodites in *S. acaulis* have a large variation in the length of styles and papillas on their gynoecium, and this length has a positive relationship with fruit set (Hermanutz & Innes 1994). It has been shown that increasing stigmatic surface increases pollen capture (Cruden et al. 1984; Dulberger & Horovitz 1984; Galen & Plowright 1985; Shykoff 1992). Such variation in papilla and style length suggests that hermaphrodites differs in their female expression, where plants with short styles and papillas have reduced female reproduction. This can indicate that these hermaphrodites will evolve toward female sterility (Hermanutz & Innes 1994).

Plants of intermediate hermaphroditic gender expressions have been found in populations of *Silene acaulis* and other gynodioecious species (Dulberger & Horovitz 1984; Hermanutz & Innes 1994; Shykoff 1988; Ågren & Willson 1991). This shows that there is a fitness trade-off in sexual expression. Studies show that hermaphrodites that specialize on female reproduction, with high fruit production, have low male reproductive success (Alatalo & Molau 2001), while hermaphrodites that specializes on male reproduction have a low female reproductive success (Alatalo & Molau 2001; Hermanutz & Innes 1994).

Gynodioecy has been viewed as an intermediate step in the evolution towards a dioecious gender expression (Barrett 2002; Charlesworth 1999; Dufay & Billard 2012). This evolution can occur when female plants are superior in fruit set, and selection on hermaphrodites may lead toward a male or male-like form (Charlesworth & Charlesworth 1978), with female sterility. This shift for hermaphrodites towards a male gender expression may also occur if hermaphrodites with long styles and high female productivity have a higher rate of self-pollination and experience inbreeding depression (Hermanutz & Innes 1994). Selfed seeds of *Silene acaulis* showed a lower seedling survivorship than outcrossed seeds (Shykoff 1988),

and can be outcompeted by seeds from female plants. The evolution of dioecy can also be expected with high levels of geitonogami (Barrett 2003). It can therefore give a higher reproductive outcome when plants allocates all recourses to the production of only pollen or ovules.

## Conclusion

This study shows that there is sexual dimorphism in *Silene acaulis* at Finse. Female plants had a significantly higher fruit set than hermaphrodites. Hermaphrodites seems to mainly function as males, with pollen dispersal as their reproductive strategy, in this study area. The advantage of being able to self-pollinate in hermaphrodites in years of low pollen availability, as often occurs in artic and alpine areas, can be the reason for the maintenance of hermaphrodites and gynodioecy in *S. acaulis* (Alatalo & Molau 2001), and prevent the evolution of complete dioecy at Finse.

This study contributes to knowledge of sexual dimorphism in alpine plant species. Further research could include investigation of nectar production of the two morphs, if this is correlated to flower size and how this affects pollinator behaviour. Another interesting aspect could be to look at the male reproductive success to see which floral traits that increase male reproductive success, and if these are opposite or similar to the morphological traits which assures female reproductive success.



