Living on the edge: conservation genetics of seven thermophilous plant species in a high Arctic archipelago

Siri Birkeland¹,²,³*, Idunn Elisabeth Borgen Skjetne¹,²*, Anne Krag Brysting², Reidar Elven³ and Inger Greve Alsos¹,⁴

¹ The University Centre in Svalbard, P.O. Box 156, Longyearbyen, NO 9171, Norway
² Centre for Ecological and Evolutionary Synthesis, Department of Biosciences, University of Oslo, P.O. Box 1066 Blindern, Oslo, NO 0316, Norway
³ Natural History Museum, University of Oslo, P.O. Box 1172 Blindern, Oslo, NO 0318, Norway
⁴ Tromsø University Museum, UiT The Arctic University of Norway, P.O. Box 6050 Langnes, Tromsø, NO 9037, Norway

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†These authors contributed equally to this work.

Abstract. Small, isolated and/or peripheral populations are expected to harbour low levels of genetic variation and may therefore have reduced adaptability to environmental change, including climate warming. In the Arctic, global warming has already caused vegetation change across the region and is acting as a significant stressor on Arctic biodiversity. Many of the rare plants in the Arctic are relicts from early Holocene warm periods, but their ability to benefit from the current warming is dependent on the viability of their populations. We therefore examined Amplified Fragment Length Polymorphism (AFLP) data from regional red listed vascular plant species in the High Arctic archipelago of Svalbard and reference populations from the main distribution area of: (1) Botrychium lunaria, (2) Carex capillaris ssp. fuscidula, (3) Comastoma tenellum, (4) Kobresia simpliciuscula ssp. subholarctica, (5) Ranunculus wilanderi, (6) Sibbaldia procumbens and (7) Tofieldia pusilla. In addition, we gathered population size data in Svalbard. The Svalbard populations had low genetic diversity and distinctiveness and few or no private markers compared to populations outside the archipelago. This is similar to observations in other rare species in Svalbard and the genetic depletion may be due to an initial founder effect and/or a genetic bottleneck caused by late Holocene cooling. There seems to be limited gene flow from other areas and the Svalbard populations should therefore be considered as demographically independent management units. Overall, these management units have small and/or few populations and are therefore prone to stochastic events which may further increase vulnerability to inbreeding depression, loss of genetic variation, and reduced evolutionary potential. Our results support theory predicting lower levels of genetic diversity in small, isolated and/or peripheral populations and may be of importance for management of other rare plant species in the Arctic.

Keywords: Amplified fragment length polymorphism (AFLP); Arctic; climate change; conservation genetics; edge populations; islands; regional red list; Svalbard.
Introduction

Small, isolated and/or peripheral populations may harbour low levels of genetic variation due to genetic drift, inbreeding, bottlenecks and founder effects (Ellstrand and Elam 1993; Frankham 1996; Cole 2003; Frankham et al. 2010). For island populations, reduction of genetic variation is expected to be greater the lower the number of founders, the smaller the population sizes, the lower the immigration rates, the smaller the island size, and the greater the distance to the mainland (Jaenike 1973; Frankham 1997). Similarly, the central–marginal hypothesis also predicts a decline in within-population genetic diversity and increase in genetic differentiation towards range margins, although observed differences from empirical studies are generally small and not consistent (Gaston 2003; Eckert et al. 2008; Hardie and Hutchings 2010). Small, isolated and/or peripheral populations are therefore expected to have reduced adaptability to environmental change (Frankham 1997; Frankham 2005). Low levels of genetic variation also make such populations susceptible to genetic threats like inbreeding depressions and further loss of genetic variation through genetic drift, which can interact with environmental stressors and increase extinction risk (Frankham 1997). Thus, levels of genetic variation are key information when trying to understand and predict the response of small, isolated and/or peripheral populations to future environmental change. Genetic data may also give valuable information about species history (e.g. population fragmentation, bottlenecks, refugia and range shifts; Young et al. 1996; Petit et al. 2003; Meirmans et al. 2011), and is also essential for delineating conservation units like evolutionarily significant units (ESUs) and management units (MUs) (Moritz 1994; Sherwin and Moritz 2000; Funk et al. 2012). An ESU can be defined as one or several populations that are especially important for maintaining the evolutionary potential of a species due to high genetic and ecological distinctiveness (Moritz 1994; Sherwin and Moritz 2000; Funk et al. 2012). At a lower level, an ESU is often built up of demographically independent populations called management units, which, in contrast to ESUs, can be delineated solely on the basis of neutral markers (Funk et al. 2012). Management units are important for the long-term persistence of the species and are often useful for short-term management goals like monitoring habitat and population status (Funk et al. 2012).

The Arctic has been warming at approximately twice the global rate since the 1980s (Anisimov et al. 2007), and we are now experiencing vegetation change across the region (Larsen et al. 2014) seen as phenology changes (Menzel et al. 2006; Ovaskainen et al. 2013; Zeng et al. 2013), increased photosynthetic activity (Xu et al. 2013), and species shifting their ranges towards higher latitudes (Parmesan and Yohe 2003; Root et al. 2003; Chen et al. 2011). The rapid rise in temperature is expected to continue throughout the century (IPCC 2013), and the question is how Arctic ecosystems will respond to this climate change. In this context, Arctic islands may provide important study systems and sentinels. Island populations have a much higher risk of extinction than mainland populations, and the possibility of range displacement may be limited (Frankham 1997). This regards especially species which are already rare and thus more prone to stochastic events (genetic, demographic and environmental stochasticity as well as random catastrophes, Shaffer 1981; Lande 1988; Lande 1993). Increased knowledge on such species may help to make more effective decisions for biodiversity conservation.

The remote High Arctic archipelago Svalbard (74–81° N and 10–35° E) is among the best studied regions in the Arctic, with detailed knowledge of the local distribution of species (Elven et al. 2011; Alsos et al. 2016a). About one fourth of the 184 native vascular plant species in Svalbard are on the regional red list (Henriksen and Hilmo 2015), and many of these are relatively warmth-demanding compared to the more common plant species (Engelskåen et al. 2003; Elven et al. 2011; Henriksen and Hilmo 2015; Alsos et al. 2016a). It is believed that the thermophilous (i.e. warmth-loving) species of Svalbard might be relicts of larger populations established between 9000 and 4000 years ago (Alsos et al. 2002; Engelskåen et al. 2003; Alsos et al. 2007; Gussarova et al. 2012), as an early Holocene warm period is well documented in a number of proxy records from the Svalbard and western Barents Sea region (Birks 1991; Birks et al. 1994; Hald et al. 2004; Alsos et al. 2016b). However, for species with only one or a few populations, more recent dispersal might be just as likely (Gussarova et al. 2012). Despite its remote location, long distance dispersal to Svalbard has been frequent (Alsos et al. 2007, 2015), but restricted seed production, especially in the thermophilous species, limits dispersal within the archipelago today (Cooper et al. 2004; Alsos et al. 2007, 2013). As the temperature rises, it could be anticipated that warmth-demanding species will become increasingly common, and cold-adapted species will become increasingly rare. However, an increase in temperature might come with several additional changes like reduced snow cover and thawing of permafrost (McBean et al. 2005). The loss of snow cover will not only expose plants to harmful sub-zero ambient temperatures and large temperature fluctuations, but may also lead to damage by winter desiccation, repeated freeze–thaw cycles and
We therefore believe that population size data on the rare and warmth-demanding plant species on Svalbard may prove valuable in monitoring ecosystem change. In addition, the warmth-demanding plant species may turn out to play an important role in ecosystem adaptation, but this will depend on the genetic state of the populations (i.e. that they are not too genetic depauperate and subject to inbreeding depression) as well as other ecological requirements and competitive abilities (Walker 1995; Callaghan et al. 2005; Crawford 2008).

In this study, we gather population size data and examine Amplified Fragment Length Polymorphism (AFLP) data from several red listed vascular plant species in Svalbard. Based on the regional red list from 2006 (Kålås et al. 2006; [see Supporting Information—Table S1]), seven study species were chosen as they all were in need of more data to ensure informed conservation decisions. Our aim is to (i) evaluate their vulnerability in terms of population size and genetic diversity in Svalbard, (ii) examine their genetic relationships to populations outside Svalbard and (iii) determine if the Svalbard populations constitute management units with special conservation value.

