Optimal habitats enhance establishment, but do not influence gender frequencies or genetic diversity of *Silene acaulis* in Svalbard (Norway)

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PREFACE

This master thesis project was a collaboration between the Department of Biosciences, University of Oslo (UiO) and the Department of Arctic Biology, The University Centre in Svalbard (UNIS). I would like to thank The Research Council of Norway for funding my field work through Arctic Field Grant 2013 and Jan Christensens legat for financial support to sequencing expenses.

It is a great adventure coming to an end handing in this thesis. From the day I stepped into Anne's office telling her about my dream of doing a master project in Svalbard, through the first meeting with dark season ice cold field work, amazing and intense summer field cruises, culminating in a wonderful spring of long days at UNIS, spectacular trips, mushing and new friends that I hope will stay for life.

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ABSTRACT

Plant reproduction in the Arctic is challenging, and tight energy budgets, short growing season and the limited number of pollinators favour self-pollination. Self-pollination over time will, however, have a negative effect through reduced genetic variation and increased risk of inbreeding depression. In gynodioecious species, hermaphrodites and male-sterile females co-occur. This breeding system allows for selfing by hermaphrodites, but enforces outcrossing through females. To maintain the gender polymorphism, females must be fitter than hermaphrodites. This female advantage is predicted to cause improved establishment performance of female offspring, in particular under harsher environmental conditions.

This study aims to evaluate how changes in habitat optimality, represented by vegetation cover at different successional stages, affect population dynamics in the gynodioecious pioneer species, *Silene acaulis* (L) Jacq. in the high arctic archipelago Svalbard (Norway). Open pioneer habitats are predicted to be optimal for this species. As a result of increased inter-specific competition, *S. acaulis* populations in suboptimal closed habitats are expected to show i) decreased establishment performance and ii) increased female frequency due to female advantage, which further should affect iii) patterns of genetic diversity and structure.

Two approaches were taken to test these issues further. A large-scale study including 17 populations (4136 individuals) from the south-western to northern parts of Spitsbergen assessed establishment patterns and female frequency of *S. acaulis* in different habitat types on a broad scale. A small-scale study including two populations (1036 individuals), one in an open habitat and one in a closed habitat, assessed these issues in more detail, including analyses of microsatellite genetic diversity and structure (224 individuals). Demographic patterns were assessed through generalized linear modelling (GLM) and significance tests, while genetic diversity and structure were investigated by estimation of inbreeding coefficients, heterozygosity measures, analysis of molecular variance (AMOVA), Mantel's tests and principal component analysis (PCA).

Average plant cushion size was significantly smaller in open habitats compared to suboptimal closed habitats. Female frequencies were slightly but not significanty higher in suboptimal closed habitats. Inbreeding levels were fairly high, and not significantly different between the two habitat types. Low levels of genetic differentiation were detected between and within sites and plots.

The results show that closed habitats reduce general establishment performance, while no significant association with habitat type is detectable for female frequency and genetic

diversity measures. It is suggested that establishment performance is affected by environmental conditions changing over local scales, while gender frequency is mainly influenced by large-scale climatic conditions, which are severe for all sites included in this study. Overall high inbreeding levels are suggested to reflect pollinator deficiency in this high arctic system, leaving considerable spatial and temporal seed dispersal as the most likely cause for low differentiation levels between and within the two populations.

Keywords: Establishment performance, female advantage, gender frequency, gynodioecy, habitat optimality, population genetics, *Silene acaulis*, Svalbard, vegetation cover

INTRODUCTION

Arctic environments are highly heterogeneous and dynamic, with great variation in environmental conditions at fine scales and low predictability between years in environmental variables such as length of growing season, temperatures and freeze-thaw events (Jónsdóttir 2005; Convey 2012). In such extreme environments, reproductive systems play a crucial part in adaptation (Molau 1993; Khodachek 1995), particularly in plants where the reproductive phase represents the only opportunity during the life cycle for genes to move among conspecific populations (Barrett 2003).

Successful sexual reproduction by outcrossing requires more energy and is more time consuming than self-pollination (selfing) or asexual reproduction (Solbrig 1976). It is often assumed that asexual reproduction and selfing is the primary mode of reproduction in arctic plants that need to reproduce during short and cold arctic summers (Billings & Mooney 1968; Bliss 1971; Peck et al. 1998). Sexual recruitment by outcrossing followed by successful germination of seedlings is predicted to depend on stochastically favourable conditions (Billings & Mooney 1968; Bell & Bliss 1980). However, selfing and asexual reproduction strategies reduce novel genetic variation and the adaptive potential of the generation, and increase the risk of inbreeding (Glémin et al. 2006). During the last decades, studies from the High Arctic have suggested that sexual reproduction might be more common among arctic plant species than previously assumed (Philipp et al. 1990; Wookey et al. 1995; Gabrielsen & Brochmann 1998; Steltzer et al. 2008; Müller et al. 2011). Sexual reproduction systems such as gynodioecy and dioecy have been found to be particularly abundant among early-flowering arctic species (Molau 1993). These early-flowering species show various mechanisms to increase outcrossing rates, such as avoidance of self-pollination and high abortion rates of

ovules and embryos. In late-thawing habitats, where time is limited, such mechanisms cannot be supported and clonal reproductive systems are more common (Molau 1993).

Pros and cons for gynodioecy as an adaptive strategy in arctic plants

A relatively common reproductive strategy of arctic plants is gynodioecy (Molau 1993). Gynodioecy is the co-occurrence of hermaphrodites and male-sterile females within natural populations (Darwin 1877). This breeding system allows for selfing by hermaphrodites, but enforces outcrossing through females. A reproductive strategy that enables selfing in self-compatible hermaphrodites secures successful population recruitment in unfavourable years or habitats (Jarne & Charlesworth 1993). Selfing relies on a single individual and has low dependency on pollen transmitting agents such as wind and insects (Solbrig 1976). High selfing rates in a population may, however, lead to inbreeding depression and decreased fitness of the resulting offspring (Charlesworth & Charlesworth 1987), while outcrossed progeny may obtain increased fitness through heterosis, that is, enhanced viability and reproduction as a consequence of increased heterozygosity (Hamilton 2009). Increased levels of heterozygosity enhance the genetic diversity of a population, which may lead to increased variation in ecologically important plant traits, and is expected to facilitate adaptive evolution and population persistence in highly variable environments (Steltzer et al. 2008). Species with high outcrossing rates are also expected to have less genetically differentiated populations, as the extensive gene flow will counteract genetic drift within populations and thus maintain the genetic diversity of the species (Hamrick & Godt 1996; Muir et al. 2004; Mable & Adam 2007). Species with high selfing rates, on the other hand, are predicted to have highly differentiated populations (Abbott & Gomes 1989; Hamrick & Godt 1996), with correspondingly low levels of heterozygosity and increased linkage disequilibrium between loci (Nordborg 2000), as a result of increased genetic drift and, potentially, fixation of alleles (Loveless & Hamrick 1984; Duminil et al. 2009). This is expected to decrease the genetic diversity and hence the adaptability of the species, but could be advantagous if environmental conditions are temporarily stable and the fixated gene combination optimal under current conditions (Peck et al. 1998).

Although outcrossing could be less favourable if it breaks up well-adapted gene combinations in more stable environments (Lande & Shannon 1996), it seems to be a more favourable strategy in the long run (e.g. Silvertown 2008). The outcrossing hypothesis for gynodioecious species proposes that females are maintained because all their offspring result

from outcrossing, whereas some of the progeny of hermaphrodites are a result of selfing, and thus are more vulnerable to decreased fitness due to inbreeding depression (Sun & Ganders 1986). Female fecundity advantage (hereafter referred to as female advantage) through higher fruit set, greater seed production and better seed germination is a general trend in gynodioecious species (Shykoff et al. 2003). This leads to expectations of increased female frequency in gynodioecious plant populations that are faced with more suboptimal conditions (Delph 2003). Populations in suboptimal sites are thus driven towards higher levels of outcrossing. Combined with earlier onset of reproduction, that is, flowering earlier in life, which is observed in stressful environments for many species (e.g. Sultan 2000), this could result in the production of overall more seeds with higher fitness, and might thus be a good strategy for successful dispersal out of suboptimal habitats and into more favourable habitats. It has consistently been suggested that one key to the success of plant species found in Svalbard today is their ability to "move out of trouble" (e.g. Brochmann & Steen 1999).

A recent review of the topic states that the causes of female advantage in gynodioecious species might be more diverse than simply the reduced inbreeding depression of female offspring (Dufay & Billard 2012). Alternative causes proposed include the reallocation of resources from the male towards the female function, and sex differences in interactions with herbivores (hermaphrodite-biased predation). Hence, further understanding of the links between female advantage and gynodioecious reproductive systems requires thorough knowledge about sex ratio variation, gender-specific establishment performance and how these two factors affect population genetic patterns under changing conditions.

Silene acaulis as a gynodioecious study system

The arctic-alpine, gynodioecious plant species *Silene acaulis* (L.) Jacq. (Caryophyllaceae) provides a suitable study system to investigate the links between female advantage and population dynamics in heterogeneous arctic environments. *Silene acaulis* is a diploid ($2n$ = 24), herbaceous, perennial plant species with a circumpolar, arctic-alpine distribution, and typical habitats are elevated sea terraces, wind-exposed ridges, rocky slopes and open tundra (Jones & Richards 1962; Aiken et al. 2007). The species reproduces sexually, clonal reproduction by re-rooting of branches is not known and one cushion usually consists of one individual (Jonsell et al. 2001; Aiken et al. 2007). The cushion grows progressively in size as the plant ages, and several studies have calculated growth rates that can be applied as proxies for plant age (Benedict 1989; McCarthy 1992; Morris & Doak 1998).

Each cushion usually produces many flowers, which are pink-purple, slightly fragrant and insect pollinated. In general, pollinator activity is low in the Arctic, and the high arctic archipelago Svalbard lacks efficient pollinators such as bees and bumblebees, but various *Diptera* probably act as important pollinators (Coulson 2007). The favourable microclimate created by *S. acaulis* cushions has been shown to facilitate invertebrate communities (Molenda et al. 2012), which further could aid pollination. Localized seed dispersal with clustering of offspring around maternal plants has been evident in several studies of this species (e.g. Gehring & Delph 1999; Klaas & Olson 2006). Seeds are dispersed through a ballistic mechanism, which is most efficient for short-distance dispersal and typically leads to a normal distribution of seeds around the parent (Howe & Westley 2009). However, several studies have suggested that long-distance dispersal of seeds by wind is frequent in the Arctic, even for species without typical adaptations for wind dispersal (e.g. Savile 1972; Alsos et al. 2007).

Sex determination in *S. acaulis* is most likely regulated through an interaction between maternally inherited cytoplasmic male sterility (CMS) genes and their associated nuclear male fertility restorer genes (Schnable & Wise 1998; Klaas & Olson 2006). That is, the match between female cytoplasmic type and male nuclear type decides whether the offspring resulting from a particular fertilization ends up being a male-sterile female, or a hermaphrodite with flowers exhibiting both male and female functions. Functional males have been recorded in some *S. acaulis* populations (e.g. Philipp et al. 1990; Hermanutz & Innes 1994). Partial maternal inheritance of sex expression, that is, that sexual phenotype variation is mainly controlled by the cytoplasmic genotype, has been reported for several species with cytonuclear gender determination (e.g. Manicacci et al. 1997; Laporte et al. 2001). Females are thus generally expected to produce more female than hermaphroditic offspring, and vice versa, but sufficiently high frequencies of compatible restorer genes and new CMS types might counteract this (Charlesworth & Laporte 1998; Delph & Kelly 2013).

Several studies have shown a female advantage in *S. acaulis*. Female plants have higher fruit set compared to hermaphrodites (Shykoff 1988; Delph et al. 1999; Delph & Carroll 2001). Females have also been found to produce a greater number of seeds (Shykoff 1988), seeds with higher seed germination rate (Keller & Schwaegerle 2006) and seedlings with greater juvenile survivorship relative to hermaphrodites (Shykoff 1988). Furthermore, the overall seed production throughout the total life span of the plant can be several times higher in females compared to hermaphrodites (Morris & Doak 1998). One study also found that

females provided a more effective gametophytic selection as their stigmatic area was larger, stigmas became receptive earlier and pollinators spent more time probing females, thus giving a greater potential for producing high quality offspring compared to hermaphrodites (Shykoff 1992).

Several causes of the apparent female advantage in *S. acaulis* have been proposed. One is a combination of maternal sex and the classical outcrossing hypothesis (Keller & Schwaegerle 2006). Female advantage is suggested to result from higher performance and abundance of female genotypes in combination with reduced selfing and inbreeding depression of female offspring. Another potential cause of female advantage is maternal effects, that is, higher maternal resource allocation to seeds in females relative to hermaphrodites (Shykoff 1988). A third explanation is the pleiotrophy hypothesis, which predicts a negative effect on reproductive traits of hermaphrodites due to nuclear restorer alleles or alleles hitch-hiking with them (Delph & Mutikainen 2003; Delph 2004).

Sex ratio variation has been observed in many *S. acaulis* populations, with female frequencies ranging from 0.08 (Klaas & Olson 2006) to 0.87 (this value is recalculated to represent female frequencies relative to the number of reproducing individuals in the population, from data given in Philipp et al. (1990)). Increased female frequencies have been observed in correlation with a range of variables, for example, decreasing fitness of hermaphrodite seeds (Delph & Carroll 2001), increasing altitude (Alatalo & Molau 1995) and increasing severity of habitats (Philipp et al. 1990). These observations lead to expectations of increased female frequencies in populations in severe habitats with hostile conditions, including harsh climate, low nutrient levels and other factors that might challenge reproduction and establishment in a *S. acaulis* population.

Silene acaulis is a pioneer species that thrives in open, poorly vegetated habitats of early successional stages where inter-specific competition is presumably low (Griggs 1956; Benedict 1989; McCarthy 1992). Hence, densely vegetated non-disturbed habitats (closed habitats), presumably with increased competition, should represent more suboptimal habitats with lower establishment rate and increased female frequencies due to female advantage. Such closed habitats will usually represent later successional states. Successional processes with directional change and little or no species replacement are usually present in the high arctic bioclimatic subzones B and C (Bliss & Peterson 1991; bioclimatic subzones: Walker et al. 2005), for example, along proglacial chronosequences in north-west Svalbard (Hodkinson et al. 2003). Here *S. acaulis* appears in sites several decades before other later successional

species such as *Dryas octopetala*. *Silene acaulis* usually does not occur in the extreme polar desert (bioclimatic subzone A) where directional succession patterns are limited (Bliss & Peterson 1991).

Aims and hypotheses

The primary aim of this study was to assess how suboptimal habitats, represented by higher vegetation cover that presumably increases competition, affect population dynamics in *Silene acaulis*, in particular i) establishment performance, ii) gender frequencies and iii) population genetic patterns. These three main topics were evaluated by testing five hypotheses:

i. To test how variation in habitat optimality influences establishment performance, abundance and size distribution of *S. acaulis* were assessed in two habitat types: open pioneer communities (open habitats) and communities with higher vegetation cover (closed habitats).

   H_1: Better establishment performance, that is, higher population density and abundance of small individuals, will be found in open habitats than in suboptimal closed habitats, as *S. acaulis* is thought to be a pioneer species preferring open habitats.

   H_2: If closed habitats in general represent later successional stages, an overall size distribution skewed towards larger individuals and more pronounced spatial clustering of individuals around larger cushions will be found in closed habitats compared to open habitats, as fewer establishment events are expected and offspring are likely to establish close to the maternal plant.

ii. To study how variation in habitat optimality influences gender-specific establishment and onset of reproduction in *S. acaulis*, female frequency and flowering frequency in different size classes were assessed in open and closed habitats.

   H_3: Higher female frequency will be found in closed habitats compared to open habitats, as female advantage providing a shift towards more females is expected under suboptimal conditions.

   H_4: Higher flowering frequency in lower size classes will be found in closed habitats compared to open habitats, as earlier onset of reproduction is expected under suboptimal conditions.

iii. The potential variation in establishment performance and female frequency with variation in habitat optimality is expected to influence levels of genetic diversity and structure between populations. To evaluate this, various measures of genetic diversity, genetic

differentiation and fine-scale genetic patterns were assessed in one open habitat population and one closed habitat population.

H_5: If open habitats are more optimal, there will be greater establishment success in such habitats, and the female advantage should be less pronounced. Consequently, more recruitment from selfing will occur in open habitats, leading to higher levels of homozygosity, more linkage disequilibrium and higher relatedness between individuals and thus more grouping of genotypes.