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## Appendix I

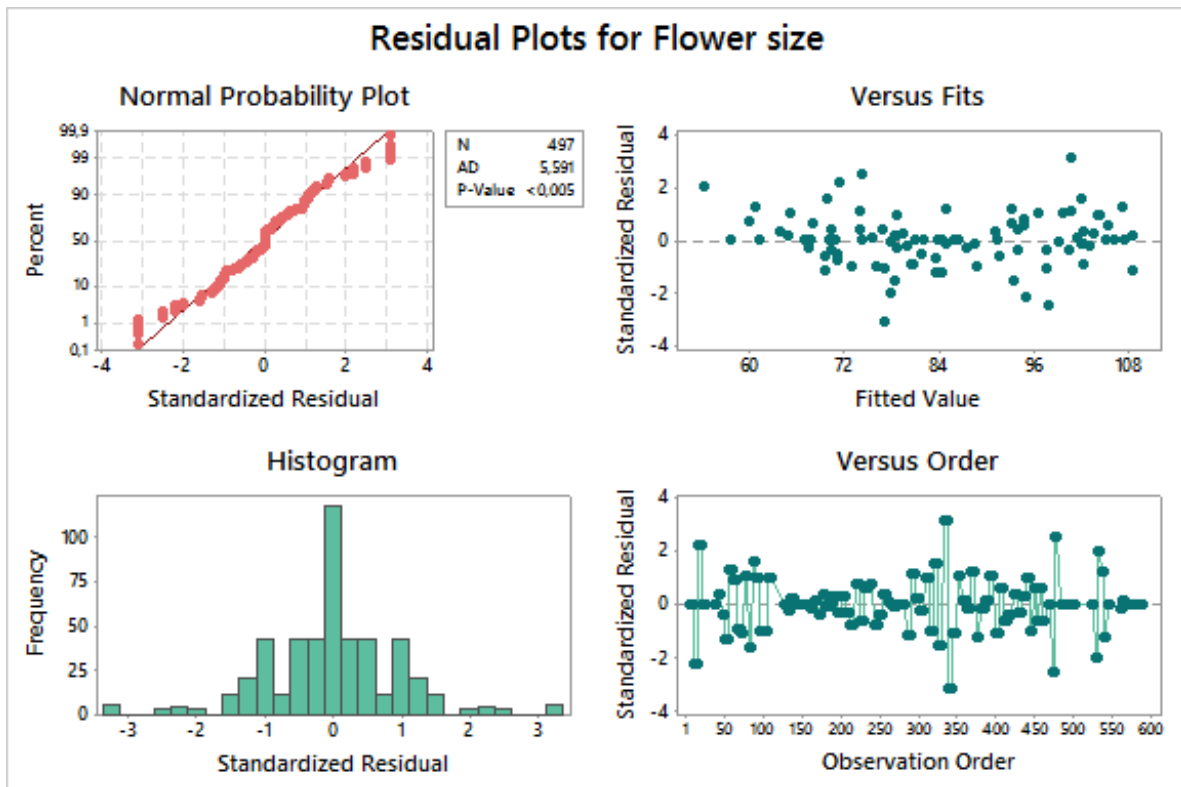


Figure 11) Residual plots for flower size.

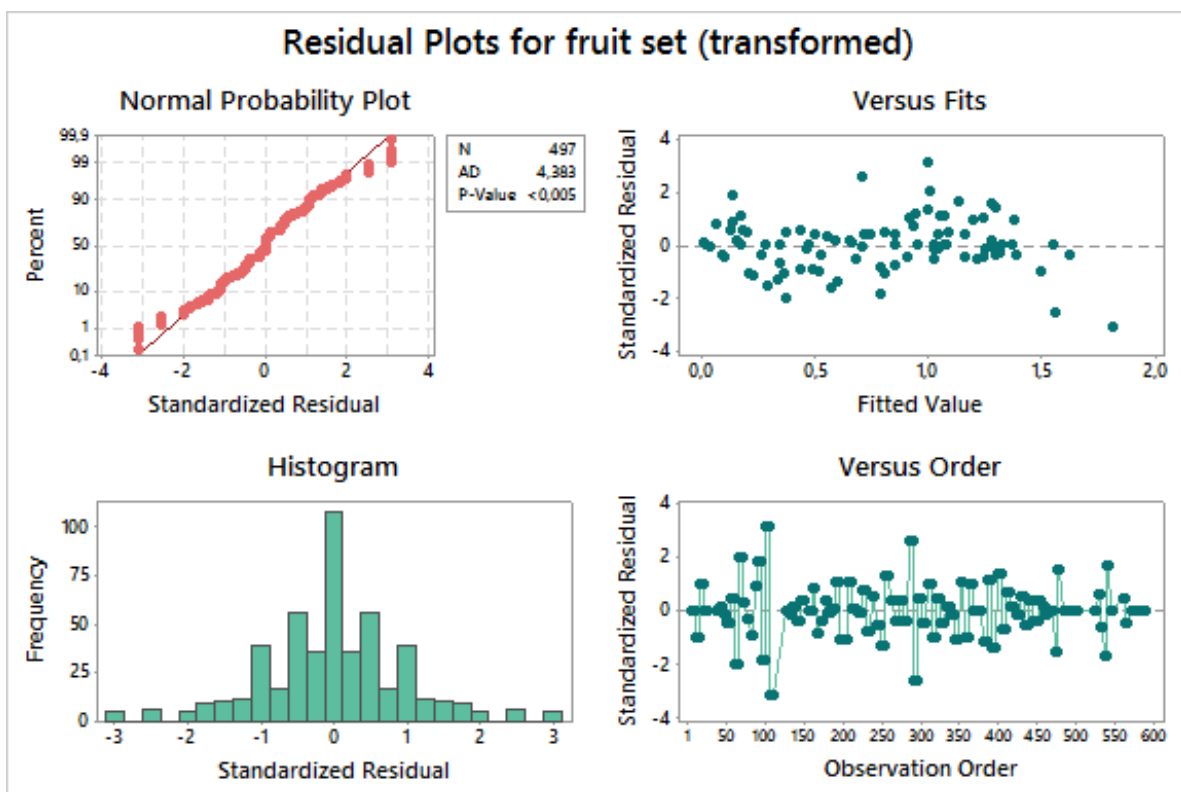


Figure 12) Residual plots for fruit set.