Methods

Study species

The seven study species are: (1) Botrychium lunaria, (2) Carex capillaris ssp. fuscidula, (3) Comastoma tenellum, (4) Kobresia simpliciuscula ssp. subholarctica, (5) Ranunculus wilanderi, (6) Sibbaldia procumbens and (7) Tofieldia pusilla (Fig. 1; [see Supporting Information—Tables S1 and S2]). All species are seed plants, except B. lunaria, which is a pteridophyte. Furthermore, all are herbaceous plants with larger distributions outside Svalbard. However, R. wilanderi is considered an endemic microspecies for the archipelago (Elven et al. 2011). Most species are diploid [see Supporting Information—Table S2], and are therefore not expected to harbour hidden genetic variation in the form of fixed heterozygosity which is so common in many Arctic plants (Brochmann and Steen 1999; Brochmann and Brysting 2008).

Population size estimation in Svalbard

To estimate population sizes in Svalbard, we either counted all visible individuals, or extrapolated the total population size from the number of individuals counted in a smaller area. Tussocks or clusters of clonal plants were treated as single individuals if they were separated by more than five centimetres, although we cannot be entirely sure that they were not connected belowground.

All previously recorded localities for the seven species were revisited (Table 1). In addition, we searched for the plants in areas that could provide suitable habitat (within bioclimatic subzone C, the Middle Arctic Tundra Zone; Elvebakk 2005; Walker et al. 2005).

Plant material

Plant material for AFLP fingerprinting was collected from most visited Svalbard localities (Table 2). In addition, reference material was sampled from other Arctic-alpine populations within the species’ distribution ranges (Table 2). However, for Kobresia simpliciuscula ssp. subholarctica we were only able to obtain material from a different subspecies, the European ssp. simpliciuscula (Elven et al. 2011). Also note that material from two assumed subspecies is included for Carex capillaris: ssp. fuscidula and ssp. capillaris (Table 2). The Svalbard population is believed to belong to the circumpolar-alpine ssp. fuscidula (Elven et al. 2011). From each Svalbard population and each reference population, fresh and healthy leaves from (if possible) ten plants were collected 2–10 m apart, and immediately stored in silica gel. A closely related species (two for Tofieldia pusilla) was also sampled for all study species to serve as outgroup in the neighbour-joining analysis (see below, Table 2). Herbarium vouchers from most populations are deposited in the herbariums at the University of Oslo (O) and the University of Tromsø (TROM). Plant material and AFLP data for Sibbaldia procumbens have previously been published in Allen et al. 2015 and Alsos et al. 2015, respectively, but then as part of other research questions.

DNA isolation

Approximately 20 mg of silica dried leaves were placed in 2 ml tubes with two tungsten carbon beads and crushed at 20 Hz for 2–8 min on a mixer mill (MM03, Retsch GmbH & Co, Haan, Germany). To obtain optimal purity and concentration of DNA, two to three different extraction protocols were tested on a few individuals of each species, and the best protocol was used further. DNA from the individuals of Botrychium lunaria, Carex capillaris, Kobresia simpliciuscula and Sibbaldia procumbens was isolated using the acidic DNA isolation protocol by Ziegenhagen et al. (1993) with the following modifications: The silica dried leaves were crushed to powder as explained above, without the use of liquid nitrogen. The samples were quickly spun down before a preheated (65 °C) extraction buffer was added. The first centrifugation step was increased to 15 min at 13 000 rpm, the second centrifugation step was increased to 20 min at 13 000 rpm and the last centrifugation step was increased to 15 min at 13 000 rpm. In addition, an extra
Figure 1. Distribution maps showing main STRUCTURE groups and genetic diversity in the seven study species. The pie charts represent the genetic groups delineated by STRUCTURE (averages over multiple runs) and the size of each pie chart is proportional to the $D$ value of each population. $D$ values of zero are indicated with a cross and squares are used instead of circles for populations with too small sample size to calculate genetic diversity. Species distributions are drawn after the maps of Hultén and Fries (1986) and are indicated in transparent dark grey (larger areas and small dots). (a) Tofieldia pusilla, (b) Botrychium lunaria, (c) Sibbaldia procumbens, (d) Carex capillaris (both subspecies), (e) Comastoma tenellum, (f) Kobresia simpliciuscula (both subspecies) and (g) Ranunculus wilanderi. Photos: Öluf Bima Magnúsdóttir, Inger Greve Alsos (Alsos et al. 2016a) and Siri Birkeland.
| Species                        | Locality                      | Pop. ID | Date visited*                      | Distribution area (m²) | No. of individuals | Fertile | Comments                                                                 |
|-------------------------------|-------------------------------|---------|-----------------------------------|------------------------|-------------------|---------|---------------------------------------------------------------------------|
| Botrychium lunaria            | Bockfjorden, Haakon VII Land  | BI01    | 2/8-2009                          | 33                     | 21                | Yes     |                                                                            |
| Carex capillaris ssp. fuscidula| Bockfjorden, Haakon VII Land  | Cc01    | 1/8-2009                          | 60,000                 | >2000             | Yes     |                                                                            |
| Comastoma tenellum            | Ossian Sarsfjellet, Haakon VII Land | Ct01 | 20/7-2009                          | 51                     | 171               | Yes     |                                                                            |
|                              | Flatøyrdalen, Ny-Fries Land   | Ct03    | 24/8-2010                          | 300                    | 300-400           | Yes     |                                                                            |
|                              | Ringhornalen, Ny-Fries Land   | Ct04    | 25/8-2010                          | 20                     | >50               | Yes     | Population discovered for the first time by authors of the present study. |
| Kobresia simpliciuscula ssp. subholarctica | Gipsvika, Bünsow Land | Ks01    | 5/7-2009                          | 246                    | 20**              | No      |                                                                            |
|                              | Adolfbukta, Dickson Land/Bünsow Land |             |                                    |                        |                   |         |                                                                            |
|                              | Mimerdalen, Dickson Land      |         |                                    |                        |                   |         |                                                                            |
|                              | Ossonian Sarsfjellet, Haakon VII Land | Ks02 | 19/7-2009                          | 800                    | 60**              | No      |                                                                            |
|                              | Blomstrandøya, Haakon VII Land | Ks03    | 19/7-2009                          | 200                    | 14**              | Yes     |                                                                            |
|                              | Flatøyrdalen A, Ny Friesland  | Ks04    | 24/7-2010                          | 75                     | 9**               | ?       |                                                                            |
|                              | Flatøyrdalen B, Ny Friesland  |         |                                    |                        |                   |         |                                                                            |
|                              | Reinsbuksdalen, Ny Friesland  |         |                                    |                        |                   |         |                                                                            |
|                              | Lernström fjellet A, Ny Friesland |       |                                    |                        |                   |         |                                                                            |
|                              | Lernström fjellet B, Ny Friesland |       |                                    |                        |                   |         |                                                                            |
|                              | Lemström fjellet A, Ny Friesland |       |                                    |                        |                   |         |                                                                            |
|                              | Ranunculus wilanderi          |         |                                    |                        |                   |         |                                                                            |
|                              | Spibaldia procumbens          |         |                                    |                        |                   |         |                                                                            |
Table 1. Continued

| Species                  | Locality                          | Pop. ID | Date visited* | Distribution area (m²) | No. of individuals | Fertile | Comments                                                                 |
|--------------------------|------------------------------------|---------|---------------|------------------------|-------------------|---------|--------------------------------------------------------------------------|
| *Tofieldia pusilla*      | Blomnesletta, Dickson Land         | Tp01    | 6/7-2009      | 600                    | 51                | Yes     | Population discovered for the first time by authors of the present study. |
| *Tofieldia pusilla*      | Kapp Nathorst, Dickson Land        | Tp04    | 24/7-2009     | 403                    | ca. 1000          | Yes     |                                                                         |
| *Tofieldia pusilla*      | Kapp Wijk, Dickson Land            | Tp03    | 24/7-2009     | 800                    | >146              | Yes     |                                                                         |
| *Tofieldia pusilla*      | Blomstranddøya, Haakon VII Land    | Tp02    | 19/7-2009     | 63                     | 24                | Yes     |                                                                         |
| *Tofieldia pusilla*      | Bockfjorden, Haakon VII Land       | Tp05    | 1/8-2009      | 6                      | 100               | Yes     |                                                                         |
| *Tofieldia pusilla*      | Ossian Sarsfjellet, Haakon VII Land| Tp05    | 2/8-2009      | 1                      | 6                 | Yes     |                                                                         |
| *Tofieldia pusilla*      | Flatøyrdalen, Ny-Fries Land        | Tp16    | 24/8-2010     | 300                    | 9                 | Yes     |                                                                         |
| *Tofieldia pusilla*      | Ringhornsdalen, Ny-Fries Land      | Tp17    | 25/8-2010     | 1000                   | 100               | Yes     | Population discovered for the first time by authors of the present study. |