MATERIALS AND METHODS

Study area

Svalbard (Norway) is an archipelago located in the High Arctic (74-81°N, 10-30°E). Environmental conditions including climate, edaphic properties and vegetation cover are highly variable among sites. Walker et al. (2005) defined through 'The Circumpolar Arctic vegetation map' five bioclimatic subzones, of which three are found in Svalbard (following Elvebakk 1999). These range from barren land with low nutrient availability, < 5% cover of vascular plants, and a mean July temperature of 1-3°C (bioclimatic subzone A), to habitats of typical tundra vegetation with higher nutrient levels, 5-50% cover of vascular plants also including shrub vegetation, and a mean July temperature of 6-7°C (bioclimatic subzone C).

This study took two approaches. The first was a large-scale study, assessing demographic patterns of populations sampled at 17 sites in 11 locations from the south-western to northern parts of Spitsbergen (Table 1; Fig. 1). The habitats ranged from exposed ridges with low vegetation cover in bioclimatic subzone B, to sheltered densely vegetated slopes in bioclimatic subzone C. As demographic analyses of plant populations require a minimum sample size to capture the actual variation in demographic parameters in the population, only sites with reasonable abundance of *S. acaulis* individuals were selected.

The second approach was a small-scale study, aiming to investigate on a detailed scale the demographic patterns and genetic structure of two populations situated 9 km apart, in close vicinity to Longyearbyen (the largest settlement in Svalbard; Table 1). One site comprised an exposed, elevated sea terrace at Hotellneset (located by the fjord in the outer parts of Adventdalen), with limited vegetation and soils dominated by biological soil crust. Common plant species included *Salix polaris*, *Saxifraga oppositifolia*, *Luzula confusa*, *Silene acaulis* and various mosses. This area had a high density of *S. acaulis*, in terms of both abundance and cover of individuals. The other site was situated on the slope of a sheltered

Table 1. Overview of sampling locations for demography data in *Silene acaulis* in Svalbard, Norway. GPS-coordinates are given in UTM format, zone 33. Small-scale populations where detailed spatial and genetic analyses were performed are marked with *. Bioclimatic subzones are defined after Walker et al. (2005).

Location	Pop ID	UTM E	UTM N	Sampling date	Collectors §	Bioclimatic subzone	Habitat description
Ankerfjella	ANKR	441992	8720562	10.08.13	AB-201; MES	B	Exposed, southwest facing ridge in plain area. Bare ground consiting of coarse gravel and rocks are dominating. *Salix polaris*, biological soil crust and mosses are frequent, and *Saxifraga oppositifolia* present to some extent.
Ankerfjella	ANKZ	441992	8720562	10.08.13	AB-201; MES	B	Exposed, southwest facing, gradual slope. *Salix polaris*, biological soil crust and lichens are dominating. *Dryas octopetala, Saxifraga oppositifolia* and mosses are also frequent.
Blommesletta	BLMS	496520	8727117	20.07.13	MES; IHK; MK; AB-326	C	Exposed, southwest facing, elevated sea terrace. Biological soil crust and lichens are dominating. *Salix polaris*, mosses, *Saxifraga oppositifolia* and *Silene acaulis* are also frequent.
Blomstrandhalvøya	BLOZ	440292	8768961	14.07.13	MES; IHK; MK; AB-326	B	Sheltered, southeast facing, densely vegetated, dry slope. *Dryas octopetala*, *Cassiope tetragona* and mosses are dominating. Much organic litter and disturbance from reindeer and frost heave activity.
Bockfjorden	BOC	466738	8819670	08.07.13	MES; MC	C	Sheltered, northeast facing, elevated sea terrace. Bare ground of black, coarse-grained sand, gravel and small rocks are dominating. Mosses are also frequent.
Colesbukta	COLR	501221	8670602	13.07.13	MES; IHK; MK; AB-326	C	Sheltered, southwest facing, dry ridge. *Salix polaris* and biological soil crust are dominating. *Luzula confusa* are frequent and *Dryas octopetala* present to some extent.
Endalen*	END	517425	8679402	16.06.13 - 17.08.13	MES; IHK; JB	C	Sheltered, southeast facing, densely vegetated slope. *Dryas octopetala* and mosses are dominating species. *Salix polaris* and biological soil crust are also frequent.
Engelskbukta	ENGR	431851	8755284	19.07.13	MES; IHK; MK; AB-326	B	Exposed, southwest facing, dry ridge. *Salix polaris, Saxifraga oppositifolia* and biological soil are dominating. *Dryas octopetala* are frequent and *Cassiope tetragona* present to some extent.
Engelskbukta	ENGZ	431277	8755393	19.07.13	MES; IHK; MK; AB-326	B	Exposed, southwest facing, vegetated plain. *Salix polaris* and biological soil crust are dominating. *Saxifraga oppositifolia* and *Luzula confusa* are frequent, and *Bistorta vivpara* present to some extent.
Hotellneset*	HOT	511548	8686224	16.06.13 - 17.08.13	MES; IHK; JB	C	Exposed, northeast facing, elevated sea terrace. Biological soil crust is dominating. *Luzula confusa*, mosses, *Salix polaris, Saxifraga oppositifolia* and *Silene acaulis* are also frequent species.
Midtrehuken	MIDR	493890	8619280	11.08.13	AB-201	B	Exposed, southwest facing ridge in moraine area. Biological soil crust, lichens and rocks are dominating. *Salix polaris* and mosses are frequent, and *Saxifraga oppositifolia* present to some extent.
Midtrehuken	MIDZ	493890	8619280	11.08.13	AB-201	B	Exposed, southwest facing, vegetated slope in moraine area. Mosses, lichens and biological soil crust are dominating. *Salix polaris* and *Dryas octopetala* are also frequent.
Ringhorndalen	RINC	522801	8807362	07.07.13	MES; JB; MC	C	Sheltered, south facing, densely vegetated slope. *Dryas octopetala* and *Bistorta vivipara* are dominating species. Biological soil crust, *Carex* sp., *Salix polaris, Silene acaulis* and organic litter are also frequent.
Ringhorndalen	RINO	521833	8805969	07.07.13	MES; JB; MC	C	Sheltered, southwest facing, gradual slope. Bare ground consisting of fine-grained sand and grasses are dominating. *Bistorta vivipara, Salix polaris* and *Saxifraga oppositifolia* are present to some extent.
Ringhorndalen	RINS	521372	8806290	17.07.13	MES; IHK; MK; AB-326	C	Sheltered, south facing, vegetated slope. Sandy soil with *Dryas octopetala* and *Bistorta vivipara* dominating. *Cassiope tetragona* is surrounding the area.
Ringhorndalen	RINZ	521372	8806290	17.07.13	MES; IHK; MK; AB-326	C	Sheltered, south facing, vegetated slope. Sandy soil with *Dryas octopetala* and grasses dominating. Some biological soil crust also present.
Signehamna	SIG	427089	8801232	18.07.13	MES; IHK; MK; AB-326	B	Sheltered, east facing, highly disturbed, steep slope. Mosses, lichens and organic litter are dominating. *Salix polaris* and *Luzula* sp. are also frequent.

§ AB-201 = UNIS bachelor course 2013; AB-326 = UNIS master course 2013; IHK = Ingvil Henden Kålås; JB = John Bills; MES = Mildrid Elvik Svoen; MC = Microfun cruise 2013; MK = Manoj Kumar

side valley to Adventdalen, Endalen, dominated by dense *Dryas octopetala* heath vegetation, graminoids and mosses, with *Salix polaris* and biological soil crust being other frequent vegetation elements. This area had a lower density of *S. acaulis* individuals. The two small-scale study populations were also included in the 17 populations assessed in the large-scale study.

Figure 1. Map of sampling sites for the 17 *Silene acaulis* populations included in this study. The sampling sites are divided between 11 locations (see Table 1) across south-western to northern parts of Spitsbergen (Svalbard, Norway). Sites are marked with population ID (Pop ID; see Table 1).

Sampling design in large-scale study

Silene acaulis is known to have a recurrent patchy distribution of individuals at a rather confined scale (Gehring & Delph 1999). Within a limited area representative of the overall population density of *S. acaulis* at each site, plots of 2 x 2 m were randomly set out until the number of sampled individuals approached the desired sample size of 300. This sample size was considered sufficient to represent the true variation of demographic parameters in the given population. At some sites, a fixed number of ten plots was set out regardless of resulting sample size (these populations were sampled as part of student projects in the UNIS courses AB-201 and AB-326; Kumar et al. 2013; Johansen et al. 2013). Cushion size was measured as two mutually orthogonal cushion diameters (in north-south and east-west directions), and all individuals were assigned to one of three possible gender categories: females (Fs), hermaphrodites (Hs) and individuals with unknown gender (Us). Females and

hermaphrodites will from this point be defined as the two genders (used synonymously with sex) of *S. acaulis* individuals, even though hermaphrodites possess both female and male reproductive functions. Individuals infected with the anther-smut *Microbotryum violaceum* were assigned to the group of Us irrespective of their expressed gender phenotype, as this fungal parasite is known to cause abnormal flower development, which might bias sex ratios (Hermanutz & Innes 1994). Vegetation analyses were performed either using the point intercept method (Bråthen & Hagberg 2004), or through visual cover estimation of vascular plants, mosses, lichens, bare ground and biological soil crust. When the point intercept method was used, 0.5 x 0.5 m frames were randomly distributed in a subset of the sampling plots, and all vascular and cryptogam plant species that were hit by 25 evenly distributed points across each frame were recorded (allowing multiple hits at one point). Soil samples were taken in a subset of the sampling plots (after removing the uppermost vegetation layer).

The term 'population' will be used here when referring to a group of individuals that were sampled from one site, as each site comprised a distinct habitat within the given location. However, the different populations (as defined) are not necessarily reproductively isolated, and might be subpopulations in larger meta-populations. Two sites in Ringhorndalen with population ID (Pop ID) RINC and RINO were sampled before onset of flowering, and thus lack gender observations. These two populations were excluded from all analyses including gender as a parameter.

Sampling design in small-scale study

Within a limited area representative of the overall population density of *S. acaulis* at each of the two sites, three plots were set out to obtain a balanced study design with a minimum number of sampled individuals (ca. 500). The plot size varied between the two sites depending on the population density of *S. acaulis* (10 x 10 m in Endalen and 2 x 2 m at Hotellneset), but between-plot distances (20-60 m) were the same at both sites.

Within each plot, all *S. acaulis* individuals were labelled and mapped in a Cartesian coordinate system using meter as length unit. Cushion size and gender were recorded in the same manner as in the large-scale study. Leaf samples for genetic analyses were collected in plastic tubes from a random subset of 50 individuals within each plot (300 individuals in total for both sites) and frozen at -80°C the same day.

Vegetation analyses were performed using the point intercept method. Four 0.5 x 0.5 m frames were placed randomly within each plot, and all vascular and cryptogam plant species

that were hit by 25 evenly distributed points across each frame were recorded (allowing multiple hits at one point). Visual estimation of vegetation cover was also performed for each frame. One soil sample was taken in connection to each frame (after removing the uppermost vegetation layer). Data collection was performed throughout the period from June 16 to August 17, completing the specific parts of the sampling scheme (i.e. size measurements, gender determination, leaf sample collection, vegetation analyses and soil sampling) for all individuals at both sites within one week.

Size classes

Proxies for plant age can be estimated from growth rates (e.g. Benedict 1989). However, growth rates are closely related to conditions in the habitat where the studies are conducted, and the application of calculated growth rates based on individuals from other areas and habitat types must be performed with caution (Morris & Doak 2005). In this study, a modified version of the size class categories presented by Morris & Doak (1998) was used (Table Apx1). The modifications were based on later adjustments of the size classes by W. Morris and collaborators (personal correspondence). Modifications adding size ranges to the smallest size classes were also made, as only size measurements and not rosette counts were part of the sampling design of the current study.

Soil sample analyses

All soil samples were sieved using a 2 mm sieve to remove any larger objects (stones, roots, etc.). A mix of 2 parts soil and 4 parts MilliQ water in a 100 mL plastic bottle was shaken vigorously for 30 min before incubation at 4°C for overnight sedimentation. pH and conductivity were measured after sedimentation using a VWR SympHony SP70C pH-meter (VWR international, Radnor, PA, USA). The remaining samples (pre-dried at 105°C overnight) were further weighed in crucibles and dried at 105°C for 60 min, before two hours of burning at 500°C in a Nabertherm Controller B170 muffle furnace. The burnt samples were weighed again and the organic content of the soil (soil organic matter) was estimated as loss on ignition, defined as the percentage of sample weight lost after burning. Approximately 2 to 4 mg of the soil samples (pre-dried at 105°C overnight) were added to a thin foil cup before packing and sealing of the cups to remove all air. Total C and N content was calculated when the sealed cups with soil were burnt at 1000°C and the released gases were measured using an EA 1110 CHNS-O elemental analyser (CE Instruments Ltd, Wigan, UK).

DNA extraction

DNA was extracted from frozen leaf samples using the DNeasy Plant Mini Kit (QIAGEN GmbH, Germany) with its corresponding Quick-StartProtocol. Two tungsten carbide beads (3 mm) were added to each tube and the frozen samples were crushed using a Retsch MN400 shaker for 1 min at 20.0-23.0 1/s, before 400 μL Buffer AP1 was added and the samples shaken for another 30 s at the same frequency. The remaining protocol was followed without any modifications until the two elution steps at the end, where 50 μL Buffer AE was added instead of 100 μL to increase the final concentration of the extracted DNA.

All extracted samples were run on a 1.5% agarose gel to secure that the extractions were successful. Concentrations of the extracted DNA were measured with a Thermo Scientific NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and all samples were diluted to working solutions of 5 μmol/μL concentration.

Microsatellite analyses

Fifty primer sequences for microsatellite loci were developed by ecogenics (Zürich-Schlieren, Switzerland) using 454 sequencing. These sequences were derived from a *S. acaulis* individual collected in Endalen, Svalbard. The ability of the primers to amplify a product was further tested at The University Centre in Svalbard (UNIS) by Müller et al. (in prep), using another *S. acaulis* individual also collected in Endalen. Primer pairs that amplified a PCR product were further tested for polymorphism using eight individuals from Svalbard; three individuals collected from one population at Polheim, one individual collected at Edgeøya, one individual collected in Endalen (F0) and three individuals grown from seeds (F1) collected from the sampled individual in Endalen (F0). Each primer pair was tested using the M13-tailing method (Schuelke 2000) with the fluorescent colour 6-FAM. The 14 primer pairs that amplified polymorphic fragments across these eight individuals were applied in this study (Table 2). Fluorescent primers (Life Technologies/Applied Biosystems, Carlsbad, CA, USA) tagged with 6-FAM (blue), NED (yellow), VIC (green), or PET (red) were multiplexed into four mixes to enable co-runs of PCR and fragment analysis for up to four primer pairs at a time. Primer pair combinations and concentrations in the mixes followed the recommendations of Müller et al. (in prep).

The following PCR protocol was used (volumes correspond to n = 1 samples): 1.0 μL HotStarTaq buffer (QIAGEN GmbH), 4.35 μL MilliQ water, 1.0 μL 2mM deoxyribonucleotides (dNTPs), 1.6 μL primer mix (see Table 2 for primer multiplexing and

Table 2. Overview of microsatellite markers for *Silene acaulis* developed by Müller et al. (in prep) and applied in this study of *S. acaulis* populations in Svalbard, Norway. Primer ID denotes primer name from Müller et al. (in prep). Locus denotes primer name used in data matrixes of this study. Size bp denotes the base pair size range of alleles for the given locus in this study. Primer mix assign which primers were multiplexed in PCR and fragment analysis. Dye denotes the fluorescent colours that the primers were tagged with. Concentration [µmol/µL] denotes the concentration of the given marker in the PCR mix.