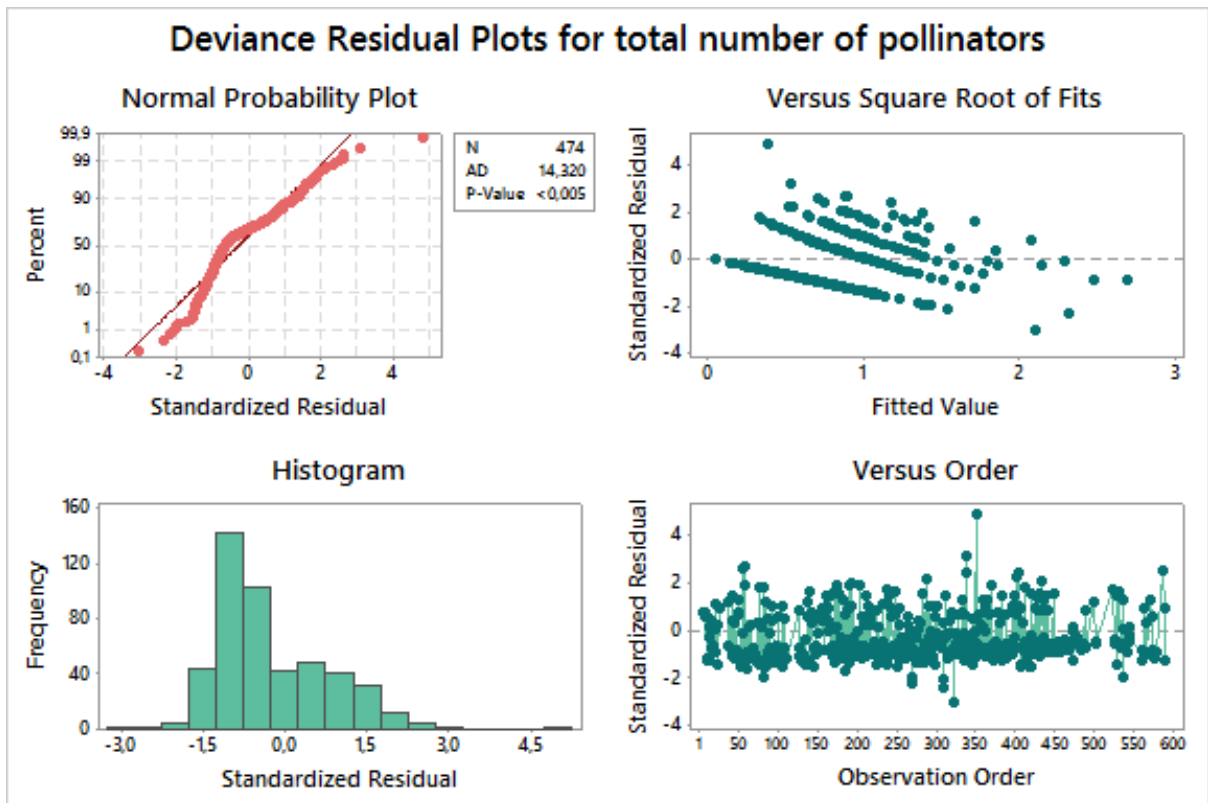


Figure 13) Residual plots for number of pollinators.