Sum: ca. 1500

Headings: Species and, if applicable, subspecies name [Species]; name of locality and region in Svalbard [Locality]; population ID used in genetic analyses (Table 2) [Pop. ID]; date for population inspection [Date visited]; extent of area where the species occurred [Distribution area (m²)]; counted/estimated number of individuals/ramets [No. of individuals]; whether fertile individuals were present at the time of the visit [Fertile].

*The populations were visited as part of the present study unless otherwise is stated.

**Tussocks.
Table 2. Sampling information and results for AFLP analyses, ordered from highest to lowest $D$-value for each species.

| Taxon | Pop ID | Country1 | Region | Locality       | Latitude (N) | Longitude (E) | Year | Collector(s)2 | n | D     | % polym | DW | Min-max | Private |
|-------|--------|----------|--------|----------------|--------------|---------------|------|---------------|---|-------|---------|-----|---------|---------|
| Botrychium lunaria |        |          |        |                |              |               |      |               |   |       |         |     |         |         |
| Bl06  | AT     | Salzburg | Lungau | 47.159191     | 13.380276    | 2009          | AT  | 10            | 0.144 | 35.2 | 0.561  | 8–9 | 0       |         |
| Bl12  | IT     | Abruzzo  | Roccacas, Monte Pratello | 41.799996    | 13.983328    | 2009          | PK  | 10            | 0.142 | 40.8 | 0.653  | 10  | 1       |         |
| Bl13  | CH     | Bern     | Wilderswil, Schynige Platte | 46.652501    | 7.913885     | 2009          | PK  | 10            | 0.132 | 33.8 | 0.636  | 8–9 | 2       |         |
| Bl05  | NO     | Troms    | Henrikheia, Tansvikdalen | 69.705651    | 19.240658    | 2009          | TA  | 9             | 0.123 | 22.5 | 0.659  | 4–5 | 3       |         |
| Bl08  | NO     | Hedmark  | Folldal gruver | 62.141457    | 9.993884     | 2010          | RE, SB | 10          | 0.122 | 23.9 | 0.388  | 6–8 | 0       |         |
| Bl14  | NO     | Troms    | Kåfjord | 69.385958     | 21.050901    | 2009          | RE  | 5             | 0.124 | 21.1 | 0.446  | 5   | 0       |         |
| Bl07  | IT     | Piemonte | Rifugio Mongioie | 44.164222    | 7.782733     | 2009          | AT  | 10            | 0.117 | 28.2 | 0.577  | 8–9 | 1       |         |
| Bl04  | IS     | Suðurland | Geysir | 64.313761     | −20.30295    | 2009          | IEBS, SB | 10          | 0.109 | 8.5  | 0.709  | 2   | 0       |         |
| Bl09  | NO     | Hardaland | Finse | 60.601938     | 7.5038925    | 2010          | IEBS, SB | 10          | 0.089 | 23.9 | 0.428  | 8–9 | 1       |         |
| Bl11  | FO (DK) | Eysturoy | Strendur | 62.096476    | −6.770503    | 2010          | IEBS, JD | 10          | 0.068 | 21.1 | 0.491  | 3–5 | 1       |         |
| Bl15  | IS     | Austurland | Skjalfell | 64.015988    | −16.772     | 2010          | ØBM | 3             | 0.061 | 7.0  | 0.463  | 3   | 0       |         |
| Bl02  | GL (DK) | Sermersooq | Tasiiloq | 65.611727    | −37.62076    | 2009          | IEBS, SB | 7           | 0.054 | 11.3 | 0.313  | 4–5 | 0       |         |
| Bl10  | IS     | Suðurnes | North of Kleifarvatn | 63.869997    | −22.55834    | 2010          | RE  | 10            | 0.027 | 8.5  | 0.246  | 1–2 | 0       |         |
| Bl03  | IS     | Suðurland | Laugarvatn | 64.213454    | −20.77142    | 2009          | IEBS, SB | 2           | 0.018 | 1.4  | 0.185  | 1   | 0       |         |
| Bl01  | S (NO) | Haakon VII Land | Backfjorden | 79.388374    | 13.439305    | 2009          | IEBS, IGA, RE, SB | 3          | 0.000 | 0.0  | 0.555  | 1   | 0       |         |
| ssp. boreale |        |          |        |                |              |               |      |               |   |       |         |     |         |         |
| Bb02  | NO     | Hedmark  | Folldal | 69.507657    | 21.30714     | 2009          | RE  | 4             | 0.199 | 36.1 | 1.038  | 4   | 0       |         |
| Carex capillaris |        |          |        |                |              |               |      |               |   |       |         |     |         |         |
| ssp.fuscidula |        |          |        |                |              |               |      |               |   |       |         |     |         |         |
| Cc08  | NO     | Troms    | Nordreisa II | 69.606346    | 22.154588    | 2009          | RE  | 4             | 0.157 | 27.7 | 1.012  | 4   | 0       |         |
| ssp.fuscidula |        |          |        |                |              |               |      |               |   |       |         |     |         |         |
| Cc07  | NO     | Troms    | Nordreisa I | 69.525222    | 19.16828     | 2009          | RE  | 3             | 0.120 | 18.1 | 1.015  | 3   | 0       |         |
| ssp.fuscidula |        |          |        |                |              |               |      |               |   |       |         |     |         |         |
| Cc06  | NO     | Troms    | Troms  | 68.83144      | −22.45722    | 2010          | RE  | 7             | 0.119 | 26.5 | 0.978  | 7   | 0       |         |
| ssp.fuscidula |        |          |        |                |              |               |      |               |   |       |         |     |         |         |
| Cc05  | IS     | Suðurnes | Grindavik | 64.211609    | −20.77471    | 2009          | IEBS, SB | 10          | 0.049 | 16.9 | 0.924  | 6–8 | 1       |         |
| Cc02  | IS     | Suðurland | Laugarvatn | 79.385909    | 13.440977    | 2009          | IGA, RE, SB | 10          | 0.044 | 13.3 | 1.022  | 8–10 | 1       |         |
| Cc01  | S (NO) | Haakon VII Land | Backfjorden | 45.941387    | 7.655009     | AT            | 8       | 0.139 | 34.9 | 0.978  | 7   | 1       |         |
| Cc03  | IT     | Valle d’Aosta | Walliser Alpen | 45.983891    | 7.792214     | 2009          | PK  | 7             | 0.109 | 28.9 | 1.082  | 8   | 1       |         |
| Cc10  | CH     | Valais  | Zermatt | 62.33944      | 10.207651    | 2010          | IEBS, SB | 9           | 0.104 | 24.1 | 1.333  | 3   | 0       |         |
| Cc09  | NO     | Hedmark  | Folldal | 73.999998     | −22.00002    | 2010          | OG  | 6             | 0.142 | 33.7 | 2.499  | 6   | 0       |         |

Uncertain  | Cc11  | GL (DK) | A. P. Olsen Land | Zackenberg | 45.983891 | 7.792214 | 2009 | PK  | 7 | 0.109 | 28.9 | 1.082 | 8   | 1       |         |