Primer ID	Locus	Primer sequence 5' - 3'	Repeat type §	Size bp	TA°	Primer mix	Dye	Concentration [µmol/µL]
Silaca 03*	P03	F GCGGATCTTGCTTGTGACG R TTTCTACTAGTGCCCGCAG	$(GTT)_6$,$(GTT)_8$, $(GTT)_5$,$(GTT)_6$	236-242	56	1	6-FAM	0.120
Silaca 25	P25	F AGCACAACTACACACACACG R TGGCGCATACCTTCATTCC	$(ATT)_8$	172-184	56	1	VIC	0.080
Silaca 38	P38	F CTTAGGCTTGTAACGCGGAG R CCCATGGACGGTTCTAAAGG	$(AAC)_8$	132-147	56	1	NED	0.080
Silaca 40	P40	F ACCAGCATGCAATATGAATGGG R AACAACCGCCTTCCTCAG	$(ATT)_{12}$	156-183	56	1	PET	0.120
Silaca 07	P07	F TGACTGGAAGTTAAGTGTGGTTC R AGAGAGTATGGTAGGTGGGG	$(TAA)_8$	205-226	56	2	6-FAM	0.120
Silaca 29	P29	F GCCAAAACACGAAAACCCG R TGGTGGTTCTGTGGTGGAG	$(ATT)_6$	200-206	56	2	VIC	0.080
Silaca 32	P32	F GATTCATGTTAGCCGACCCC R TGCTGCAGTATTAGTGTTTGTG	$(TGT)_8$	144-177	56	2	NED	0.080
Silaca 18	P18	F ACAAGTCGGATCAAGTGTTGG R GCTCAACAGACCGGAATGC	$(AAAT)_6$	165-173	56	2	PET	0.120
Silaca 23	P23	F CCAGCAACACCAGCAGAAG R CCATGGAACATGTGTATGGAGC	$(ATG)_6$	236-245	56	3	6-FAM	0.120
Silaca 44	P44	F AGTAGTTATACAAGTGGTGGTGG R TCCTCTATGAACTCGCTGCC	$(ATT)_{10}$	210-216	56	3	VIC	0.080
Silaca 08	P08	F CACTACTCAGAAAAGGTCAATTGTG R GGGAATCCAAGAAGGTGGC	$(TTA)_6$	215-239	56	3	NED	0.080
Silaca 34	P34	F TCACCGATGGTCGTCAAGG R AGGCTCTCAACTAGGATTCGG	$(CAA)_7$	171-196	56	3	PET	0.120
Silaca 50	P50	F AAGACTCGGGAGAAACCAC R CTCTTGACTCTCTACCTCCCC	$(ATC)_6$	233-239	56	4	6-FAM	0.120
Silaca 36	P36	F ACCCTCCTTACGTTCCTAATTC R ATGTAGGCGTGACGAAGGC	$(TCC)_8$	165-180	56	4	VIC	0.080

§ Based on genomic DNA sequences analyzed on a Roche 454 GS-FLX platform

° Annealing temperature (°C)

*Marker that was excluded in the final analyses

primer concentrations), 0.05 μL HotStarTaq polymerase (QIAGEN GmbH) and 2 μL diluted sample DNA. The PCRs were conducted with an AB GeneAmp PCR System 9700 v. 3.09 under conditions of initial denaturation at 95°C for 15 min, followed by 30 alternating cycles of denaturation, annealing and elongation at 95°C, 56°C and 72°C, respectively, for 30 s each. The final elongation step after finishing the last cycle was held for 2 min at 72°C, before the temperature decreased to a final hold temperature of 10°C. Random subsets of the PCR products were run on a 2% agarose gel to secure that the PCRs had worked properly before sending them for further analyses. Two negative controls were included on each 96-well plate.

The PCR products were diluted ten times before they were sent to the DNA sequencing lab at the University Hospital for North Norway (UNN) in Tromsø for fragment analysis. The fragment analyses were run on an Applied Biosystems 3130xl Genetic Analyzer using GS500LIZ (Life Technologies/Applied Biosystems) as size standard. The returning fragment length peaks were assessed using the program GENEIOUS 7.1.3 (Biomatters Ltd., Aukland, New Zealand). Microsatellite genotypes were scored through an automated software function and the outcome edited manually to ensure that the resulting genotypes were reasonable (e.g. that a true homozygote was not misinterpreted to be a heterozygote because of stutter peaks or noise), resulting in a genotype matrix containing allele sizes (base pair length).

Ninety-six replicate PCRs were run (corresponding to approximately 8% of the PCR runs) and additionally, 50 to 100 PCRs were replicated through re-runs of PCRs for individuals and primers that were unsatisfactorily amplified the first time (giving a total of > 12.5% replicates). Replicates were compared to original runs to confirm that genotypes corresponded, and then removed from the genotype matrix. Individuals with missing data for five or more (> 31%) markers and markers with missing data for 37 or more (> 16%) individuals were also removed from the final matrix. These thresholds were arbitrarily chosen trying to balance between improving the quality of the genotype matrix and keeping the sample size and number of loci as high as possible.

Statistical data analyses

Demographic and genetic data matrixes

Three matrixes, one including demographic data for all 17 large-scale populations, one including gender data and additional demographic data for 15 large-scale populations (excluding RINO and RINC) and one including demographic data and spatial coordinates for

the two small-scale populations, were applied in the statistical analyses of demographic population properties. The genotype matrix with allele sizes was used as an input file for the software CONVERT 1.13 (Glaubitz 2004), which was applied to create appropriate input files for statistical analyses of genetic population properties in ARLEQUIN 3.5.1.3 (Excoffier & Lischer 2010), STRUCTURE 2.3.4 (Pritchard et al. 2000) and various packages implemented in R 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria). The input files for the GENALEX 6.5 (Peakall & Smouse 2012) and COANCESTRY 1.0.1.5 (Wang 2010) softwares were prepared manually from the original genotype matrix. Subsets for Endalen and Hotellneset were created through CONVERT, or within the appropriate software when possible. The genetic matrix including both small-scale populations is referred to as the 'small-scale dataset', while subsets for Endalen and Hotellneset are referred to as the 'Endalen subset' and the 'Hotellneset subset'. One matrix including the spatial coordinates of all genotyped individuals was used in analyses that combined genetic and spatial information of individuals.

Description of sampling sites and definition of open versus closed habitats

Ordination analyses are used in ecology to provide a low-dimensional representation of the main structure in a matrix of species abundances that have been recorded in a set of sites or plots (Son & Halvorsen 2014). Two different types of ordination method were applied in this study to visualize the differentiation between the two habitats (open and closed) of the small-scale study: detrended correspondence analyses (DCA; Hill & Gauch 1980) and global non-metric multidimensional scaling (GNMDS; Kruskal 1964). The vegetation scores obtained from the point intercept hits were transformed before application through an intermediate weighting method (Son & Halvorsen 2014), giving unhit species that were present but not hit a score of 1 (minimum) and species that were hit 25 times a score of 5 (maximum). The transformation of hit values was performed by applying $y_j = 2 + \ln(x_j)$, where x_j is the number of hits for a species j, and y_j is the transformed hit value (personal correspondence with Rune Halvorsen). The DCA was carried out through the *decorona()* function implemented in the *vegan* package (Oksanen et al. 2012) in R with default settings. Site scores of the two first ordination axes were obtained and plotted against each other in an ordination diagram, revealing the main structures of species abundance among the vegetation frames. Edaphic variables were fitted onto the ordination diagram through the *envfit()* function implemented in the *vegan* package, to relate the abundance structure to measured edaphic properties. Main vegetation elements causing variation along the two first ordination axes were also fitted onto

the ordination diagram using the same function. The GNMDS was performed using the *vegdist()*, *isoMDS()* and *initMDS()* functions implemented in the *vegan* and *MASS* packages (Venables & Ripley 2002) in R with the following settings: Bray-Curtis dissimilarities, number of axes = 3, number of random starts = 100, maximum number of iterations = 1000 and stress convergence criterion = 10^{-7}. The minimum stress solution was detected, and the two first ordination axes obtained and plotted against each other in an ordination diagram. Edaphic variables and vegetation elements were fitted onto the ordination diagram in the same manner as for the DCA. Correspondence between the first and second ordination axes between the DCA and GNMDS ordinations was tested using Kendall's tau through the *cor.test()* function implemented in the *stats* package (R Core Team 2012) in R.

A threshold value separating the two vegetation cover categories: open and closed (used synonymously with open and closed habitat types), was defined based on vegetation cover values of the two small-scale habitats, Hotellneset (open) and Endalen (closed). Vegetation cover values, based on cover estimates of vascular plants from different vegetation cover estimation methods, were assigned to all sites. The threshold value was then used to assign each site to one of the two vegetation cover groups.

Establishment performance

Abundance and size distribution of *S. acaulis* in the two habitat types were assessed at both geographical study scales. Calculations of population density, size averages and corresponding standard deviations (SD) at various hierarchical levels (including small-scale and large-scale populations) were performed through the *mean()* and *sd()* functions implemented in the *base* and *stats* packages (R Core Team 2012) in R. A one-sided Wilcoxon rank sum test was performed though the *wilcox.test()* function implemented in the *stats* package in R with default settings to test if population densities were significantly higher in open habitats compared to closed habitats. Comparison of size averages in different habitat types was performed using generalized linear modelling (GLM) through the *glm()* function implemented in the *stats* package in R with default settings. Size values were square root transformed to acquire a better fit of the data to the normal distribution.

Fine-scale spatial patterns were assessed in the small-scale populations through estimates of Ripley's K function (Ripley 1976), using a matrix with Cartesian coordinates and demographic parameters for all sampled individuals in the two populations. Ripley's K function estimates the spatial distribution of points (individuals) by assessing the number of

additional random points within a distance r from a typical random point in an observed point distribution, and compares the observed spatial distribution of individuals $K_{iso}(r)$ to the expectations for a random spatial distribution $K_{pois}(r)$. Estimates of Ripley's K function were obtained through the *Kest()* function implemented in the *spatstat* package (Baddeley & Turner 2005) in R, choosing Ripley's isotropic edge correction method (iso; $K_{iso}(r)$) and comparing it to the theoretical value for a stationary Poisson process (theo; $K_{pois}(r)$). A Wilcoxon rank sum test was performed through the *wilcox.test()* function with default settings on deviations of $K_{iso}(r)$ from $K_{pois}(r)$ (adjusted for maximum value of r) at plot level, to test if the amount of spatial clustering was significantly different between the two sites.

Gender-specific establishment and onset of reproduction

Sex ratios, gender-specific size distributions and flowering frequencies in different size classes were inspected at both study scales to assess gender-specific establishment performance and onset of reproduction in the two habitat types. Sex ratios of the populations were estimated through calculation of female frequencies, measured as the proportion of females relative to the total number of reproducing individuals, that is, individuals sexed as females (Fs) or hermaphrodites (Hs), in the population. A Pearson's Chi-squared test was performed on numbers of Fs and Hs in Endalen and Hotellneset through the *chisq.test()* function implemented in the *stats* package in R, to check if there was a significant difference in female frequency between the two small-scale populations. Correspondingly, a two-sided Wilcoxon rank sum test was performed though the *wilcox.test()* function implemented in the *stats* package in R with default settings, to test if there was a significant difference in female frequency between open and closed habitat sites in the large-scale populations.

To assess relationships between size and gender at various hierarchical levels (including site, plot and/or habitat type in small-scale and large-scale populations), GLM was performed through the *glm()*function implemented in the *stats* package in R with default settings and square root transformed size values. Size estimates and standard errors (SE) for gender groups were back-transformed to cm before further application in tables and comparisons.

Flowering frequencies in different size classes were measured as the proportion of reproducing individuals relative to the total number of individuals within the given size class in a population. A two-sided Wilcoxon signed rank test was performed though the *wilcox.test()* function implemented in the *stats* package in R with paired test settings, to check

if flowering frequencies in different size classes were significantly different between open and closed habitats.

Genetic diversity and structure

Analyses of molecular variance (AMOVA; Excoffier et al. 1992) assign the relative amount of genetic variation to different hierarchical levels, and were performed in ARLEQUIN with 999 permutations, including variation at three hierarchical levels: variation between sites, variation among plots within sites and variation within plots. The analyses were carried out on the small-scale dataset including both Endalen and Hotellneset individuals, and for the Endalen and Hotellneset subsets separately (then only including the last two levels of variation).

Bayesian clustering analyses by STRUCTURE were used to identify the appropriate number of genetic clusters, K, and assign each individual to one or several (if genotypes are admixed) of these clusters (Pritchard et al. 2000). Such analyses were performed for the small-scale dataset, and for Endalen and Hotellneset subsets independently, using the following settings: max K = 10, max runs per K = 10, burnin = 100000, # reps = 1000000, noadmix = 0 and linkage = 0. STRUCTURE results were uploaded and evaluated in STRUCTURE HARVESTER (Earl & vonHoldt 2011), a software package which presents graphs of mean likelihood and variation per K, in addition to the ad hoc statistic delta K (Evanno et al. 2005). Most emphasis was placed on delta K when selecting the probable number of Ks, as this method predicts well the real number of clusters (Evanno et al. 2005). A random STRUCTURE run of the chosen number of Ks was further selected and run through DISTRUCT (Rosenberg 2004) to visualize the results.

Principal component analysis (PCA) is a multivariate ordination method that assesses the main structures of a matrix in order to find groups of similar individuals (Hotelling 1933). PCAs were run on the separate Endalen and Hotellneset subsets, and within each site individuals were coloured according to sampling plot, using the *dudi.pca()* and *s.class()* functions implemented in the *ade4* package (Chessel et al. 2004) in R with default settings. Correspondingly, PCAs were run including individuals within one sampling plot at a time, and the two first ordination axes were obtained and plotted against each other in an ordination diagram. The various plots were inspected to check if genetic groups corresponding to the sampling plots or any other genetically similar groups could be detected.

Mantel's tests assessing the correlation between two matrixes (Mantel 1967) were performed on corresponding distance matrixes of genotype and spatial coordinate data for the two small-scale populations. Tests were carried out including individuals within site and within plots using the *mantel.randtest()* function implemented in the *ade4* package in R with default settings.

F_{ST} values assessing the level of differentiation among subpopulations (Hamilton 2009) were calculated using GENALEX for pairwise comparison of the difference between Endalen and Hotellneset.

Observed heterozygosity (H_o) and expected heterozygosity (H_e) can be used as indicators of random mating and gene dispersal in (or between) populations (Hamilton 2009). Measures of H_o and H_e were obtained through the *adegenet* package (Jombart 2008) in R. This package stores individual genotypes in an object called 'genind'. The *summary()* function implemented in the *base* package of R was applied on the genind object to extract values of H_o and H_e per locus for the small-scale data set, and for the Endalen and Hotellneset subsets. A two-sided Wilcoxon signed rank test was performed though the *wilcox.test()* function with default settings, to check if H_o was significantly different from H_e within site. Further, a two-sided Wilcoxon signed rank test was performed with the paired test settings, to test whether the deviation of H_o from H_e per locus were significantly different between Endalen and Hotellneset.

Average linkage disequilibrium (LD) values between loci (Hamilton 2009) were calculated at plot level in ARLEQUIN, and averaged for each site using the *mean()* and *sd()* functions implemented in the *base* package of R. A one-sided Wilcoxon rank sum test was performed on plot values though the *wilcox.test()* function with default settings to see if linkage disequilibrium values were significantly higher at Hotelnesset than in Endalen.

Relatedness and inbreeding estimates were performed using the triadic likelihood estimator (TrioML; Wang 2007) implemented in COANCESTRY. This method uses a third individual as control (reference) when estimating the pairwise relatedness (r) between two individuals, and can also be applied to calculate individual inbreeding coefficients (F) based on multilocus genotypes. Both estimates have a value range of [0,1]. The analyses in COANCESTRY were run with the following settings: accounting for inbreeding, # reference individuals = 224, # bootstraps = 100 and # threads = 10. Identical analyses were run using the moment estimators QuellerGt (Queller & Goodnight 1989) and LynchRd (Lynch & Ritland 1999), and their correlations with the TrioML method were tested through an

implemented correlation test function in the software to check the robustness of the TrioML results. The implemented bootstrapping test of group differences was also applied to test if the estimates of r and F were significantly different between sites, among plots between and within sites, among individuals within plots and between plots (within sites) and between gender groups. The number of bootstrap samples was set to 10000 for these tests.

RESULTS

Habitat differentiation

Vegetation cover values (based on cover of vascular plants) were 0.49 for Endalen and 0.39 for Hotellneset. Ordination analyses of vegetation data through the DCA and GNMDS methods gave well corresponding results, confirmed by the Kendall's rank correlation tau that detected high correlation between both first ordination axes ($\tau = 0.83$, $T = 252$, $p < 0.001$) and second ordination axes ($\tau = 0.83$, $T = 253$, $p < 0.001$) of the two methods. Only the DCA results are reported (Fig. Apx1). The two habitats in the small-scale study were well separated along the first ordination axis, while plots within sites were separated mainly along the second axis. Presence of *Dryas octopetala*, *Minuartia* sp. and lichens were the main vegetation elements differentiating the two sites, with higher abundance of *D. octopetala* and lichens in Endalen and *Minuartia* sp. at Hotellneset. Presence of *Luzula confuza* and biological soil crust additionally separated plots within sites. Nutrition levels (amount of nitrogen in the soil) and, to some extent, the amount of organic matter in the soil and pH increased when moving from plots in Endalen to plots at Hotellneset, and were the main edaphic parameters differentiating the two sites (Fig. Apx1). Soil organic matter and pH were also important for differentiation along the second axis.

Vegetation cover values ranged from 0.14 to 0.39 for open habitat sites and from 0.40 to 0.76 for closed habitat sites, while no trends were apparent for edaphic parameters between sites of the two habitat types (Table Apx2).

Large-scale demography

Demographic data of 4136 individuals from 17 populations were collected (Table 3). The number of sampled individuals within one site varied from 80 to 545, and the number of plots within a site ranged from 3 to 40.

Table 3. Demograhic data for 4136 *Silene acaulis* individuals from small-scale and large-scale populations in Svalbard, Norway. Fs denotes females, Hs hermaphrodites and Us individuals of unknown gender. Fem freq denotes female frequency calculated as the proportion of females relative to the total number of reproducing individuals in the population. For explanation of Pop ID, see Table 1.