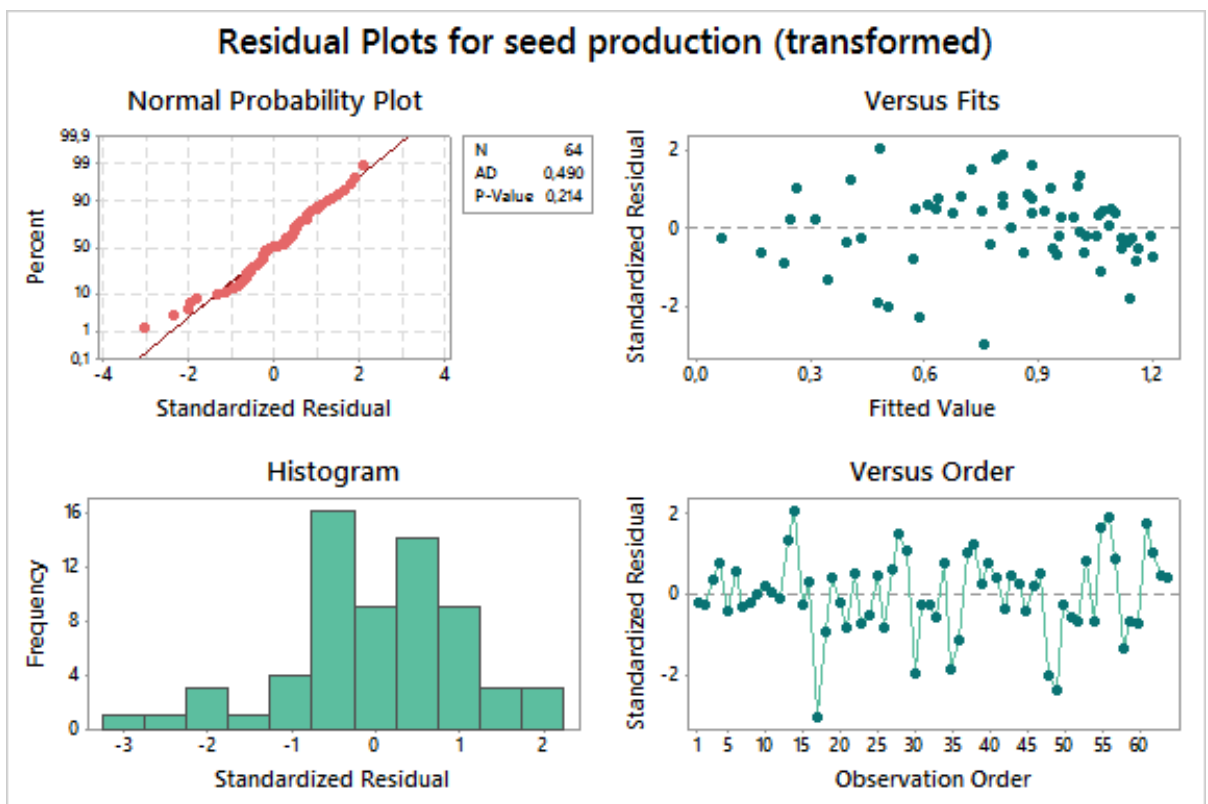


Figure 14) Residual plots for seed production in the supplemental pollination experiment.

## Appendix II

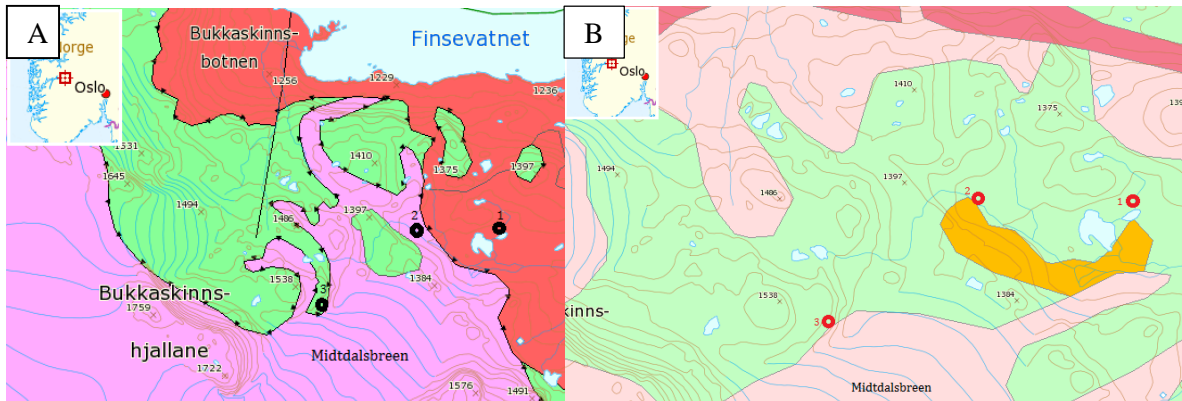


Figure 15) Maps of my study area, with indication map of Norway. a) Map of bedrock with the three populations marked with black circles, green indicates phyllite, pink indicates charnockite and anorthosite, and red indicates anorthosite, and b) map of soil with the three plots marked with red circles. Green indicates moraine materials, pink indicates naked bedrock and orange indicates glacier river materials.







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