Continued
Table 2. Continued

| Taxon                  | Pop ID | Country | Region      | Locality           | Latitude (N) | Longitude (E) | Year | Collector(s)  | n  | D     | % polym. | DW    | Min–max | Private |
|------------------------|--------|---------|-------------|--------------------|--------------|---------------|------|---------------|----|-------|----------|-------|---------|---------|
| **Sparganium**, *=* Camx krausei | Gk01   | S (NO)  | James I Land | Kapp Smith         | 78.66421     | 15.113933    | –    | IGA           | 8  | –     | –        | –     | –       | –       |
|                         | Gk06   | CH      | Valais      | Zermatt            | 45.983329    | 7.7833381    | 2009 | PK            | 10 | 0.103 | 27.4     | 1.042 | 9       | 3       |
|                         | Gk09   | AT      | Styria      | Sölkkpass          | 47.269996    | 14.080002    | –    | Schönswetter et al. (2004) | 5  | 0.088 | 16.7     | 1.038 | 5       | 1       |
|                         | Gk10   | RU      | Polar-Ural  | Stanztegog mountain | 66.905802   | 65.728889    | 2004 | AT, IGA       | 10 | 0.042 | 10.7     | 1.035 | 4–9     | 2       |
|                         | Gk07   | CH/IT   | Splügenpass  |                    | 46.499998    | 9.3300063    | –    | Schönswetter et al. (2004) | 5  | 0.038 | 7.1       | 1.035 | 4–5     | 3       |
|                         | Gk02   | NO      | Trøms       | Trømsø             | 69.783802    | 19.435459    | 2009 | TA            | 9  | 0.036 | 15.5     | 1.040 | 5–7     | 0       |
|                         | Gk04   | S (NO)  | Ny-Fries Land | Ringhornsdalen     | 79.335782    | 16.128264    | 2010 | AKB, IGA      | 10 | 0.028 | 8.3       | 1.036 | 5–7     | 0†      |
|                         | Gk01   | S (NO)  | Haakon VII Land | Ossian Sarsfjellet | 78.928385    | 12.44045     | 2009 | IGA, IEBS, RE, SB | 7  | 0.024 | 7.1       | 1.036 | 2–3     | 0†      |
|                         | Gk11   | US      | Alaska      | Seward Peninsula   | 65.259997    | –166.35      | –    | Schönswetter et al. (2004) | 5  | 0.019 | 4.8       | 1.038 | 2–4     | 5       |
|                         | Gk05   | NO      | Hedmark     | Folldal            | 62.192569    | 9.778011     | 2010 | AKB, IEBS, RE, SB | 10 | 0.018 | 6.0       | 1.036 | 2–4     | 0       |
|                         | Gk03   | S (NO)  | Ny-Fries Land | Flatøyrdalen      | 79.289322    | 16.054733    | 2010 | AKB, IGA, PBE, RE | 8  | 0.011 | 2.4       | 1.032 | 1–4     | 0†      |
|                         | Gk08   | FR/IT   | Col du Petit St. Bernard | | 45.669984    | 6.869996     | –    | Schönswetter et al. (2004) | 2  | –     | –        | –     | –       | –       |
| **Gentianella**, *=* campestris | Gc01   | NO      | Trøms       | Trømsø             | 69.719201    | 19.102329    | 2009 | TA            | 5  | –     | –        | –     | –       | –       |
|                         | Gc02   | IS      | Suðurland   | Geysir             | 2009 IEBS, SB | 5               | –     | –            | –  | –     | –        | –     | –       | –       |
| **Kobresia**, *=* simplicissima | Ks02   | S (NO)  | Haakon VII Land | Ossian Sarsfjellet | 78.928538    | 12.459776    | 2009 | IGBS, IGA, ÖBM, RE, SB | 10 | 0.016 | 3.0       | 0.375 | 2–3     | 1       |
|                         | Ks04   | S (NO)  | Ny-Fries Land | Flatøyrdalen      | 78.289322    | 16.028505    | 2010 | AKB, IGA, PBE, RE | 9  | 0.009 | 1.5       | 0.365 | 1–2     | 1       |
|                         | Ks01   | S (NO)  | Ønskog land  | Gipsvik            | 78.453216    | 16.535147    | 2009 | IEBS, IGA, SB  | 9  | 0.000 | 0.0       | 0.277 | 1       | 0       |
|                         | Ks03   | S (NO)  | Haakon VII Land | Blomstrand        | 78.973786    | 12.184115    | 2009 | IGBS, IGA, ÖBM, RE, SB | 10 | 0.000 | 0.0       | 0.277 | 1       | 0       |
|                         | Ks05   | NO      | Sør-Trøndelag | Røros             | 62.60159     | 11.654586    | 2009 | RE            | 5  | 0.120 | 9.1       | 1.228 | 3       | 2       |
|                         | Ks06   | NO      | Hedmark     | Folldal            | 62.317515    | 9.853130     | 2010 | AKB, IEBS, RE, SB | 8  | 0.020 | 3.0       | 0.819 | 2–3     | 0       |
| **Kobresia myosuroides** | Km02   | IS      | Suðurland   | Geysir             | 2009 IEBS, SB | 5               | –     | –            | –  | –     | –        | –     | –       | –       |
| Species                | Code | Location                        | Collector | Year  | Locality Details                      | Code | N  | PM | MA  | NM  | Type |
|-----------------------|------|---------------------------------|-----------|-------|---------------------------------------|------|----|----|-----|-----|------|
| Ranunculus wilanderi  | Rw01 | Dickson Land, Kapp Thordsen      | Thordsen  | 2009  | 78.459342 15.545322                   | IGA  | 11 | 0.001 | 0.9 | 1-2 |       |
| Ranunculus auricomus  | Rw02 | Dickson Land, Kapp Thordsen      | Thordsen  | 2008  | 78.459055 15.52601                    | IGA  | 8  |       |     |     |       |
| Sibbaldia procumbens  | Ro01 | NO Hedmark, Follidal             |          | 2010  | AkB, RE, SB                            | IGA  | 5  |       |     |     |       |
|                       | Sp01 | GL (DK) Qaasuitsup, Blomsteraleden, Qeqertarsuaq (Disko Island) |          | 2006  | KBW                                   | IGA  | 4  | 0.239 | 25.2 | 1.024 | 4    |
|                       | Sp02 | GL (DK) Sermersooq, Kulusuk      |          | 2009  | IEBS, SB                              | IGA  | 4  | 0.108 | 11.7 | 0.300 | 4    |
|                       | Sp03 | RU Khanty-Mansiya, The Ural Mountains |          | 2004  | IGA, AT                               | IGA  | 5  | 0.100 | 12.6 | 1.406 | 5    |
|                       | Sp04 | IS Vestfjør, Brekkudalur, Onundarfjordur |          | 2010  | RE                                    | IGA  | 10 | 0.095 | 14.4 | 0.391 | 10   |
|                       | Sp05 | GL (DK) Sermeæsoq, Tasiilaq      |          | 2009  | IEBS, SB                              | IGA  | 10 | 0.090 | 15.3 | 0.290 | 6-8  |
|                       | Sp06 | US Alaska, Undaska              |          | 2007  | IEBS, SB                              | IGA  | 5  | 0.087 | 10.8 | 0.753 | 5    |
|                       | Sp07 | NO Hordaland, Finse             |          | 2010  | IEBS, SB                              | IGA  | 10 | 0.068 | 11.7 | 0.314 | 8-9  |
|                       | Sp08 | CA Yukon, North Yukon           |          | 2006  | BB                                    | IGA  | 5  | 0.065 | 8.1  | 0.383 | 5    |
|                       | Sp09 | NO Hedmark, Follidal             |          | 2010  | IEBS, SB                              | IGA  | 10 | 0.063 | 9.0  | 0.296 | 6-8  |
|                       | Sp10 | FO (DK) Eystror, Strendur       |          | 2010  | IEBS, JD                              | IGA  | 8  | 0.052 | 7.2  | 0.307 | 8    |
|                       | Sp11 | IT Piemonte, Coll di'Agnello     |          | 2009  | AT                                    | IGA  | 10 | 0.043 | 10.8 | 0.429 | 4-6  |
|                       | Sp12 | J (NO) South-West Jan Mayen     |          | 2011  | GA                                    | IGA  | 3  | 0.032 | 2.7  | 0.283 | 2    |
|                       | Sp13 | CH Valais, Aletschgletscher     |          | 2009  | PK                                    | IGA  | 10 | 0.027 | 5.4  | 0.604 | 5-7  |
|                       | Sp14 | AT Salzburg, Lungau, Oberes Murtal, Radstadt Tauern |          | 2009  | AT, KM                                | IGA  | 10 | 0.023 | 4.5  | 0.367 | 3-4  |
|                       | Sp15 | IT Valle d'Aosta, Breuil-Cervinia, Plan Maison |          | 2009  | AT                                    | IGA  | 10 | 0.018 | 2.7  | 0.284 | 3-4  |
|                       | Sp16 | IS Austurland, Skaffafell        |          | 2010  | ÖBM                                   | IGA  | 11 | 0.015 | 4.5  | 0.314 | 2-4  |
|                       | Sp17 | CA Nunavut, Kitikmeot region    |          | 2009  | IEBS, IG, RE, SB                      | IGA  | 25 | 0.000 | 0.0  | 0.300 | 1    |
|                       | Sp18 | CA Nunavut, Kitikmeot region    |          | 2009  | IEBS, IG, RE, SB                      | IGA  | 1  | 1    | 1    | 1    | 0    |
|                       | Sp19 | CA Nunavut, Kitikmeot region    |          | 2009  | IEBS, IG, RE, SB                      | IGA  | 1  | 1    | 1    | 1    | 0    |
| Sibbaldia cuneata     | Sc01 |                                 |          | 2009  | RE                                    | IGA  | 2  |       |     |     |       |
| Tofieldia pusilla     | Tp07 | GL (DK) Sermersooq, Tasiilaq    |          | 2009  | IEBS, SB                              | IGA  | 10 | 0.214 | 53.1 | 0.290 | 9-10 |