Pop ID	Vegetation cover §	Plot size (cm)	# plots	# individuals (genetic analyses)	Average size (cm) ± SD	Smallest individual (cm)	Largest individual (cm)	Smallest flowering (cm)	Largest flowering (cm)	# Fs	# Hs	# Us	Fem freq	Average size Fs ± SD	Average size Hs ± SD	Average size Us ± SD
Small-scale populations																
END	Closed	10x10	3	491 (112)	7.87 ± 6.13	0.50	33.75	0.50	33.75	147	130	214	0.53	10.50 ± 6.37	9.69 ± 6.15	4.96 ± 4.53
HOT	Open	2x2	3	545 (112)	4.26 ± 4.58	0.50	30.00	1.75	30.00	71	72	402	0.50	9.59 ± 5.74	8.98 ± 4.63	2.49 ± 2.61
Large-scale populations																
ANKR	Open	2x2	10	123	6.85 ± 5.48	1.00	34.50	2.00	25.50	31	25	68	0.55	7.17 ± 4.63	9.55 ± 6.00	5.71 ± 5.35
ANKZ	Open	2x2	10	190	10.65 ± 7.52	0.50	41.50	2.00	31.30	14	43	134	0.25	11.69 ± 4.84	14.11 ± 7.49	9.42 ± 7.44
BLMS	Open	2x2	6	337	5.23 ± 6.83	0.50	42.00	0.50	42.00	99	41	197	0.71	10.07 ± 9.02	8.43 ± 7.61	2.13 ± 1.93
BLOZ	Closed	2x2	19	112	21.99 ± 13.57	1.00	62.50	1.75	62.50	35	19	58	0.65	23.03 ± 15.24	23.42 ± 10.46	20.90 ± 13.54
BOC	Open	2x2	5	288	3.18 ± 5.11	0.50	33.25	3.00	33.25	29	16	243	0.64	10.21 ± 6.63	15.31 ± 8.65	1.54 ± 1.68
COLR	Closed	2x2	5	150	7.91 ± 6.74	0.50	41.00	2.00	41.00	55	52	43	0.51	8.80 ± 7.51	10.00 ± 6.49	4.24 ± 4.12
ENGR	Open	2x2	10	434	8.03 ± 5.58	1.00	18.75	1.00	18.75	230	176	28	0.57	6.18 ± 2.99	6.34 ± 2.63	3.40 ± 1.88
ENGZ	Open	2x2	10	80	12.86 ± 8.56	1.75	35.50	3.00	35.50	37	14	29	0.73	12.20 ± 7.28	13.89 ± 9.31	9.86 ± 13.21
MIDR	Open	2x2	10	154	7.09 ± 5.86	0.50	31.50	2.75	31.50	12	10	133	0.55	11.42 ± 7.10	10.98 ± 6.65	9.86 ± 6.41
MIDZ	Closed	2x2	10	181	7.99 ± 5.60	0.75	39.50	4.00	39.50	23	18	141	0.56	10.07 ± 5.15	13.82 ± 7.99	6.91 ± 4.73
RINC*	Closed	2x2	8	287	9.65 ± 7.57	0.50	39.00	-	-	-	-	-	-	-	-	-
RINO*	Closed	2x2	38	223	13.79 ± 12.13	1.00	65.00	-	-	-	-	-	-	-	-	-
RINS	Closed	2x2	20	117	11.30 ± 12.64	0.50	65.00	2.25	65.00	35	22	60	0.61	17.81 ± 14.61	9.44 ± 6.05	8.19 ± 11.90
RINZ	Closed	2x2	7	178	9.42 ± 8.31	0.50	55.00	1.75	36.00	83	41	54	0.67	10.72 ± 6.67	9.12 ± 6.45	7.65 ± 11.16
SIG	Open	2x2	40	246	11.77 ± 8.42	0.50	42.25	2.00	42.25	72	33	141	0.69	13.07 ± 8.62	15.13 ± 7.47	10.32 ± 8.25

§ Refers to the two vegetation cover categories defined in this study, see Materials and methods; Sampling design in large-scale study

* No gender data were available for these populations, consequently they were excluded from all gender analyses

Establishment performance

Population densities of *S. acualis* were generally higher in open habitats (n per m^2 ± SD = 11.11 ± 13.82) compared to closed habitats (n per m^2 = 4.17 ± 3.11), and according to the Wilcoxon rank sum test the association between open habitats and higher population densities was close to significance (W = 52, p = 0.07). The size distribution of *S. acaulis* individuals in open habitats were more left-skewed than in closed habitats (Fig. 2A and B). GLM confirmed that the expected cushion size (± SE) in open habitats (5.18 cm ± 0.00) was significantly lower than in closed habitats (8.53 cm ±0.00; t = 16.9, p < 0.001, AIC = 13338). However, the variation among sites was high within both habitat types (Table 3).

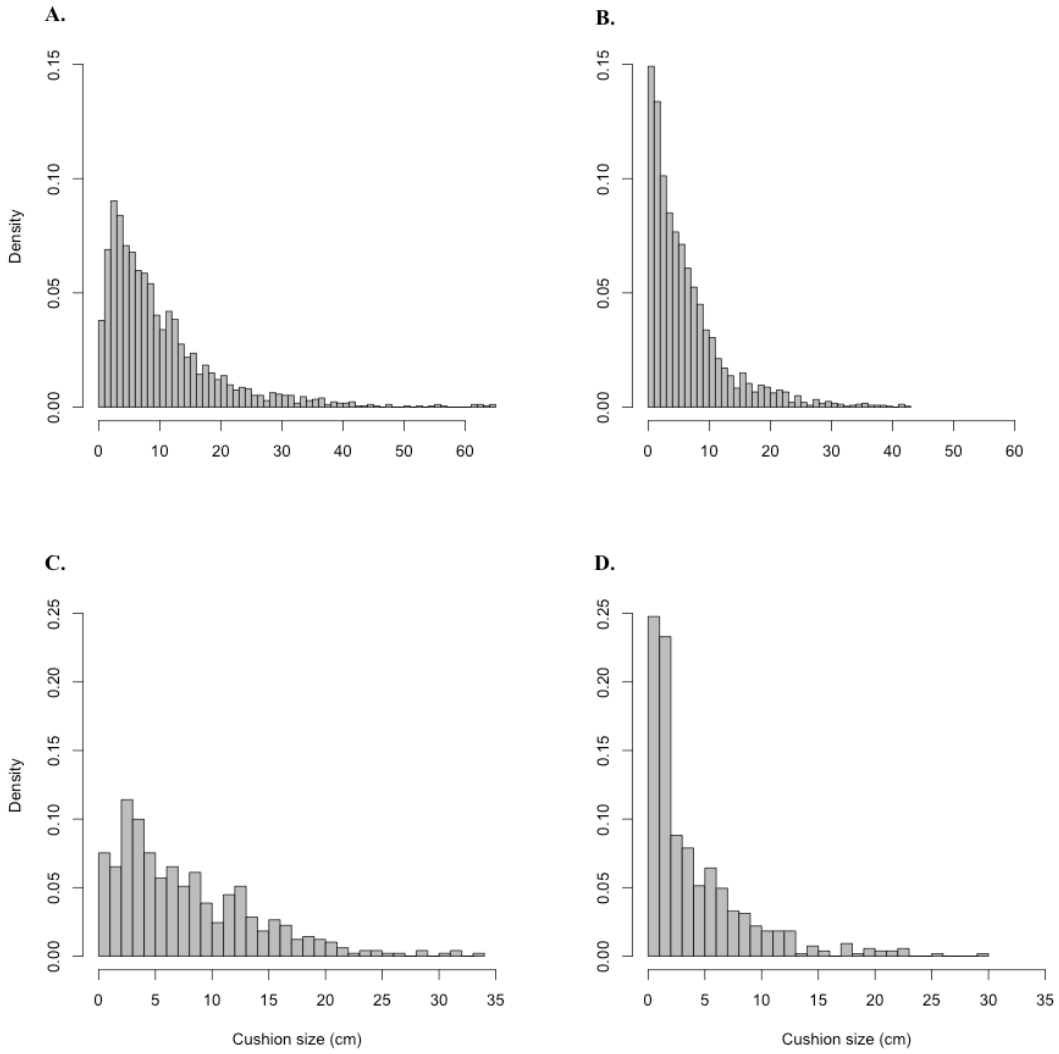

Figure 2. Density distribution of *Silene acaulis* cushion size (cm) in populations sampled in Svalbard, Norway. Averages for all 17 large-scale populations are shown in **A.** for closed habitats (eight populations) and **B.** for open habitats (nine populations), while small-scale populations are shown in **C.** Endalen (closed habitat) and **D.** Hotellneset (open habitat).

Gender-specific establishment and onset of reproduction

The overall female frequency (± SD) for all 17 populations was 0.58 ± 0.12. The average female frequency for all populations in closed habitats was 0.59 ± 0.15, while it was slightly lower (0.57 ± 0.06) when considering only open habitat populations (Table 3). The Wilcoxon rank sum test performed over all site values identified no significant difference in the female frequency between open and closed sites (W = 25, p = 0.86).

Average sizes of Fs and Hs were significantly larger than the average size of Us, but Fs and Hs were not significantly different in size from each other (Table 4A). However, all three gender groups had significantly larger average size in closed habitat populations compared to open habitat populations (Table 4A).

Flowering frequencies in different size classes were not different in open and closed habitat populations (Fig. 3A; Wilcoxon signed rank test, V = 28, p = 0.55).

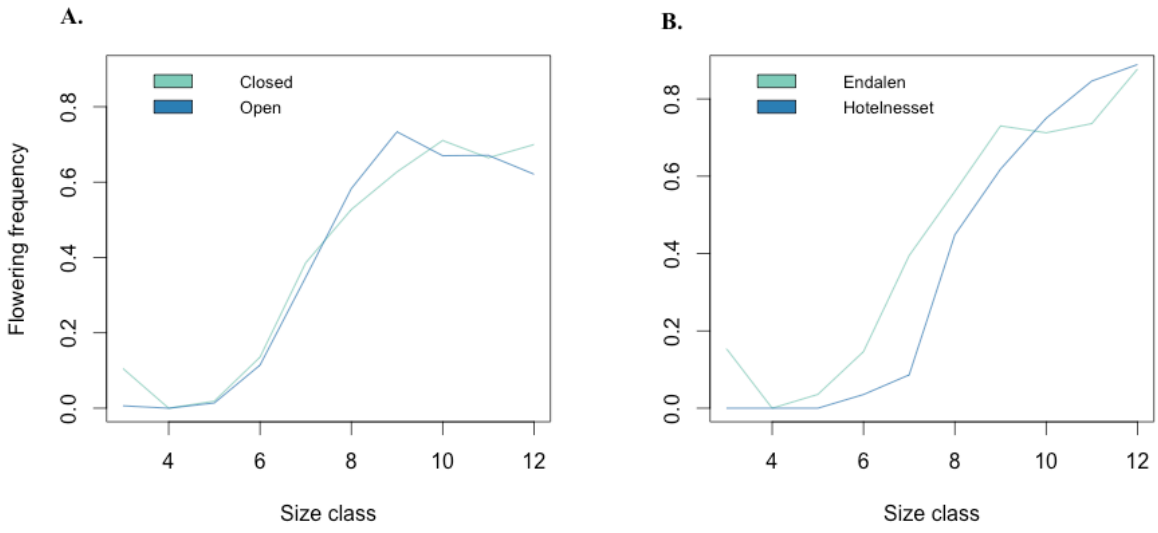

Figure 3. Flowering frequency in *Silene acaulis* size classes in **A.** closed habitat populations and open habitat populations (large-scale study), and **B.** Endalen (closed habitat) and Hotellneset (open habitat; small-scale study), Svalbard, Norway. Flowering frequencies are measured as the proportion of reproducing individuals relative to the total number of individuals within the given size class in a population.

Small-scale demography

In the small-scale study, 491 individuals were sampled from three 10 x 10 m plots in Endalen, and 545 individuals from three 2 x 2 m plots at Hotellneset (a total of 1036 individuals; Table 3).

Establishment performance

The demographic patterns found in the more detailed small-scale study were congruent with

Table 4. Generalized linear modelling (GLM) of assosiations between size and gender in the **A.** large-scale and **B.** small-scale study of *Silene acaulis* populations in Svalbard, Norway. The complete dataset includes individuals from both closed and open habitat populations (Endalen and Hotellneset in the small-scale study). END and HOT denotes Endalen and Hotellneset. Gender groups are assigned as females (Fs), hermaphrodites (Hs) and individuals with unknown gender (Us). The modelling was performed using the *glm()* function implemented in R 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria) on square root transformed size values applying normal distribution as distribution family. Significant p-values are marked with *.

Dataset	Group 1	Average size group 1 ± SE (cm)	Group 2	Average size group 2 ± SE (cm)	Difference (group 1 - group 2)	AIC §	t value	p value	
A. Large-scale study									
Complete	Fs	8.97 ± 0.13	Hs	8.93 ± 0.00	0.04	11106	-0.11	0.91	
	Fs	8.97 ± 0.13	Us	4.06 ± 0.00	4.91	11106	-22.36	< 0.01	*
	Hs	8.93 ± 0.00	Us	4.06 ± 0.00	4.87	11106	-19.92	< 0.01	*
Closed	Fs	10.65 ± 0.00	Hs	9.72 ± 0.00	0.93	10955	-1.69	0.09	
	Fs	10.65 ± 0.00	Us	5.91 ± 0.00	4.74	10955	-11.49	< 0.01	*
	Hs	9.72 ± 0.00	Us	5.91 ± 0.00	3.81	10955	-8.65	< 0.01	*
Open	Fs	7.98 ± 0.00	Hs	8.43 ± 0.00	-0.45	10955	1.15	0.25	
	Fs	7.98 ± 0.00	Us	3.40 ± 0.00	4.58	10955	-18.30	< 0.01	*
	Hs	8.43 ± 0.00	Us	3.40 ± 0.00	5.03	10955	-17.57	< 0.01	*
Between groups	Fs closed	10.65 ± 0.00	Fs open	7.98 ± 0.00	2.67	10955	-6.10	< 0.01	*
	Hs closed	9.72 ± 0.00	Hs open	8.43 ± 0.00	1.29	10955	-2.56	0.01	*
	Us closed	5.91 ± 0.00	Us open	3.40 ± 0.00	2.51	10955	-10.78	< 0.01	*
B. Small-scale study									
Complete	Fs	9.30 ± 0.00	Hs	8.68 ± 0.00	0.62	2618	-1.23	0.22	
	Fs	9.30 ± 0.00	Us	2.69 ± 0.00	6.61	2618	-20.96	< 0.01	*
	Hs	8.68 ± 0.00	Us	2.69 ± 0.00	5.99	2618	-18.89	< 0.01	*
Endalen	Fs	9.53 ± 0.00	Hs	8.82 ± 0.01	0.71	2553	-1.19	0.23	
	Fs	9.53 ± 0.00	Us	4.10 ± 0.00	5.43	2553	-12.01	< 0.01	*
	Hs	8.82 ± 0.01	Us	4.10 ± 0.00	4.72	2553	-10.28	< 0.01	*
Hotellneset	Fs	8.81 ± 0.01	Hs	8.45 ± 0.01	0.36	2553	-0.45	0.65	
	Fs	8.81 ± 0.01	Us	2.05 ± 0.00	6.76	2553	-14.43	< 0.01	*
	Hs	8.45 ± 0.01	Us	2.05 ± 0.00	6.40	2553	-13.92	< 0.01	*
Between sites	Fs END	9.53 ± 0.00	Fs HOT	8.81 ± 0.01	0.72	2553	-0.99	0.32	
	Hs END	8.82 ± 0.00	Hs HOT	8.45 ± 0.01	0.37	2553	-0.52	0.60	
	Us END	4.10 ± 0.00	Us HOT	2.05 ± 0.00	2.05	2553	-8.45	< 0.01	*

§ Akaike information criterion

the patterns found in the large-scale study. The density of individuals was markedly higher in the open habitat at Hotellneset, where the plot size giving ca. 500 sampled individuals per site was 25 times smaller (2 x 2 m) than in the closed habitat in Endalen (10 x 10 m). The size distribution of the Hotellneset individuals was more left-skewed than that of Endalen (Fig. 2C and D). GLM confirmed that the expected cushion size (± SE) of individuals was significantly greater in Endalen (6.72 cm ± 0.00) compared to Hotellneset (3.34 cm ± 0.00; $t = -12.11$, $p <$

0.01, AIC = 2976). This was also the overall trend when comparing the two sites at plot level (data not shown).