Continued
| Taxon                | Pop ID | Country | Region    | Locality           | Latitude (N) | Longitude (E) | Year | Collector(s) | n | % polym. | DW | Min–max | Private |
|---------------------|--------|---------|-----------|--------------------|--------------|---------------|------|--------------|---|----------|-----|---------|---------|
| Tofieldia calyculata| Tco01  | CA      | Yukon     | Yukon/North Territories | 67.038211 | 116.21555     | LG   |               | 8 | 0.059    | 0.028 |         |         |
| Tofieldia coccinea  | Tco01  | CA      | Yukon     | Yukon/North Territories | 67.038211 | 116.21555     | LG   |               | 8 | 0.059    | 0.028 |         |         |

Heidings population identity [Pop ID]; n number of individuals sampled for AFLP analyses \( [n] \), average proportion of pairwise differences \( [D] \), proportion of polymorphic markers \( [% \text{polym.}] \), frequency down weighted marker value \( [\text{DW}] \), minimum to maximum number of AFLP multilocus phenotypes \( [\text{min–max}] \), and number of private AFLP markers \( [\text{Private}] \). Mean \( [\text{l}] \) and standard deviation \( [\text{r}] \) for \( [D] \), \[% \text{polym.}] \), and \[\text{DW}] \) given below each species. Populations from Svalbard are indicated in bold. Outgroup for neighbour-joining analyses marked in grey.

1 Country: AT, Austria; CA, Canada; CH, Switzerland; DE, Germany; DK, Denmark; FO, Faroe Islands; FR, France; GL, Greenland; IS, Iceland; IT, Italy; J, Jan Mayen; NO, Norway; RU, Russia; S, Svalbard; US, United States of America.

2 Collectors: AKB, Anne Krag Brysting; AT, Andreas Tribsch; BA, Brian Apland; BB, Bruce Bennett; BK, Brad Krieckhaus; GA, Geir Arnesen; IGA; Inger Greve Alsos; IA, Ida Apland; IEBS, Idunn Elisabeth Borgen Skjetne; JD, Jan Djurhuus; KBW, Kristine Bakke Westergaard; KM, Karin Moosbrugger; LA, Liudmila Aleksandrovna Sergienko; LG, Lovisa Gustafsson; ML, Maarten J. J. E. Loonen; OG, Olivier Gilg; OBM, Olof Birna; Olafsson; PBE, Pernille Bronken Eidesen; PK, Patrick Kuss; RE, Reidar Elven; SB, Siri Birkeland; TA, Torbjørn Alm; UL, Unni Lundgren.

3 Comastoma tenellum plant material from the Alps and Alaska is from Schönswetter et al. (2004) and plant material and AFLP data for S. procumbens has previously also been published in Allen et al. (2015) and Aksnes et al. (2015a), respectively, but then as part of other research questions.

4 Two private markers were found in Svalbard as a whole when grouping Ct01, Ct03, and Ct04.

5 Two private markers were found in Svalbard as a whole, when grouping Ct01, Ct03, and Ct04.

6 Comastoma tenellum plant material from the Alps and Alaska is from Schönswetter et al. (2004) and plant material and AFLP data for S. procumbens has previously also been published in Allen et al. (2015) and Aksnes et al. (2015a), respectively, but then as part of other research questions.
purification step was added after the last centrifugation: 1 ml ice-cold 70% ethanol was added to each sample, centrifuged for 2 min at 13 000 rpm, and then removed. This step was repeated before the samples were left over night to dry. The final DNA pellet was dissolved in 100 μl TE-buffer and 1 μl RNase was added before the incubation at 37 °C. DNA from individuals of Comastoma tenellum and Ranunculuswilanderi was isolated using the Qiagen DNeasy™ Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer’s protocol. DNA from Tofieldia pusilla individuals was isolated using the E.Z.N.A.™ SP Plant DNA Mini Kit, following the protocol for dry specimens (Omega Bio-Tek, Norcross, USA). The protocol was modified by adding a freezing step (at −80 °C for 10 min) prior to cell lysis. To increase the final DNA concentration of C. tenellum and T. pusilla samples, the amount of AE buffer was reduced to 30–50 μl, the first eluate (i.e. DNA dissolved in AE buffer) was re-eluted in a second elution step, and incubation was done at 65 °C. The DNA concentration of the samples was measured using a spectrophotometer (NanoDrop™ 1000, Thermo Fisher Scientific, Wilmington, USA). Samples isolated with the Ziegenhagen protocol were diluted so that the final DNA concentrations were approximately the same within each species (60 ng/μl). The Qiagen DNeasy Plant Mini Kit and the E.Z.N.A. Plant DNA Mini Kit gave concentrations of approximately 20 ng/μl, which were kept undiluted.

**AFLP analysis**

Amplified Fragment Length Polymorphism (AFLP) was used to generate dominant molecular markers from the sampled individuals (Vos et al. 1995). The AFLP procedure was modified slightly from Jørgensen et al. (2006): 2 μl DNA isolate was used in the restriction-ligation step, and the amount of AmpliTaq polymerase (Applied Biosystems/Life Technologies, Carlsbad, CA, USA) used in the pre-selective amplification of fragments was increased to 0.075 μl. PCR conditions during the elongation step were modified to 2 and 1 min at 72 °C for the pre-selective and selective amplification of fragments, respectively. All reactions were carried out on an Eppendorf Thermal Cycler (Mastercycler® ep gradient S, Hamburg, Germany). A series of primer tests were performed prior to the final selective amplification step on a selection of samples from different geographic regions [see Supporting Information—Table S3]. Finally, 3–4 primer pairs were chosen for each species [see Supporting Information—Table S3]. The 6-FAM EcoRI-primer and all non-labelled primers and adaptors were ordered from MWG (Ebersberg, Germany) or IDT (Leuven, Belgium), while the other fluorescent-labelled primers were ordered from Applied Biosystems/Life Technologies.

A set of negatives, replicates and duplicates was included in all final AFLP runs to check for contamination and replicability (Bonin et al. 2004). The fluorescently labelled AFLP fragments were detected on an ABI3730 DNA Analyser (Applied Biosystems/Life Technologies). For each sample, 2 μl from a mix of co-loaded selective products (3 μl FAM, 3 μl NED, 3 μl PET and 2 μl VIC) were mixed with 0.3 μl GeneScan™ 500 (−250) LIZ size standard and 11.7 μl Hi-Di™ formamide (both from Applied Biosystems/Life Technologies). Selective products of Sibbaldia procumbens were run with only 8.85 μl HiDi formamide and 0.15 μl LIZ size standard. The plate was vortexed, spun down and denatured at 95 °C for 5 min. After denaturation, the plate was immediately put on ice for a few minutes and then run on the ABI Analyzer.

AFLP profiles were visualized using GeneMapper ver. 4.0 (Applied Biosystems). Unambiguously scorable fragments (peaks) in the size range of 50–500 bp were scored as absence/presence, following the approach of Whitlock et al. (2008), and their R-based interactive scripting program AFLPscore ver. 1.4., using the filtering option for locus selection and relative threshold for phenotype calling. Error rate estimation was calculated as the average percentage of differences between replicate pairs (i.e. mismatch error rate; Bonin et al. 2004). For each primer combination, the thresholds for locus selection and phenotype calling that resulted in the highest number of highly reproducible markers were chosen. Fragments with a frequency lower than the error rate were re-checked and removed if no clear peak was present. Fragments missing in only a few individuals were also re-checked and corrected if scored incorrectly.

**Statistical analyses of AFLP data**

The percentage of polymorphic AFLP markers was calculated both at species level [see Supporting Information—Table S3] and at population level. Monomorphic markers at species level were excluded from further analyses. Within-population genetic diversity was estimated as the average proportion of pairwise differences between individuals, D (Nei 1973; Kosman 2003) and the percentage of polymorphic markers. The minimum and maximum number of AFLP multilocus phenotypes was calculated for each population. The minimum number of AFLP multilocus phenotypes included only multilocus phenotypes which were identical across all markers, whereas the maximum number of multilocus phenotypes allowed for a number of pairwise differences equal to the error rate. To address the genetic distinctiveness of the Svalbard populations, ‘frequency down weighted marker values’ (DW) were...
calculated according to Schönswetter and Tribsch (2005) (except for populations with less than two sampled individuals). Private AFLP markers (i.e. markers unique to the Svalbard populations) were also recorded. All calculations listed above, as well as most data format conversions, were performed using the AFLPdat R-script ver. 2010 (Ehrich 2006) in R ver. 3.2.1 (R Core Team 2015).

Genetic groups were delineated for each species (except Ranunculus wilanderi) using STRUCTURE ver. 2.3.3 (Pritchard et al. 2000), run through the Biportal (now the Lifeportal) of the University of Oslo. We applied the no-admixture model on the AFLP data, which was treated as diploid multi-locus genotypes, using the recessive allele model for dominant markers (Falush et al. 2007). The number of possible groups, K, was set to range from one to the total number of sampling localities for each species. Ten independent runs were carried out for each number of K. A burn-in period of \(10^5\) iterations was followed by \(10^6\) iterations. The results of the independent runs were summarized using the R-script STRUCTURE-sum ver. 2011 (Ehrich et al. 2007) and the most appropriate number of genetic groups, K, was determined according to recommendations in Evanno et al. (2005; i.e. as the K with the highest value of delta K), but posterior probabilities (Pritchard et al. 2000) and similarity coefficient estimates (Nordborg et al. 2005) were also considered. To reveal hierarchical genetic structure in the data, separate STRUCTURE analyses were run on the group(s) to which the Svalbard individuals were grouped by the first STRUCTURE analysis for species with moderate to strong geographic structure. Finally, supplementary principal coordinates analyses (PCO) (Davis 1986) and neighbour-joining analyses (Saitou and Nei 1987) were performed to evaluate the results obtained by the STRUCTURE analyses. PCO and neighbour-joining analyses were performed in PAST ver. 2.13 (Hammer et al. 2001) using the Dice similarity coefficient (Dice 1945). Most results from the PCO and neighbour-joining analyses are not presented, as they were largely congruent with the STRUCTURE results. However, the neighbour-joining and PCO analyses gave support for a separate Greenlandic group in Carex capillaris (for PCO plot, [see Supporting Information—Figure S4]), contradicting the results from STRUCTURE. Due to its uncertain affiliation, the Greenlandic population was omitted from further analyses (i.e. the hierarchical STRUCTURE analysis, AMOVA analyses and the assignment tests).

To determine the partitioning of genetic variation among populations and among genetic groups revealed by the STRUCTURE analyses, AMOVAs (analyses of molecular variance) were run in Arlequin ver. 3.5 (Excoffier et al. 2005). A fixation index, the \(F_{ST}\) analogue for dominant markers (\(F_{ST};\) Excoffier et al. 1992), was calculated based on the number of pairwise differences between individuals.

The source area(s) of the Svalbard populations (except for Ranunculus wilanderi and Kobresia simpliciscula) was inferred by performing multi-locus assignment tests in AFLPOP ver. 1.1 (Duchesne and Bernatchez 2002). Geographically consistent genetic groups or subgroups (i.e. obtained by the STRUCTURE analyses) were considered as potential source areas. If no geographic genetic structure was revealed by the STRUCTURE and additional PCO and neighbour-joining analyses, geographic regions were considered as potential source areas. We used a log likelihood difference of one as a threshold for allocation. With this threshold, the likelihood for an AFLP phenotype to be found in its most likely source region had to be 10 times higher, or more, than for the second most likely source region.

As in Alsos et al. (2007), we examined the genetic founder and bottleneck effects in relation to adaptation to the current climatic conditions in Svalbard. We used six different measures to quantify the genetic founder/bottleneck effects (Alsos et al. 2007; [see Supporting Information—Table S5]). To quantify the adaptation to the current climatic conditions in Svalbard, we used two measures of temperature requirement and rated their rarity (Alsos et al. 2007; [see Supporting Information—Table S5]). The measures of genetic founder/bottleneck effects and climatic adaptation were summarised in two separate principal component analyses (PCA), using R ver. 3.2.1 (R Core Team 2015). The first principal components from the two analyses were then plotted against each other, showing the genetic founder/bottleneck effects for the species in relation to their adaptation to the current climatic conditions in Svalbard. Finally, a simple linear regression was performed to find the correlation coefficient between the two variables (i.e. climatic adaptation and founder/bottleneck effects). Ranunculus wilanderi was omitted from the analysis due to limited AFLP data. In addition to the study species, we included 12 species with already published AFLP data from Svalbard (Alsos et al. 2007; Westergaard et al. 2011; Gussarova et al. 2012; [see Supporting Information—Table S5]).

**Results**

**Number of populations, population sizes and red list categories**

The number of populations found in Svalbard (Table 1) ranged from one (Botrychium lunaria, Carex capillaris ssp. fuscicula, Ranunculus wilanderi and Sibbaldia...
procumbens) to ten (Tofieldia pusilla); all populations were situated within the warmest bioclimatic subzone in Svalbard (the Middle Arctic Tundra Zone). Two populations of T. pusilla and one of Comastoma tenellum were previously unknown. The population sizes ranged from less than five individuals (T. pusilla, Ossian Sarsfjellet) to more than 2000 (C. capillaris ssp. fuscidula, Bockfjorden). These new population size data led to a downgrading of C. capillaris ssp. fuscidula, T. pusilla, S. procumbens, C. tenellum and R. wilanderi in the 2010 Red List [see Supporting Information—Table S1]. However, T. pusilla was upgraded from ‘Least Concern’ to ‘Near Threatened’ in the 2015 Red List due to a higher weighting of fragmentation of its range. The same year, an adjustment to the IUCN criteria also led to a further downgrading of C. tenellum (now ‘Vulnerable’) and C. capillaris (now ‘Near Threatened’). At present, five of the seven study species are considered threatened in Svalbard [see Supporting Information—Table S1], mostly due to restricted extent of occurrence (criterion B1), limited area of occupancy (criterion B2) and/or a low number of reproducing individuals (criterion D1) (Henriksen and Hilmo 2015).