Inspections of the spatial distribution of individuals at plot level revealed that more spatial clustering was present in Endalen compared to Hotellneset. Estimates of Ripley's K-function, $K_{iso}(r)$, were generally higher than expected for a random spatial distribution, $K_{pois}(r)$, in the plots in Endalen (Fig. Apx2A, B and C), while this was not the case for the plots at Hotellneset (Fig. Apx2D, E and F). However, the difference between sites was not significant when performing a Wilcoxon rank sum test on deviations of $K_{iso}(r)$ from $K_{pois}(r)$ (adjusted for maximum value of r) at plot level ($W = 9$, $p = 0.1$).

Gender-specific establishment and onset of reproduction

The female frequency was higher in Endalen (ratio = 0.53) than at Hotellneset (ratio = 0.50; Table 3), but this difference was not significant when performing a Pearson's Chi-squared test on number of Fs and Hs at the two sites ($\chi^2 = 0.32$, $df = 1$, $p = 0.57$).

GLM revealed that the average size of the Us was significantly lower compared to those sexed as females or hermaphrodites when considering the complete dataset including both Endalen and Hotellneset individuals (Table 4B). However, no such significant association was found when comparing the size of Fs and Hs. A corresponding pattern was found when investigating the relationship between size and gender in Endalen and Hotellneset separately (Table 4B). The size averages of all three gender groups were higher in Endalen compared to Hotellneset, but unlike the large-scale populations this difference was only significant for the Us (Table 4B).

The frequency of flowering individuals appeared higher in lower size classes in Endalen compared to Hotellneset (Fig. 3B). However, the Wilcoxon signed rank test revealed that the difference between the two sites was not significant ($V = 37$, $p = 0.10$).

Small-scale population genetics

The final genotype matrix consisted of 224 individuals; 112 individuals per site with 36 to 38 individuals per plot. All 14 microsatellite loci were polymorphic across the 224 individuals included in the genetic analyses, but one locus was excluded due to high levels of missing data. The number of alleles per locus ranged from 2 to 7 with an average of 4.3 alleles per locus. The percentage of missing data within individuals ranged from 0% to 30.8%, within

plots from 1.2% to 8.1% and within loci from 0.9% to 15%, with an average of 4.9% missing data per locus.

Genetic diversity and structure

Genetic analyses revealed low differentiation overall at all hierarchical levels. The AMOVA with three levels assigned 6.1% of the variation between sites, 1.2% among plots within sites and 92.8% within plots. Slightly less variation was explained among plots within sites (0.1%) when performing hierarchical AMOVAs on Endalen and Hotellneset subsets separately. Bayesian clustering analyses performed in STRUCTURE and PCA analyses corresponded well with the AMOVA results. STRUCTURE identified weak clustering corresponding to sites and no clustering corresponding to plots within sites (Fig. Apx3), while PCA of fine-scale genetic structure found little genetic differentiation among individuals of different sampling plots in both sites, with a cumulative inertia for the two first principal component axes (i.e. amount of variation explained by the given axes relative to the total explainable variation) of 17.58% in Endalen and 13.50% at Hotellneset (Fig. 4). When including only individuals within one sampling plot at a time, the two first ordination axes explained somewhat more variation, with

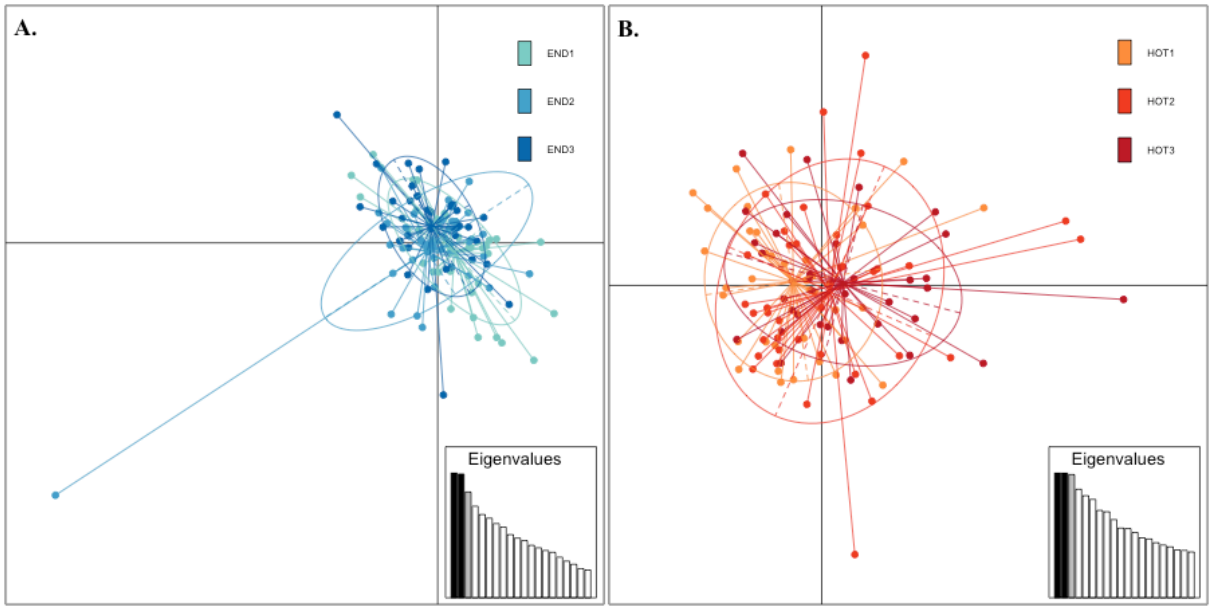

Figure 4. Principal component analysis (PCA) of genetic variability among *Silene acaulis* individuals in Svalbard, Norway, within **A.** Endalen and **B.** Hotellneset. First (horizontal) and second (vertical) principal component axes are plotted, and individuals grouped after sampling plots (corresponding to colour). The cumulative inertia for the two first principal component axes (i.e. amount of variation explained by the given axes relative to the total explainable variation) was 17.58% for the Endalen subset and 13.50% for the Hotellneset subset. Eigenvalues corresponding to the two first principal components are filled in black. Points are connencted to the group means of sampling plots with corresponding colours, while the ellipses show the 95% inertia for the given sampling plot. The outlaying individual in END2 (**A.**) was checked, but no genotyping errors were detected and the outcome of the PCA was not changed when excluding this individual.

cumulative inertias ranging from 22.98% to 32.14% (Fig. Apx4). Mantel's tests displayed no positive relationship between geographic and genetic distances, in either Endalen (observation (obs) = -0.006, expectation (exp) = -0.001, variance, (var) = 0.001, p = 0.55), or at Hotellneset (obs = 0.017, exp = -0.002, var = 0.001, p = 0.31). Tests including only individuals within plots were also non-significant for all plots at both sits (data not shown). The F_{ST} was 0.033 when performing a pairwise comparison of the Endalen and Hotellneset populations.

Expected heterozygosity (H_e) ranged from 5.9% to 55.9% for different loci, with an average of 37.8%, while observed heterozygosity (H_o) ranged from 4.2% to 58.2% per locus, with an average of 25.5%. The Wilcoxon signed rank test found that H_o was significantly different from the expected heterozygosity (H_e) when including both sites (average deviation (dev) ± SD = 0.12 ± 0.10, V = 91, $p < 0.001$), when only the Endalen subset was considered (dev = 0.10 ± 0.08, V = 90, $p < 0.001$) and when only the Hotellneset subset was examined (dev = 0.12 ± 0.12, V = 88, p = 0.001; Fig. 5). The Wilcoxon signed rank test revealed that the deviation of H_o from H_e per locus was not significantly higher at Hotellneset than in Endalen (W = 84, p = 0.52). The average linkage disequilibrium (LD) was higher at Hotellneset (LD ± SD = 1.85 ± 0.71) than in Endalen (LD = 1.79 ± 0.76), but not significantly (Wilcoxon rank sum test, W = 5, p = 0.5).

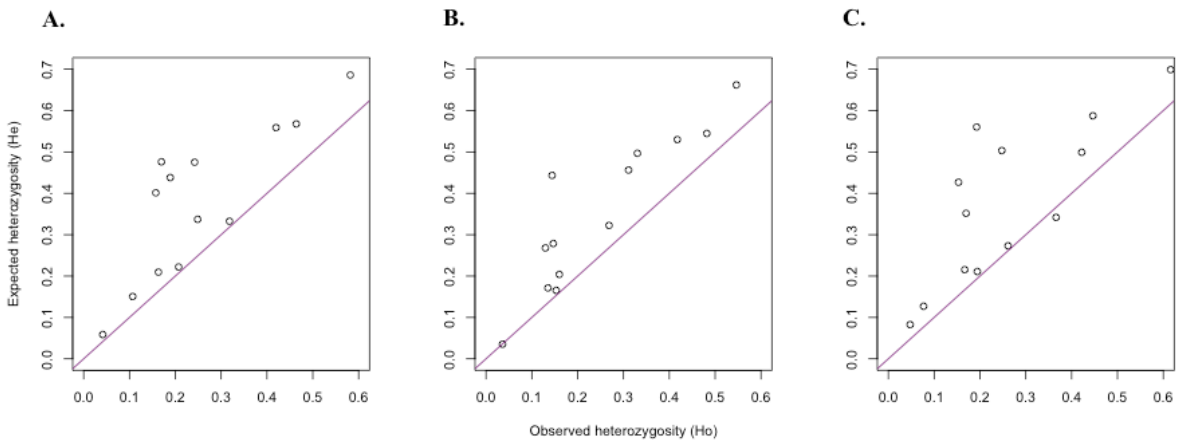

Figure 5. Observed heterozygosity (H_o) plotted against expected heterozygosity (H_e) for *Silene acaulis* individuals in Svalbard, Norway: **A.** including the complete small-scale dataset (average deviation (dev) ± SD=0.12 ± 0.10), **B.** only the Endalen subset (dev=0.10 ± 0.08) and **C.** only the Hotellneset subset (dev=0.12 ± 0.12). Plotted values are H_o and H_e calculated per locus.

Estimates of pairwise relatedness (r) between all individuals in the two populations applying the TrioML method gave an average relatedness estimate of 0.202 ± 0.243 SD. Average estimates when calculating pairwise relatedness of individuals within one site were 0.252 ± 0.273 for Endalen and 0.214 ± 0.241 for Hotellneset. The bootstrapping test of group

differences implemented in the software found that the average relatedness was significantly higher among individuals within sites than among individuals between sites (Table Apx3). Furthermore, the pairwise relatedness was significantly higher among individuals within plots than among individuals between plots in Endalen, while this was not the case for individuals within and between plots at Hotelnesset (Table Apx3).

The average estimated inbreeding coefficient (F), also calculated using the TrioML estimator, was overall 0.400 ± 1.188. The bootstrapping test of group differences found no significant difference between the average inbreeding coefficients of individuals in Endalen and at Hotellneset (Table Apx3).

The relationships between gender groups (Fs, Hs and Us), inbreeding, and relatedness estimates were tested through bootstrapping. Individuals of unknown gender (which are expected to be generally smaller, and thus younger, than reproductive individuals) were not more inbred than females or hermaphrodites. Neither were individuals of one gender more related to individuals of the same gender than to individuals of the opposite gender (Table Apx3).

The pairwise relatedness (r) estimates applying the TrioML likelihood estimator correlated well with the estimates using the QuellerGt moment estimator, with a correlation coefficient of 0.81. Similarly, the TrioML estimates of the inbreeding coefficient (F) correlated well with the estimates using the LynchRd moment estimator, with a correlation coefficient of 0.80. This indicates good robustness of the TrioML method applied on these data.

DISCUSSION

The primary aim of this study was to evaluate how changes in habitat optimality, represented by variation in vegetation cover, affect population dynamics in the gynodioecious pioneer species, *Silene acaulis*, where females are expected to perform better than hermaphrodites under less optimal conditions. Enhanced establishment performance of *S. acaulis* individuals was found in optimal open habitats relative to suboptimal closed habitats, while no association with habitat type could be detected for gender frequencies and genetic diversity measures. Inbreeding estimates were fairly high, suggesting high levels of selfing and inbreeding in these populations. However, low levels of genetic differentiation were detected at all levels, indicating an extensive gene flow both between and within the two populations for which genetic patterns were investigated.

Establishment performance in open and closed habitat types

Silene acaulis is a pioneer species that seems to thrive in plant communities of early successional stages where inter-specific competition is fairly low (Griggs 1956). Better establishment performance, that is, higher population density and abundance of small individuals, was therefore predicted in optimal open habitats compared to more suboptimal closed habitats of later successional stages (hypothesis $\mathbf{H_1}$). This study detected generally higher population density of *S. acaulis* in open habitats compared to closed habitats, and significantly so for the two small-scale populations. Combined with the left-skewed size distribution and significantly smaller average cushion size of individuals in open habitats compared to closed habitats, these results are clearly consistent with the hypothesis of better establishment performance in optimal open habitat sites.

In previous studies, larger individuals of *S. acaulis* have been observed in late-successional habitats (Benedict 1989; McCarthy 1992; Gehring & Delph 1999). Thus, if closed habitats in general represent later successional stages, an overall size distribution skewed towards larger individuals would be expected in closed habitats compared to open habitats ($\mathbf{H_2}$). Individuals in closed habitat sites in the current study were significantly larger than those in the open habitat sites. This right-skewed size distribution of individuals in suboptimal closed habitats is consistent with the expectation that these habitats represent later successional stages where other species gradually replace *S. acaulis*. More spatial clustering of *S. acaulis* individuals is also predicted in closed habitats of later successional stages, as fewer establishments are expected and offspring in general are known to establish close to the maternal plant (Gehring & Delph 1999). Although the result was not significant, the small-scale study showed a weak trend of greater spatial clustering of individuals in the closed habitat, adding to the evidence that closed habitats are communities in later successional stages. These results are also in agreement with other studies of plant community succession in Svalbard, where higher vegetation cover in general represents later successional stages (e.g. Hodkinson et al. 2003; Nakatsubo et al. 2005; Moreau et al. 2009).

Habitat optimality, gender-specific establishment and onset of reproduction

Female frequency and habitat optimality versus severity

Previous studies of gynodioecious species, including *S. acaulis*, have suggested a female advantage that causes shifts towards more females under harsher conditions (e.g. see review by Delph 2003). Hence, overall higher female frequencies were predicted for populations in

suboptimal closed habitats (**H_3**). Consistent with this expectation, the average female frequency of closed habitat populations were higher than the average of open habitats at both study scales, but the difference between habitat types was not significant at any scale. There was also an extensive variation in sex ratios among study sites, with female frequencies ranging from 0.25 to 0.73 in the different large-scale populations (Table 3). Previous studies of *S. acaulis* and other gynodioecious species have reported positive correlations between female frequency and environmental gradients such as increasing altitude (Alatalo & Molau 1995), latitude (Asikainen & Mutikainen 2003; Nilsson & Ågren 2006) and decreasing precipitation (Cuevas & López 2010). Philipp et al. (1990) subjectively ordered sites according to increased severity and found a corresponding increase in female frequency. Although the current study detected reduced establishment performance of *S. acaulis* in suboptimal closed habitats compared to more favourable open habitats, the closed habitats investigated in this study might not represent a critical enough increase in the perceived severity to result in a significant increase in female frequency. Insufficiently differentiated sampling sites were also suggested as an explanation for the lack of association between female frequencies and various site quality variables of *S. acaulis* populations in the Front Range of Colorado, USA (Delph & Carroll 2001).

A study of the gynodioecious pioneer species *Thymus pulegoides* detected a negative correlation between female frequency and percentage vegetation cover, that is, increasing female frequencies with decreasing vegetation cover (Stakelienė & Ložienė 2014). It was suggested that this negative correlation could be explained by higher disturbance in habitats with low vegetation cover, which might promote higher female frequencies. Disturbance was not measured or evaluated in the current study of high arctic *S. acaulis*, and the sex ratio varied considerably among sites within each of the two habitat types, particularly among open habitat populations. It is possible that high disturbance or other untested variables rather than successional processes are responsible for the low vegetation cover in some of these sites, increasing the perceived severity and harshness of environmental conditions, and therefore also the female frequency in these populations.

Environmental conditions including climate, edaphic properties, topography, exposure levels, disturbance and successional dynamics of vegetation cover and species composition are expected to vary substantially over short distances in the high arctic environment (Hodkinson et al. 2003; Jónsdóttir 2005; Walker et al. 2005). It is likely that the perceived severity of a given site is determined by a complex combination of environmental variables

with diverse associations in terms of co-variation and linearity of relationships. The resolution of the current study, a two-level severity gradient from open to closed habitats, might be too low to detect potential associations between gender frequencies and presumed shifts in inter-specific competition levels. More sophisticated analyses of sex ratio variation along various environmental gradients are thus probably required to better understand the impact of ecological conditions on female frequency in high arctic populations of *S. acaulis*.