Genetic results

The levels of genetic variation within the Svalbard populations were low for most species, with only one AFLP multilocus phenotype identified in Botrychium lunaria, Sibbaldia procumbens and probably also in Ranunculus wilanderi (Table 2). Moreover, there was a positive correlation between genetic founder/bottleneck effects and thermophily ($R^2 = 0.6964$, $n = 18$, Fig. 2). The strongest founder/bottleneck effects were found in B. lunaria, which is also the most thermophilous species [see Supporting Information—Table S5]. Strong founder/bottleneck effects and high levels of thermophily were also found in S. procumbens, Carex capillaris ssp. fuscidula and Kobresia simpliciuscula ssp. subhilarctica. Intermediate levels of founder/bottleneck effects were found in Tofieldia pusilla and Comastoma tenellum.

Tofieldia pusilla and Botrychium lunaria had a considerably higher proportion of within population genetic variation than among population genetic variation according to the AMOVA (Table 3). Furthermore, the STRUCTURE analyses delineated four genetic groups in T. pusilla ($K = 4$) and three genetic groups in B. lunaria ($K = 3$). However, T. pusilla (Fig. 1a) had the weakest geographic pattern with nearly all populations being admixed, while admixture occurred only in half of the B. lunaria populations (Fig. 1b). Neither species had unique STRUCTURE groups nor private markers in Svalbard. The assignment tests could not target source area(s) for the Svalbard population of B. lunaria. For T. pusilla, the assignment test allocated the Svalbard populations to a large unspecified European group including all sampled populations except Greenland [see Supporting Information—Table S6]. Carex capillaris and Sibbaldia procumbens both had a considerably higher proportion of among population genetic variation than within population genetic variation according to the AMOVA (Table 3). When taking the two STRUCTURE groups found in S. procumbens (Fig. 1c) into account, as much as 69.9% of the total detected genetic variation was attributed to variation among these (Table 3). In the hierarchical STRUCTURE analysis of the Eurasian group (data not shown), the Svalbard population of S. procumbens formed a group together with the Russian population and a population from Folldal, mainland Norway (hereafter called Northwest Europe). Two main STRUCTURE groups ($K = 2$) were also delineated in C. capillaris (Fig. 1d), largely corresponding to the two assumed subspecies C. capillaris ssp. fuscidula and C. capillaris ssp. capillaris. In the hierarchical STRUCTURE analysis of the ssp. fuscidula group (data not shown), the Svalbard population was separated as its own group. The assignment test confirmed Northwest Europe and Northern Norway as the source areas for the Svalbard individuals of S. procumbens and C. capillaris ssp. fuscidula, respectively. However, source area was only confirmed in half of the C. capillaris ssp. fuscidula individuals...
In both *C. capillaris* ssp. *fuscidula* and *S. procumbens*, the Svalbard population scored below average on the rarity index (DW = 1.022 and 0.300, respectively, Table 2), but one private Svalbard marker was found in *C. capillaris* ssp. *fuscidula* (Table 2).

*Comastoma tenellum* had high among population variation (Table 3) and the STRUCTURE analysis revealed three geographically consistent genetic groups: (1) Svalbard and Russia, (2) Alaska, Norway and one population from the Alps and (3) the remaining populations from the Alps (Fig. 1e). The assignment tests indicated

**Table 3. Analyses of molecular variance (AMOVAs) of AFLP multilocus phenotypes in six of the seven study species.**

| Species                        | Source of variation                                      | d.f. | % of total variation | Fixation index (\(F_{ST}\)) |
|--------------------------------|----------------------------------------------------------|------|----------------------|-----------------------------|
| *Tofieldia pusilla*            | Among all populations                                    | 17   | 35.4                 |                             |
|                                | Within all populations                                   | 127  | 64.6                 | 0.35                        |
| *Botrychium lunaria*           | Among all populations                                    | 14   | 36.1                 |                             |
|                                | Within all populations                                   | 97   | 63.9                 | 0.36                        |
| *Carex capillaris*             | Among all populations                                    | 9    | 65.9                 |                             |
|                                | Within all populations                                   | 58   | 34.1                 | 0.66                        |
|                                | Among two main genetic groups (largely corresponding to ssp. *capillaris* and ssp. *fuscidula*) | 1    | 28.0                 |                             |
|                                | Among populations within two main genetic groups (largely corresponding to ssp. *capillaris* and ssp. *fuscidula*) | 7    | 38.6                 |                             |
|                                | Within populations                                       | 53   | 33.5                 | 0.67                        |
| *Sibbaldia procumbens*         | Among all populations                                    | 17   | 78.3                 |                             |
|                                | Within all populations                                   | 133  | 21.7                 | 0.78                        |
|                                | Among two main genetic groups                            | 1    | 69.9                 |                             |
|                                | Among populations within two main genetic groups         | 16   | 15.7                 |                             |
|                                | Within populations                                       | 133  | 14.4                 | 0.86                        |
| *Comastoma tenellum*           | Among all populations                                    | 10   | 86.2                 |                             |
|                                | Within all populations                                   | 70   | 13.8                 | 0.86                        |
|                                | Among three main genetic groups                          | 2    | 57.0                 |                             |
|                                | Among populations within three main genetic groups       | 8    | 31.4                 |                             |
|                                | Within populations                                       | 70   | 11.5                 | 0.88                        |
| *Kobresia simpliciuscula*      | Among all populations                                    | 5    | 94.6                 |                             |
|                                | Within all populations                                   | 45   | 5.4                  | 0.95                        |
|                                | Among two main genetic groups (corresponding to ssp. *subholarctica* and ssp. *simpliciuscula*) | 1    | 94.5                 |                             |
|                                | Among populations within two main genetic groups (corresponding to ssp. *subholarctica* and ssp. *simpliciuscula*) | 4    | 2.9                  |                             |
|                                | Within populations                                       | 45   | 2.6                  | 0.97                        |

Headings: components of variance [Source of variation]; degrees of freedom [d.f.]; percentage of total variation [% of total variation]; fixation index for binary data [Fixation index (\(F_{ST}\))]. (main genetic groups were first of all inferred from STRUCTURE analyses (see Fig. 2), but PCO and neighbour-joining analyses were also considered).

*All P < 0.0001.

[see Supporting Information—Table S6].
Russia as source area for all Svalbard individuals [see Supporting Information—Table S6], but Svalbard constituted a separate group in the additional hierarchical STRUCTURE analysis for the Svalbard-Russia group (data not shown). Furthermore, two private Svalbard markers were also found (Table 2).

Two STRUCTURE groups were delineated for K. simpliciuscula (Fig. 1f). These two groups corresponded to ssp. simpliciuscula and ssp. subholarctica, and nearly all detected genetic variation in the data set was attributed to variation between these two subspecies (Table 3). Two of the K. simpliciuscula populations in Svalbard (Ossian Sarsfjellet and Flateyrdalen) possessed one possible private marker each (Table 2). Finally, STRUCTURE and AMOVA analyses were not performed for the endemic and genetically depauperate microspecies R. wilanderi (Fig. 1g).

Discussion

As expected from Alsos et al. (2007), we found that genetic founder/bottleneck effects are correlated with adaptation to the climatic conditions in Svalbard. Furthermore, we found that most of our study species, which are characterized by high levels of thermophily, have experienced strong genetic founder/bottleneck effects. Climatic limitations seem also to be reflected in the number, sizes and localization of the examined Svalbard populations.

Causes of low levels of genetic variation

Alsos et al. (2007) interpreted the stronger genetic founder/bottleneck effect in thermophilous plants in Svalbard as a result of restricted establishment, survival and local reproduction rather than dispersal per se. Temperature has probably been less of a limiting factor for thermophilous species arriving in the early Holocene warm period, as previously inferred for e.g. Betula nana, Campanula rotundifolia, Vaccinium uliginosum (Alsos et al. 2002), Euphrasia wettsteinii (Gussarova et al. 2012) and Salix herbacea (Alsos et al. 2009). The observed genetic patterns are therefore likely a product of subsequent bottleneck effects following climate cooling rather than an initial founder effect for this group of species. Most of our study species probably belong to the group of early Holocene arrivals, and some of them even have populations that are clearly differentiated from their source populations outside Svalbard. The Svalbard populations of Carex capillaris ssp. fuscidula and Comastoma tenel- lum were for instance identified as unique groups in the hierarchical STRUCTURE analyses and also harboured one and two private markers, respectively. Colonization during the warmer parts of the Holocene can also be inferred for Kobresia simpliciuscula ssp. subholarctica and Tofieldia pusilla as these two species have multiple populations with several AFLP multilocus phenotypes despite today’s unfavourable climate.