Even though suboptimal habitat conditions appeared to have low influence on gender-specific establishment performance in *S. acaulis*, there was an overall female biased sex ratio present in this high arctic system, reflected in the average female frequency (0.58) of all sampled populations. This range and magnitude of female frequency values are similar to those detected in other arctic and high arctic studies of *S. acaulis* (e.g. Philipp et al. 1990; Hermanutz & Innes 1994; Morris & Doak 1998; Klaas & Olson 2006), while studies from lower latitudes have found correspondingly lower female frequency values (e.g. Alatalo & Molau 1995; Maurice et al. 1998; Delph & Carroll 2001; Table 5). The large-scale extreme

Table 5. Average and maximal female frequencies recorded in studies of *Silene acaulis*. Fem freq denotes female frequency and is calculated as the proportion of females relative to the total number of reproducing individuals in the population. Individuals in the various studies are sexed as females and hermaphrodites and/or males, and belong to various subspecies of *S. acaulis*.

Study	Location	Average fem freq	Maximal fem freq
Alatalo & Molau (1995)	Northern Swedish Lapland	0.50	0.61
Delph & Carroll (2001)	Nivot Ridge, Front Range of Colorado, USA	0.30	0.39
Hermanutz & Innes (1994)	Baffin Island, Canada	0.72	0.80
Klaas & Olson (2006)	Alaska Range and White Mountains in mid-eastern Alaska	0.56	0.83
Maurice et al. (1998)	The French Alps, France	0.42	0.57
Morris & Doak (1998)	South-central Alaska	0.58	-
Philipp et al. (1990)	North-western Greenland	0.58	0.87

climatic conditions in arctic and high arctic areas (e.g. low summer temperatures and short growing season) appear to skew the magnitude of female frequencies in this species to already high levels and thus overriding the effect of other more local severity gradients. Establishment rates on the other hand seem to be affected by variation in environmental conditions at a more local scale. It is possible that the variation in large-scale climatic conditions among sites in the current study is not large enough to cause significant sex ratio variation, whereas the variation in local-scale habitat conditions is sufficient to cause changes in establishment performance. This could explain the lack of association found here between

changes in habitat optimality and gender frequencies despite the apparent negative influence of suboptimal habitat conditions on establishment performance.

Habitat optimality and onset of reproduction

Earlier onset of reproduction is a known response to stress in plants (Sultan 2000). Suboptimal conditions were therefore expected to result in more flowering individuals in lower size classes in closed habitats compared to open habitats (**H_4**). The results obtained here were not consistent with this hypothesis, as flowering frequencies in different size classes of *S. acaulis* did not differ between open and closed habitats. This suggests that changes in habitat optimality have low influence on the timing of reproductive onset, which could be related to the perennial life history of *S. acaulis*. Seasonal conditions in arctic and alpine tundras with cold and short growing seasons have been predicted to hinder all phases of sexual reproduction in long-lived tundra plants (Bell & Bliss 1980). More stable habitat conditions such as vegetation cover and inter-specific competition, although stressful, might therefore be less important than seasonal conditions for the timing of reproductive onset in perennial species. Nevertheless, closed habitats had higher average cushion size and, thus, more large individuals that would be expected to produce more seeds. This could increase the overall reproductive effort and success of these populations relative to open habitat populations, and thus aid dispersal and movement out of such suboptimal habitats into more favourable habitats (Brochmann & Steen 1999).

Genetic diversity and structure

Habitat optimality and genetic diversity

Selfed or highly inbred offspring have been found to exhibit lower establishment success under harsh conditions compared to outcrossed offspring in gynodioecious species (Sun & Ganders 1986). Higher establishment rate in open habitats was predicted to result in increased recruitment of selfed *S. acaulis* individuals compared to suboptimal closed habitats, leading to higher levels of homozygosity, more linkage disequilibrium and higher relatedness between individuals (**H_5**). However, the data obtained here indicate that, despite the enhanced establishment performance of individuals in open habitats, there was no significant difference in average inbreeding coefficients, levels of linkage disequilibrium or heterozygosity between the two small-scale sites in this study (Table Apx3). This was consistent when including all individuals and when comparing each gender group separately. There were also no signs of

higher relatedness between individuals or more grouping of genotypes in the open habitat site, Hotellnesset. Bootstrapping tests showed that individuals were significantly more closely related within plots than between plots in Endalen but not at Hotelnesset. This could, however, be an effect of smaller plot size at Hotellneset, the slightly higher level of spatial clustering of individuals in Endalen, or a potensial difference in seed dispersal distances between the two sites due to less wind, more snow acumulation and more vegetation hindering dispersal in Endalen compared to Hotellneset. No signs of more recruitment of selfed offspring were detectable when comparing the open habitat to the closed habitat, despite the prevalent enhanced establishment performance of *S. acaulis* individuals in open habitats. This indicates that selfed and inbred offspring have fairly equal establishment performance in optimal and suboptimal habitats, which is in conflict with hypothesis $\mathbf{H_5}$. The results also correspond well with the fairly equal gender frequencies detected for the two study sites.

Although average inbreeding coefficients were not significantly different between habitats (Table Apx3), they were generally high, consistent with inbreeding levels found in other self-compatible or inbred plant populations (e.g. Galeuchet et al. 2005; García-Fernández et al. 2012). Levels of linkage disequilibrium were also higher and heterozygosity levels lower than would be expected for populations with random mating patterns and high outcrossing rates (Sun & Salomon 2003; Hamilton 2009). This suggests that substantial inbreeding is taking place in these populations, either through biparental inbreeding, that is, mating between close relatives, or through selfing with good establishment of selfed offspring at both sites. Pollinator activity is expected to be low in high arctic environments (Philipp et al. 1990), and particularly so in Svalbard where efficient pollinators such as bees and bumblebees are not present (Coulson 2007). A reproductive system that allows for selfing might be advantagous under such conditions (Sun & Salomon 2003). This could serve as a balancing mechanism for hermaphrodite fitness against the female advantage, if the advantage of selfing under such conditions increases hermaphrodite fecundity relative to female fecundity. Further evaluation of this topic requires knowledge about maternal relationships and more detailed assessment of mating patterns in these populations.

Genetic differentiation and gene flow

Fewer establishment events in closed habitats might lower genetic input through seed dispersal to these populations, and over time, genetic drift might cause higher genetic

differentiation of these populations from other surrounding populations (Loveless & Hamrick 1984; Hamilton 2009). However, for species with high outcrossing rates, lower levels of population differentiation are expected, as gene flow counteracts the process of genetic drift (Hamrick & Godt 1996; Muir et al. 2004; Mable & Adam 2007). Other studies of *S. acaulis* in areas where efficient pollinators such as bees and bumblebees are present have found low genetic differentiation between populations and higher levels of differentiation at smaller scales within populations (clusters at scale ≤2m; Gehring & Delph 1999; Klaas & Olson 2006). A suggested explanation of these patterns is that gene flow occurs at two different spatial scales, with localized seed dispersal resulting in patches of family groups, and long distance pollen dispersal that is extensive enough to counteract genetic drift (Gehring & Delph 1999).

In this study, detected levels of genetic differentiation were generally low at all hierarchical levels between and within the two study sites. In particular, the PCAs revealed that genetic differentiation and grouping of genotypes was low within plots, consistent with the lack of correlation between genetic and geographic distances for genotypes found when performing Mantel's tests on the same scale. These results suggest considerable gene flow within and between plots and sites in the two study populations (situated 9 km apart), with seed and pollen dispersal distances exceeding the overall scale of the sampling area in this study (> 70 m). A possible scenario is that all populations start as open habitat populations that, with time and habitat succession, develop into closed habitat populations, where fewer seeds germinate and establish. Closed habitats are expected to have fewer establishment events and more large individuals that can produce more seeds, which may disperse and establish new populations in open habitats. Seed flow is thus dominantly from closed to open habitats, while pollen flow is expected in both directions.

High inbreeding levels indicate restricted pollen dispersal due to pollinator deficiency, which suggests that considerable seed dispersal might be the main cause for the low genetic differentiation levels between populations. It has been proposed that establishment rather than dispersal is the main limiting factor for plant colonization in the Arctic (Alsos et al. 2007), revealing a great potential for seed dispersal distances, particularly in the High Arctic with frequently strong wind and smooth snow-covered surfaces (Savile 1972). Seeds are also expected to contribute to temporal gene flow through seed banks, and *S. acaulis* seeds were consistently shown to germinate from soil samples collected in polar heath habitats in north-

west Svalbard (Cooper et al. 2004). This could also explain the low genetic differentiation found within sites and plots.

If females are the primary source of the suggested seed dispersal out of suboptimal closed habitats, the relatively high female frequency in favourable open habitat populations could potentially be explained by a deficiency of corresponding nuclear male restorer genes in these populations. Influence of spatial structure in nuclear male restorer genes and founder effects on female frequency have, for example, been suggested in the gynodioecious species *Thymus vulgaris* (Manicacci et al. 1996; 1997). Further evaluation of mating patterns, female advantage and the importance of females as source for new populations should include a combination of nuclear and cytoplasmic DNA markers. The inclusion of more populations is also required in order to better understand the gene flow and establishment dynamics between open and closed habitats in these high arctic populations of *S. acaulis*.

Conclusions

Mating patterns, establishment performance and modes of gene flow are expected to affect demographic and genetic population properties extensively. The data obtained in the current study indicate that suboptimal habitat conditions reduce general establishment performance of *S. acaulis*, while no significant association was detectable for female frequencies and genetic diversity measures, although this was initially expected for this gynodioecious species. Establishment performance appears to be affected by environmental conditions changing over local scales, while gender frequencies are mainly influenced by large-scale climatic conditions, which are very severe for all sites included in this study. Generally high inbreeding levels are suggested to reflect pollinator deficiency in this high arctic system, leaving considerable spatial and temporal seed dispersal as the most likely cause for the low differentiation levels found at all scales examined.

REFERENCES

Abbott RJ, Gomes MF. (1989). Population genetic structure and outcrossing rate of *Arabidopsis thaliana* (L.) Heynh. Heredity 62:411–418.

Aiken SG, Dallwitz MJ, Consaul LL, McJannet CL, Boles RL, Argus GW, et al., eds. (2007). Flora of the Canadian Arctic Archipelago: Descriptions, illustrations, identification, and information retrieval. NRC Research Press, National Research Council of Canada, Ottawa http://nature.ca/aaflora/data/index.htm (Accessed October 15, 2014).

Alatalo JM, Molau U. (1995). Effect of altitude on the sex ratio in populations of *Silene acaulis* (Caryophyllaceae). Nordic Journal of Botany 15:251–256.

Alsos IG, Eidesen PB, Ehrich D, Skrede I, Westergaard K, Jacobsen GH, et al. (2007). Frequent long-distance plant colonization in the changing Arctic. Science 316:1606–1609.

Asikainen E, Mutikainen P. (2003). Female frequency and relative fitness of females and hermaphrodites in gynodioecious *Geranium sylvaticum* (Geraniaceae). American Journal of Botany 90:226–234.

Baddeley A, Turner R. (2005). spatstat: An R package for analyzing spatial point patterns. Journal of Statistical Software 12:1–42.

Barrett S. (2003). Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. Philosophical Transactions of the Royal Society B: Biological Sciences 358:991–1004.

Bell KL, Bliss LC. (1980). Plant reproduction in a high arctic environment. Arctic and Alpine Research 12:1–10.

Benedict JB. (1989). Use of *Silene acaulis* for dating: the relationship of cushion diameter to age. Arctic and Alpine Research 21:91–96.

Billings WD, Mooney HA. (1968). The ecology of arctic and alpine plants. Biological Reviews 43:481–529.

Bliss LC. (1971). Arctic and alpine plant life cycles. Annual Review of Ecology and Systematics 2:405–438.

Bliss LC, Peterson KM. (1991). Plant succession, competition, and the physiological constraints of species in the Arctic. In:Arctic ecosystems in a changing climate: An ecophysiological perspective, Chapin, FS, III, Jefferies, RL, Reynolds, JF, Shaver, GR, Svoboda, J, & Chu, EW, eds. Academic Press: San Diego, pp. 111–138.

Bråthen KA, Hagberg O. (2004). More efficient estimation of plant biomass. Journal of Vegetation Science 15:653–660.

Brochmann C, Steen SW. (1999). Sex and genes in the flora of Svalbard - implications for conservation biology and climate change. Det Norske Videnskaps-Akademi. I. Matematisk-Naturvitenskapelig Klasse, Skrifter, Ny Serie 38:33–72.

Charlesworth D, Charlesworth B. (1987). Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18:237–268.

Charlesworth D, Laporte V. (1998). The male-sterility polymorphism of *Silene vulgaris*: Analysis of genetic data from two populations and comparison with *Thymus vulgaris*. Genetics 150:1267–1282.

Chessel D, Dufour AB, Thioulouse J. (2004). The ade4 package - I: One-table methods. R News 4:5–10.

Convey P. (2012). Polar terrestrial environments. In:Life at extremes: Environments, organisms, and strategies for survival, Bell, E, ed. CABI: Oxfordshire, pp. 81–102.

Cooper EJ, Alsos IG, Hagen D, Smith FM, Coulson SJ, Hodkinson ID. (2004). Plant recruitment in the High Arctic: seed bank and seedling emergence on Svalbard. Journal of Vegetation Science 15:115–124.

Coulson SJ. (2007). The terrestrial and freshwater invertebrate fauna of the High Arctic archipelago of Svalbard. Zootaxa 1448:41–58.

Cuevas E, López S. (2010). Sex ratio and sex-specific latitudinal variation in floral characteristics of gynodioecious *Kallstroemia grandiflora* (Zygophyllaceae) in Mexico. Biotropica 43:317–323.

Darwin C. (1877). The different forms of flowers on plants of the same species. Murray: London.

Delph LF. (2003). Sexual dimorphism in gender plasticity and its consequences for breeding system evolution. Evolution & Development 5:34–39.

Delph LF. (2004). Testing for sex differences in biparental inbreeding and its consequences in a gynodioecious species. American Journal of Botany 91:45–51.

Delph LF, Bailey MF, Marr DL. (1999). Seed provisioning in gynodioecious *Silene acaulis* (Caryophyllaceae). American Journal of Botany 86:140–144.

Delph LF, Carroll SB. (2001). Factors affecting relative seed fitness and female frequency in a gynodioecious species, *Silene acaulis*. Evolutionary Ecology Research 3:487–505.

Delph LF, Kelly JK. (2013). On the importance of balancing selection in plants. New Phytologist 201:45–56.

Delph LF, Mutikainen P. (2003). Testing why the sex of the maternal parent affects seedling survival in a gynodioecious species. Evolution 57:231–239.

Dufay M, Billard E. (2012). How much better are females? The occurrence of female advantage, its proximal causes and its variation within and among gynodioecious species. Annals of Botany 109:505–519.

Duminil J, Hardy OJ, Petit RJ. (2009). Plant traits correlated with generation time directly affect inbreeding depression and mating system and indirectly genetic structure. BMC Evolutionary Biology 9:177.

Earl DA, vonHoldt BM. (2011). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359–361.

Elvebakk A. (1999). Bioclimatic delimitation and subdivision of the Arctic. Det Norske Videnskaps-Akademi. I. Matematisk-Naturvitenskapelig Klasse, Skrifter, Ny Serie 38:81–112.

Evanno G, Regnaut S, Goudet J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611–2620.

Excoffier L, Lischer HEL. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564–567.

Excoffier L, Smouse PE, Quattro JM. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Gabrielsen TM, Brochmann C. (1998). Sex after all: high levels of diversity detected in the arctic clonal plant *Saxifraga cernua* using RAPD markers. Molecular Ecology 7:1701–1708.

Galeuchet DJ, Perret C, Fischer M. (2005). Microsatellite variation and structure of 28 populations of the common wetland plant, *Lychnis flos-cuculi* L., in a fragmented landscape. Molecular Ecology 14:991–1000.

García-Fernández A, Segarra-Moragues JG, Widmer A, Escudero A, Iriondo JM. (2012). Unravelling genetics at the top: mountain islands or isolated belts? Annals of Botany 110:1221–1232.

Gehring JL, Delph LF. (1999). Fine-scale genetic structure and clinal variation in *Silene acaulis* despite high gene flow. Heredity 82:628–637.

Glaubitz JC. (2004). CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Molecular Ecology Notes 4:309–310.

Glémin S, Bazin E, Charlesworth D. (2006). Impact of mating systems on patterns of sequence polymorphism in flowering plants. Proceedings of the Royal Society B: Biological Sciences 273:3011–3019.

Griggs RF. (1956). Competition and succession on a Rocky Mountain fellfield. Ecology 37:1–15.

Hamilton MB. (2009). Population genetics. 1st ed. Wiley-Blackwell: Oxford.

Hamrick JL, Godt MJW. (1996). Effects of life history traits on genetic diversity in plant species. Philosophical Transactions of the Royal Society B: Biological Sciences 351:1291–1298.

Hermanutz LA, Innes DJ. (1994). Gender variation in *Silene acaulis* (Caryophyllaceae). Plant Systematics and Evolution 191:69–81.

Hill MO, Gauch HG Jr. (1980). Detrended correspondence analysis: an improved ordination technique. Vegetatio 42:47–58.

Hodkinson ID, Coulson SJ, Webb NR. (2003). Community assembly along proglacial chronosequences in the high Arctic: vegetation and soil development in north-west Svalbard. Journal of Ecology 91:1–13.

Hotelling H. (1933). Analysis of a complex of statistical variables into principal components. The Journal of Educational Psychology 24:417.

Howe F, Westley LC. (2009). Ecology of pollination and seed dispersal. In: Plant ecology, Crawley, M J, ed. Blackwell Scientific Publications: Oxford, pp. 262-283.

Jarne P, Charlesworth D. (1993). The evolution of the selfing rate in functionally hermaphrodite plants and animals. Annual Review of Ecology and Systematics 441–466.

Johansen MK, Trillmann M, Vermeulen J. (2013). Demography of the moss campion, *Silene acaulis*, in a snowbed-ridge gradient. The University Centre in Svalbard, Longyearbyen.

Jombart T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405.

Jones V, Richards PW. (1962). Biological flora of the British Isles, no. 83: *Silene acaulis* (L.) Jacq. Journal of Ecology 50:475–487.

Jonsell B, Borgen L, Brysting AK, Dahl Å, Dahlgren G, Elven R, et al. (2001). Flora Nordica 2. Chenopodiaceae - Fumariaceae. 1st ed. The Bergius Foundation, The Royal Swedish Academy of Sciences: Stockholm.

Jónsdóttir IS. (2005). Terrestrial ecosystems on Svalbard: Heterogeneity, complexity and fragility from an arctic island perspective. Biology and Environment: Proceedings of the Royal Irish Academy 105:155–165.

Keller SR, Schwaegerle KE. (2006). Maternal sex and mate relatedness affect offspring quality in the gynodioecious *Silene acaulis*. Journal of Evolutionary Biology 19:1128–1138.

Khodachek EA. (1995). Reproductive strategies of plants in environments of the Arctic. In:Global change and Arctic terrestrial ecosystems, Callaghan, TV, Molau, U, Tyson, MJ, Holten, JI, Oechel, WC, Gilmanov, T, et al., eds. European Commission: Brussels, pp. 60–79.

Klaas AL, Olson MS. (2006). Spatial distributions of cytoplasmic types and sex expression in Alaskan populations of *Silene acaulis*. International Journal of Plant Sciences 167:179–189.

Kruskal JB. (1964). Nonmetric multidimensional scaling: a numerical method. Psychometrika 29:115–129.

Kumar M, Kålås IH, Svoen ME. (2013). Commonalities of cushion plants in the high arctic archipelago Svalbard - habitat preference and the effects of habitat succession. The University Centre in Svalbard, Longyearbyen.

Lande R, Shannon S. (1996). The role of genetic variation in adaptation and population persistence in a changing environment. Evolution 434–437.

Laporte V, Viard F, Bena G, Valero M, Cuguen J. (2001). The spatial structure of sexual and cytonuclear polymorphism in the gynodioecious *Beta vulgaris* ssp. *maritima*: I/ at a local scale. Genetics 157:1699–1710.

Loveless MD, Hamrick JL. (1984). Ecological determinants of genetic structure in plant populations. Annual Review Ecology and Systematics 15:1–31.

Lynch M, Ritland K. (1999). Estimation of pairwise relatedness with molecular markers. Genetics 152:1753–1766.

Mable BK, Adam A. (2007). Patterns of genetic diversity in outcrossing and selfing populations of *Arabidopsis lyrata*. Molecular Ecology 16:3565–3580.

Manicacci D, Atlan A, Couvet D. (1997). Spatial structure of nuclear factors involved in sex determination in the gynodioecious *Thymus vulgaris* L. Journal of Evolutionary Biology 10:889–907.

Manicacci D, Couvet D, Belhassen E, Gouyon PH, Atlan A. (1996). Founder effects and sex ratio in the gynodioecious *Thymus vulgaris* L. Molecular Ecology 5:63–72.

Mantel N. (1967). The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220.

Maurice S, Desfeux C, Mignot A, Henry J-P. (1998). Is *Silene acaulis* (Caryophyllaceae) a trioecious species? Reproductive biology of two subspecies. Canadian Journal of Botany 76:478–485.

McCarthy DP. (1992). Dating with cushion plants - establishment of a *Silene acaulis* growth curve in the Canadian Rockies. Arctic and Alpine Research 24:50–55.

Molau U. (1993). Relationships between flowering phenology and life history strategies in tundra plants. Arctic and Alpine Research 25:391–402.

Molenda O, Reid A, Lortie CJ. (2012). The alpine cushion plant *Silene acaulis* as foundation species: A bug's-eye view to facilitation and microclimate. PLoS ONE 7:e37223.

Moreau M, Laffly D, Brossard T. (2009). Recent spatial development of Svalbard strandflat vegetation over a period of 31 years. Polar Research 28:364–375.

Morris WF, Doak DF. (2005). How general are the determinants of the stochastic population growth rate across nearby sites? Ecological Monographs 75:119–137.

Morris WF, Doak DF. (1998). Life history of the long-lived gynodioecious cushion plant *Silene acaulis* (Caryophyllaceae), inferred from size-based population projection matrices. American Journal of Botany 85:784–784.

Muir G, Lowe AJ, Fleming CC, Vogl C. (2004). High nuclear genetic diversity, high levels of outcrossing and low differentiation among remnant populations of *Quercus petraea* at the margin of its range in Ireland. Annals of Botany 93:691–697.

Müller E, Cooper E, Alsos I. (2011). Germinability of arctic plants is high in perceived optimal conditions but low in the field. Botany 89:337–348.

Nakatsubo T, Bekku YS, Uchida M, Muraoka H, Kume A, Ohtsuka T, et al. (2005). Ecosystem development and carbon cycle on a glacier foreland in the high Arctic, Ny-Ålesund, Svalbard. Journal of Plant Research 118:173–179.

Nilsson E, Ågren J. (2006). Population size, female fecundity, and sex ratio variation in gynodioecious *Plantago maritima*. Journal of Evolutionary Biology 19:825–833.

Nordborg M. (2000). Linkage disequilibrium, gene trees and selfing: an ancestral recombination graph with partial self-fertilization. Genetics 154:923–929.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, et al., eds. (2012). vegan: Community Ecology Package project. R package version 2.0-5. http://vegan.r-forge.r-project.org/ (Accessed October 17, 2014).

Peakall R, Smouse PE. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. Bioinformatics 28:2537–2539.

Peck JR, Yearsley JM, Waxman D. (1998). Explaining the geographic distributions of sexual and asexual populations. Nature 391:889–892.

Philipp M, Böcher J, Mattsson O, Woodell SRJ. (1990). A quantitative approach to the sexual reproductive biology and population structure in some arctic flowering plants: *Dryas integrifolia*, *Silene acaulis* and *Ranunculus nivalis*. Meddelelser om Grønland. Bioscience 34:1–60.

Pritchard JK, Stephens M, Donnelly P. (2000). Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Queller DC, Goodnight KF. (1989). Estimating relatedness using genetic markers. Evolution 258–275.

R Core Team, ed. (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria http://www.R-project.org/ (Accessed October 9, 2014).

Ripley BD. (1976). The second-order analysis of stationary point processes. Journal of Applied Probability 13:255–266.

Rosenberg NA. (2004). distruct: a program for the graphical display of population structure. Molecular Ecology Notes 4:137–138.

Savile DBO. (1972). Arctic adaptations in plants. Canada Department of Agriculture Monograph 6:1-81.

Schnable PS, Wise RP. (1998). The molecular basis of cytoplasmic male sterility and fertility restoration. Trends in Plant Science 3:175–180.

Schuelke M. (2000). An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18:233–234.

Shykoff JA. (1988). Maintenance of gynodioecy in *Silene acaulis* (Caryophyllaceae): stage-specific fecundity and viability selection. American Journal of Botany 75:844–850.

Shykoff JA. (1992). Sex polymorphism in *Silene acaulis* (Caryophyllaceae) and the possible role of sexual selection in maintaining females. American Journal of Botany 138–143.

Shykoff JA, Kolokotronis S-O, Collin CL, López-Villavicencio M. (2003). Effects of male sterility on reproductive traits in gynodioecious plants: a meta-analysis. Oecologia 135:1–9.

Silvertown J. (2008). The evolutionary maintenance of sexual reproduction: Evidence from the ecological distribution of asexual reproduction in clonal plants. International Journal of Plant Sciences 169:157–168.

Solbrig OT. (1976). On the relative advantages of cross-and self-fertilization. Annals of the Missouri Botanical Garden 63:262–276.

Son TCV, Halvorsen R. (2014). Multiple parallel ordinations: the importance of choice of ordination method and weighting of species abundance data. Sommerfeltia 37:1–37.

Stakelienė V, Ložienė K. (2014). Gynodioecy in *Thymus pulegioides* L., *T. serpyllum* L., and their hybrid *T.* × *oblongifolius* Opiz (Lamiaceae): Flower size dimorphism, female frequency, and effect of environmental factors. Plant Biosystems 148:49–57.

Steltzer H, Hufbauer RA, Welker JM, Casalis M, Sullivan PF, Chimner R. (2008). Frequent sexual reproduction and high intraspecific variation in *Salix arctica*: Implications for a terrestrial feedback to climate change in the High Arctic. Journal of Geophysical Research 113:G03S10.

Sultan SE. (2000). Phenotypic plasticity for plant development, function and life history. Trends in Plant Science 5:537–542.

Sun G, Salomon B. (2003). Microsatellite variability and heterozygote deficiency in the arctic-alpine Alaskan wheatgrass (*Elymus alaskanus*) complex. Genome 46:729–737.

Sun M, Ganders FR. (1986). Female frequencies in gynodioecious populations correlated with selfing rates in hermaphrodites. American Journal of Botany 73:1645–1648.

Venables WN, Ripley BD. (2002). Modern applied statistics with S. 4 ed. Springer: New York.

Walker DA, Raynolds MK, Daniëls FJ, Einarsson E, Elvebakk A, Gould WA, et al. (2005). The circumpolar Arctic vegetation map. Journal of Vegetation Science 16:267–282.

Wang J. (2010). coancestry: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Molecular Ecology Resources 11:141–145.

Wang J. (2007). Triadic IBD coefficients and applications to estimating pairwise relatedness. Genetical Research 89:135–153.

Wookey PA, Robinson CH, Parsons AN, Welker JM, Press MC, Callaghan TV, et al. (1995). Environmental constraints on the growth, photosynthesis and reproductive development of *Dryas octopetala* at a high Arctic polar semi-desert, Svalbard. Oecologia 102:478–489.

APPENDIX

Table Apx1. Size class categories applied in the demographic study of *Silene acaulis* populations in Svalbard, Norway. Cushion diameter is measured as the average of north-south and east-west axes. From a central tap root, closely spaced stems spread out, each terminating in a single rosette. The size class categories are a modified version of that used by Morris & Doak (1998).

Size class	Description	# rosettes	Cushion diameter (cm)	Cushion area (cm^2)
1	Seeds in seed bank			
2	Seedlings			
3		1	< 0.50]	
4		2-5	< 0.50-0.75]	
5		6-10	< 0.75-1.25]	
6		11-19	< 1.25-2.25]	
7		≥ 20	< 2.25-3.75]	< 12.5
8			< 3.75-5.50]	< 25
9			< 5.50-7.75]	< 50
10			< 7.75-11.25]	< 100
11			< 11.25-15.75]	< 200
12			> 15.75	> 200

Table Apx2. Vegetation cover and edaphic parameters of large-scale sampling sites. Soil samples were pooled before analyses for some sites, and standard deviation is therefore not available for these sites. Analyses of soil organic matter were not performed for sites sampled as part of a student project in the UNIS course AB-201 (Johansen et al. 2013)

Pop ID	Vegetation cover	Vegetation cover value §	Amount N (%)	Amount C (%)	C:N ratio	Soil organic matter (%)	Soil pH
ANKR	Open	0.14	0.49 ± -	6.56 ± -	11.45 ± -	-	6.23 ± -
ANKZ	Open	0.36	0.80 ± -	10.35 ± -	12.48 ± -	-	6.46 ± -
BLMS	Open	0.29	0.06 ± -	8.13 ± -	13.10 ± -	20.28 ± -	7.10 ± -
BLOZ	Closed	0.41	0.41 ± 0.16	5.83 ± 2.34	14.03 ± 0.55	12.80 ± -	7.20 ± -
BOC	Open	0.06	0.01 ± 0.00	0.69 ± 0.15	69.07 ± 15.02	0.61 ± 0.19	7.01 ± 0.18
COLR	Closed	0.44	0.44 ± 0.00	5.57 ± 0.01	12.70 ± 0.06	13.16 ± -	6.30 ± -
END	Closed	0.49	0.41± 0.19	8.86 ± 3.87	22.32 ± 2.59	81.28 ± 33.25	6.38 ± 0.12
ENGR	Open	0.26	0.24 ± 0.06	3.39 ± 0.71	14.20 ± 0.31	7.91 ± -	6.40 ± -
ENGZ	Open	0.34	0.46 ± 0.07	5.86 ± 0.83	12.87 ± 0.14	13.28 ± -	6.30 ± -
HOT	Open	0.39	1.03 ± 0.29	33.92 ± 14.77	31.48 ± 6.51	34.33 ± 11.71	6.62 ± 0.34
MIDR	Open	0.13	0.69 ± -	11.37 ± -	18.72 ± -	-	7.11 ± -
MIDZ	Closed	0.40	1.08 ± -	16.80 ± -	15.88 ± -	-	6.92 ± -
RINC	Closed	0.65	0.09 ± 0.52	2.73 ± 0.52	35.40 ± 9.77	6.33 ± 1.52	7.24 ± 0.23
RINO	Closed	0.42	0.01 ± 0.00	0.70 ± 0.15	69.75 ± 14.74	0.76 ± 0.19	7.92 ± 0.86
RINS	Closed	0.40	0.07 ± 0.02	1.65 ± 0.21	24.06 ± 4.72	2.86 ± -	7.10 ± -
RINZ	Closed	0.76	0.06 ± 0.01	1.12 ± 0.13	17.55 ± 0.50	3.52 ± -	6.70 ± -
SIG	Open	0.17	0.23 ± 0.13	2.94 ± 0.13	12.85 ± 0.18	4.77 ± -	6.00 ± -

§ Estimates are based on various vegetation cover estimate methods (see Materials and methods; Sampling design in large-scale study) and the observations were performed by many different persons

Table Apx3. Bootstrapping tests of group differences in **A.** pairwise relatedness (r) and **B.** inbreeding coefficient (F) of *Silene acaulis* individuals in Endalen (END) and Hotellneset (HOT; small-scale study), Svalbard, Norway. CI95 refers to the 95% confidence intervall for the resampled distribution. Gender refers to the two defined genders of the species: females (Fs) and hermaphrodites (Hs). Us are individuals of unknown gender. The tests were performed through an implemented function in COANCESTRY 1.0.1.5 (Wang 2010). The number of bootstrapping samples was set to 10000 for all tests. Significant differences are marked with *. N.S. is non-significant differences.

Dataset	Group 1	Average group 1 ± SE	Group 2	Average group 2 ± SE	Difference (group 1 - group 2)	CI95	Significance level	
A. Pariwise relatedness (r)								
Total	Within sites	0.233 ± 0.067	Between sites	0.171 ± 0.050	0.062	[-0.006, 0.006]	> 0.95	*
	Within gender	0.223 ± 0.264	Between gender	0.216 ± 0.259	0.007	[-0.014, 0.014]	N.S.	
Endalen	Within plots	0.274 ± 0.082	Between plots	0.242 ± 0.071	0.032	[-0.014, 0.015]	> 0.95	*
	Within gender	0.262 ± 0.285	Between gender	0.255 ± 0.270	0.007	[-0.022, 0.023]	N.S.	
Hotellneset	Within plots	0.220 ± 0.060	Between plots	0.211 ± 0.057	0.009	[-0.013, 0.013]	N.S.	
	Within gender	0.204 ± 0.258	Between gender	0.193 ± 0.255	0.011	[-0.038, 0.038]	N.S.	
B. Inbreeding coefficient (F)								
Total	END	0.355 ± 0.062	HOT	0.354 ± 0.053	0.001	[-0.063, 0.064]	N.S.	
	Fs	0.356 ± 0.234	Hs	0.344 ± 0.242	0.012	[-0.090, 0.096	N.S.	
	Us	0.359 ± 1.008	Fs + Hs	0.349 ± 1.352	0.010	[-0.064, 0.064]	N.S.	
	Fs END	0.339 ± 0.249	Fs HOT	0.350 ± 0.231	-0.011	[-0.174, 0.128]	N.S.	
	Hs END	0.368 ± 0.231	Hs HOT	0.325 ± 0.244	0.043	[-0.149, 0.153]	N.S.	
	Us END	0.309 ± 0.947	Us HOT	0.419 ± 1.080	-0.110	[-0.346, 0.400]	N.S.	
Endalen	Fs	0.368 ± 0.231	Hs	0.339 ± 0.249	0.028	[-0.115, 0.114]	N.S.	
	Us	0.358 ± 0.947	Fs + Hs	0.353 ± 1.266	0.005	[-0.095, 0.095]	N.S.	
Hotellneset	Fs	0.325 ± 0.243	Hs	0.350 ± 0.232	-0.025	[-0.153, 0.171]	N.S.	
	Us	0.360 ± 1.039	Fs + Hs	0.353 ± 1.480	0.007	[-0.082, 0.074]	N.S.	

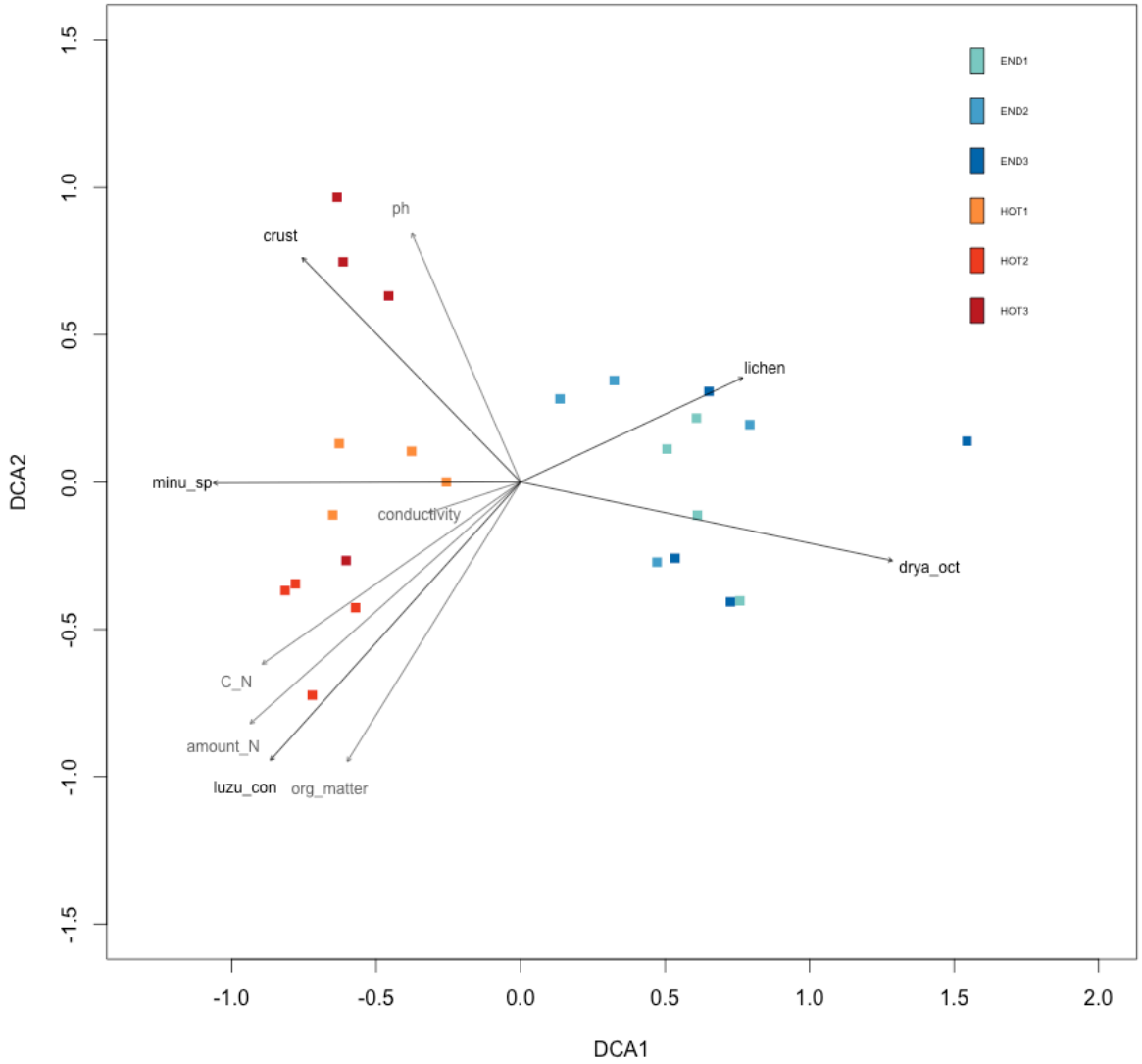

Figure Apx1. Detrended correspondence analysis (DCA) of vegetation data at plot level. Each of the three sampling plots in Endalen (END) and Hotellneset (HOT; Svalbard, Norway) is represented by four frames where vegetation cover was registered using the point intercept method (Bråthen & Hagberg 2004). The first ordination axis is represented by the x-axis (DCA1), while the y-axis represents the second ordination axis (DCA2). Overlayed vectors describe edaphic conditions (grey) and main vegetation components (black) of the frames.

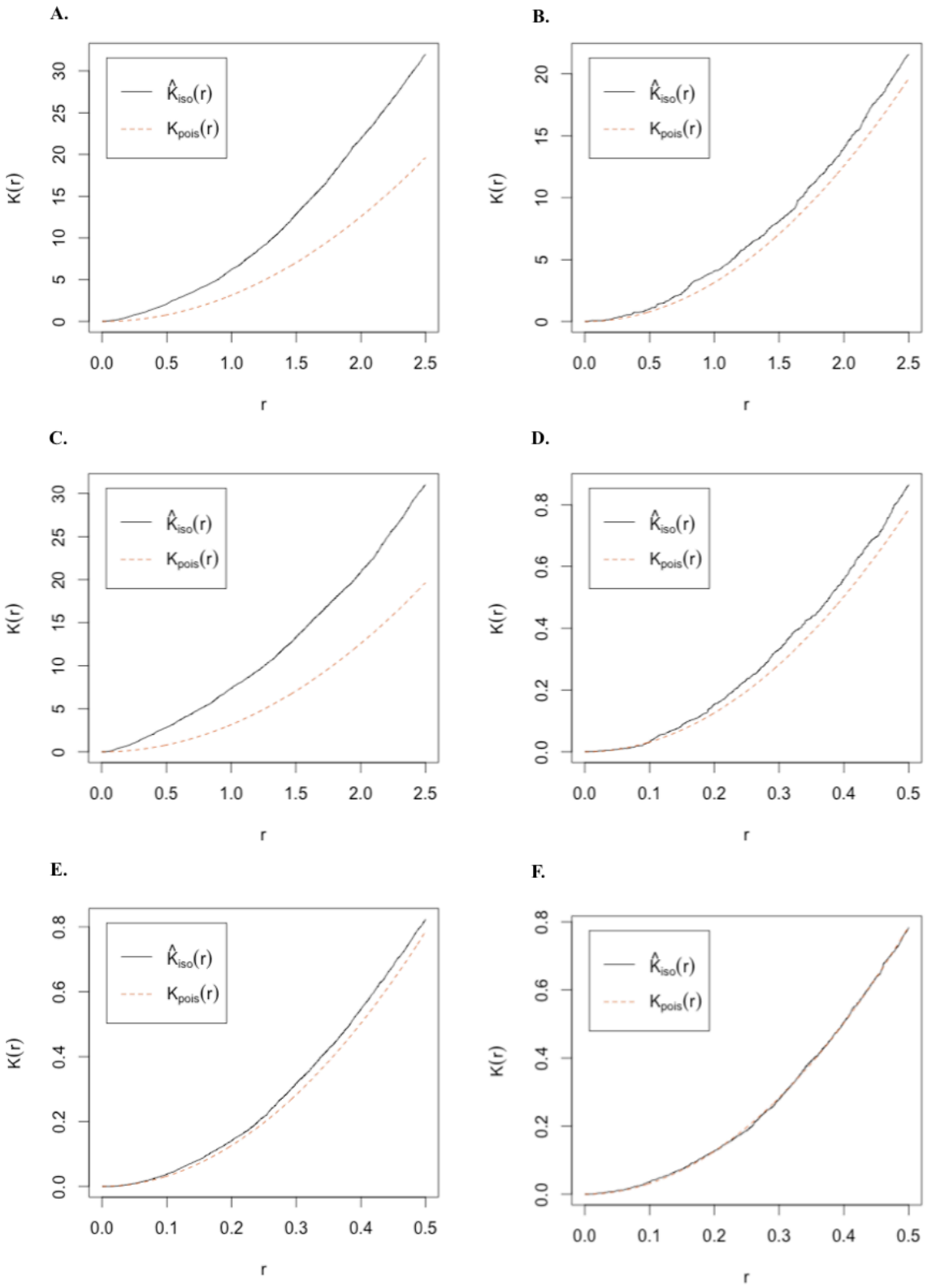

Figure Apx2. Estimates of Ripley's K-function, $K_{iso}(r)$ (black), plotted against the theoretical value expected for a random spatial distribution, $K_{pois}(r)$ (orange stippled) for *Silene acaulis* individuals in Svalbard, Norway in **A.-C.** Endalen (END) sampling plot 1-3 and **D.-F.** Hotellneset (HOT) sampling plot 1-3. Notice that the ranges of the axes in Endalen and Hotelnesset plots are different. The average deviance of $K_{iso}(r)$ from $K_{pois}(r)$, adjusted for the maximal value of r within the given plot, is **A.** 2.027 in END1, **B.** 0.383 in END2, **C.** 2.104 in END3, **D.** 0.068 in HOT1, **E.** 0.046 in HOT2 and **F.** 0.001 in HOT3.

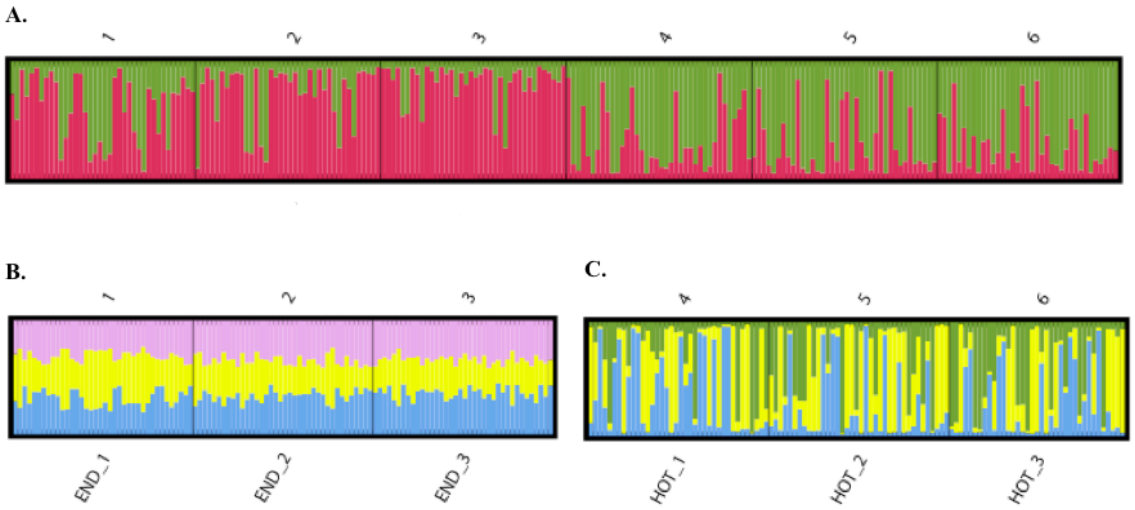

Figure Apx3. Plots of the results from STRUCTURE analyses of *Silene acaulis* populations in Svalbard, Norway: **A.** including both sites of the small-scale study (K=2), **B.** only the Endalen (END) subset (K=3) and **C.** only the Hotellneset (HOT) subset (K=3). The groups (k) are represented by different colours. The segmentation of vertical pilars refers to the percentage by which an individual was placed in the different groups.

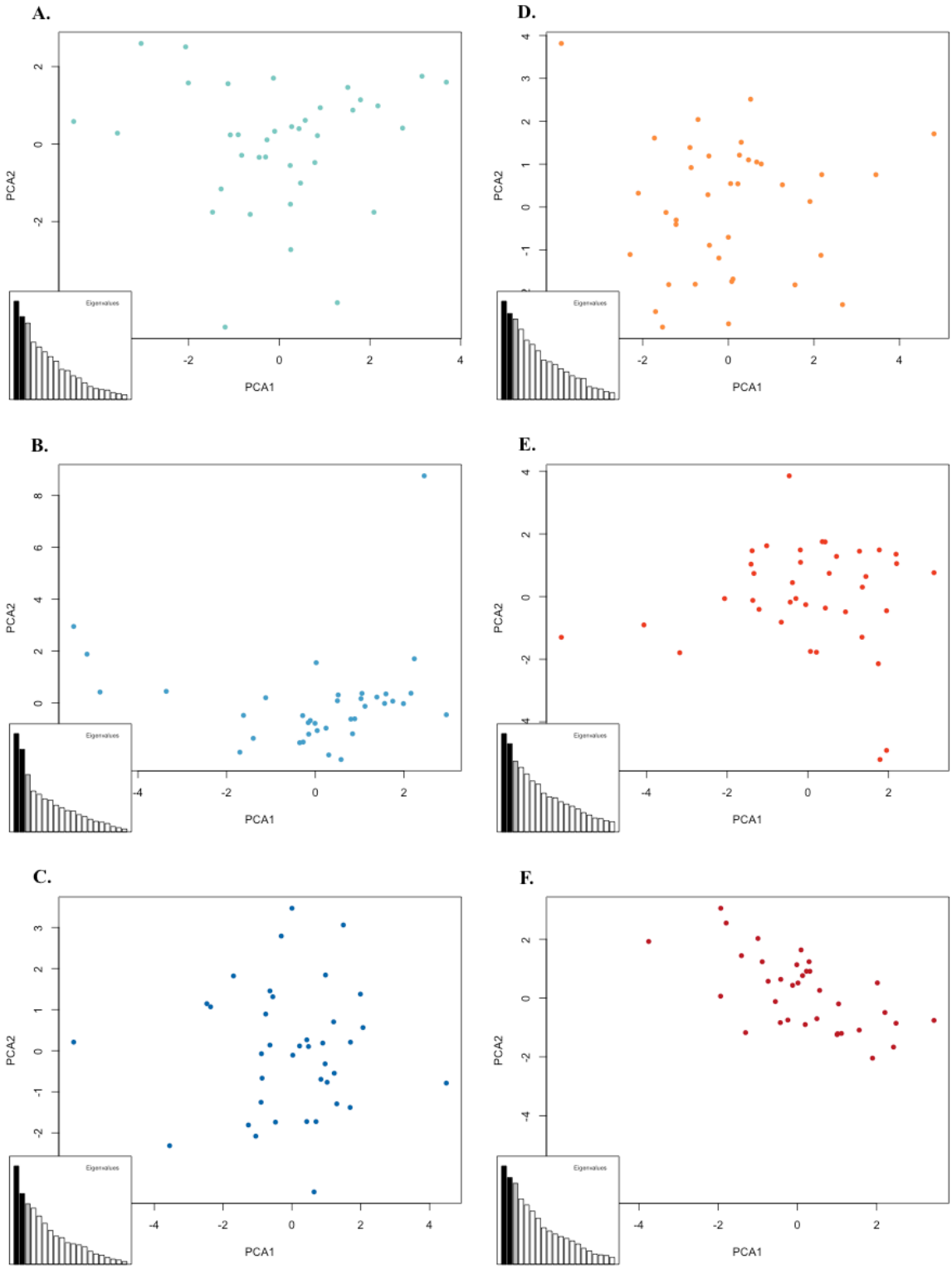

Figure Apx4. Principal component analysis (PCA) of genetic variability among *Silene acaulis* individuals in Svalbard, Norway, within different sampling plots in **A.-C.** Endalen and **D.-F.** Hotellneset. **A.** and **D.** give principal component axis 1 and 2 (PCA1 and PCA2) plotted against each other for individuals within sampling plot 1, **B.** and **E.** for individuals within sampling plot 2 and **C.** and **F.** for individuals within sampling plot 3 in Endalen and Hotellneset, respectively. The cumulative inertia for the two first principal component axes, i.e. amount of variation explained by the given axes relative to the total explainable variation, was **A.** 26.50%, **B.** 32.14%, **C.** 28.47%, **D.** 22.98%, **E.** 23.14% and **F.** 24.19%. Eigenvalues corresponding to the two first principal components are filled in black. The outlier in END2 (**B.**) was checked, but no genotyping errors were detected and the outcome of the PCA was not changed when excluding this individual.