In contrast, the single populations of Botrychium lunaria and Sibbaldia procumbens consisted only of one AFLP multilocus phenotype and were not differentiated from populations in other geographic regions. Botrychium lunaria and S. procumbens also showed the strongest genetic founder/bottleneck effects of all species included. It is somewhat surprising to observe such a strong founder/bottleneck effect in B. lunaria as we expected levels of genetic variation to be extremely low throughout the distribution range due to intragametophytic self-fertilization (see e.g. Soltis et al. 1988; Hauk and Hauffer 1999; Farrar 2006). Contrary to what we predicted, most B. lunaria populations actually contain many AFLP multilocus phenotypes and a higher proportion of within-population genetic variation relative to among population genetic variation. This pattern has, however, also been found in several other Botrychium studies that are using non-coding markers (Camacho and Liston 2001; Williams 2012). As there is generally low genetic differentiation among Botrychium populations, the explanation is probably a combination of high dispersal potential and a mainly inbreeding mating system (Soltis et al. 1988; Stensvold 2008; Williams 2012). The strong genetic founder/bottleneck effects in B. lunaria and S. procum- bens may be the result of recent founding events and the observed lack of genetic diversity might suggest that each of their populations in Svalbard was established by a single propagule.

Overall, our results strongly support that the genetic depletion of the thermophilous species in Svalbard is a result of restricted initial establishment and/or population decline following climate cooling (Alsos et al. 2002, 2015), as well as lack of sexual reproduction under the present climatic conditions (Engelskjøn et al. 2003).

Threats to the Svalbard populations

Due to low levels of genetic diversity, the thermophilous plant species in Svalbard may be vulnerable to inbreeding depressions and also have reduced evolutionary potential. This will however depend on species traits and species history. The risk of inbreeding depression may for instance be low for Botrychium lunaria as this is a pteridophyte that reproduces by intragametophytic self-fertilization and is expected to have undergone purging of deleterious recessive alleles (Farrar 2006). Similarly, Ranunculus wilanderi is apomictic and will not experience any increase in homozygosity with decreasing
population size (Richards 2003; Pellino et al. 2013). Furthermore, like many other pteridophytes, the subterranean, gametophytic phase of *B. lunaria* is also highly dependent on its mycorrhizal fungal partner (Farrar 2006; Winther and Friedman 2007). The gametophyte is therefore thought to have reduced direct interaction with the environment and evolutionary potential may not entirely depend on genetic variation in the sporophyte generation (Farrar 2006). However, based on the results presented here, most of the study species may still be prone to inbreeding depressions, further loss of genetic variation and also have reduced adaptability to future environmental change.

In addition to the abovementioned threats, demographic and/or environmental stochasticity may also be of serious concern for the thermophilous plant species in Svalbard. This regards especially the species with few and small populations. Presence of seed banks may function as a buffer against population fluctuations and extinctions, but are not reported from thermophilous species in Svalbard (Alsos et al. 2003; Cooper et al. 2004). The relative extinction risk associated with demographic and/or environmental stochasticity will also depend on the population growth rate (Lande 1993). Future climate change may stimulate population growth, but this will depend on a number of factors like e.g. current reproductive fitness and habitat preferences. Arctic wetland species like *Carex capillaris* ssp. *fuscidula*, *Kobresia simpliciuscula* ssp. *subholarctica*, *Ranunculus wilanderi* and *Tofieldia pusilla* are for instance expected to be negatively affected by changes in drainage conditions, evaporation rates and water supply (Young et al. 1997). Furthermore, competition is expected to increase with climate warming, and Arctic species with conservative nutrient-use strategies, slow growth and inflexible morphologies may become outcompeted by more responsive, faster growing, taller species immigrating from southern latitudes (Callaghan et al. 2005). Tracking of potential population size changes may give valuable insights into climate change responses and, following, future extinction risk.

**Svalbard management units and an evolutionarily significant microspecies**

The low levels of genetic diversity and distinctiveness that we recorded for the Svalbard populations of our study species are also reflected in most Arctic species studied until now, and may partly relate to the recent glaciation of the region (Eidesen et al. 2013; Stewart et al. 2016). We argue that all Svalbard populations examined in this study should be viewed as separate management units for three reasons: First, most of our study species have probably been present in Svalbard since the early Holocene warm period and for *Carex capillaris* ssp. *fuscidula* and *Comastoma tenellum* the Svalbard populations are genetically clearly differentiated from their source populations outside Svalbard (see above). Second, all examined Svalbard populations are likely demographically independent as there seems to be little current gene flow between these populations and populations outside Svalbard. This is clearly demonstrated by the strong founder/bottleneck effects. Finally, conservation of edge populations may be important for maintaining evolutionary potential as e.g. stress tolerance alleles may be more common here than in more optimal habitats (Sherwin and Moritz 2000). Considering the Svalbard populations as separate management units is also in line with the regional red list which treats Svalbard as a separate management area (Henriksen and Hilmo 2015).

Although delineating Evolutionarily Significant Units (ESUs) is beyond the scope of this study due to the lack of adaptive markers, information on *Ranunculus wilanderi* clearly suggests that it constitutes such a unit. The species is considered an endemic for the archipelago, but is just one of numerous microspecies within the *Ranunculus auricomus* complex (Jonsell 2001). Members of this complex possess the ability to produce seeds asexually by agamospermy (Jonsell 2001; Pellino et al. 2013), and reproductive isolation can therefore occur rapidly. *Ranunculus wilanderi* is nevertheless the only member of the *R. auricomus* complex present in Svalbard, it differs morphologically from other members in the *R. auricomus* complex (personal observation), and only shares its unusual habitat preference (damp moss tundra) with one other member from the complex; the diploid, and probably sexually reproducing, *Ranunculus boecheri* from eastern Greenland (Elven et al. 2011). Based on this we argue that *R. wilanderi* can be considered a separate ESU, although the relationship to other *R. auricomus* microspecies should be further examined.

**Genetic relationships of importance for conservation**

If it should become necessary to consider management strategies like translocations, information about genetic relationships will be especially important for species with historically isolated populations and little to moderate contemporary gene flow (Ottewell et al. 2016). In our case, this relates especially to *Carex capillaris* ssp. *fuscidula*, *Sibbaldia procumbens* and *Comastoma tenellum*. Although Svalbard is known to be predominantly colonized from Northern Russia and only occasionally from Northern Norway and Greenland (Alsos et al. 2007; Gussarova et al. 2012; Alsos et al. 2015), we were only
able to confirm Russia as source area for *C. tenellum*. For *C. capillaris* ssp. *fuscidula* the Svalbard population assigned to Northern Norway, but in this case no Russian populations were actually sampled or included in the analysis. The assignment test suggested Northwest Europe (including both Russian and Norwegian populations) as source area for *S. procumbens* but Allen et al. (2015) found the same Svalbard specimens of *S. procumbens* to belong to the North-American/North-Atlantic group using plastid markers—the opposite group of what is reported here. One explanation for these contradictory results might be that the current population of *S. procumbens* in Svalbard was established through multiple introductions from different sources, followed by hybridization and subsequent decline in genetic variation (Allen et al. 2015). Multiple introductions have also been suggested for several other plant species in Svalbard (Alsos et al. 2007). On the other hand, the individuals from Svalbard clearly clustered with Northwest Europe (confirmed by both STRUCTURE and PCO analyses), and also showed very little genetic differentiation from other individuals within this group. An alternative explanation may therefore be that the opposing results are caused by the use of genetic markers reflecting genetic differentiation at different time scales. Plastid markers can often be more conservative than nuclear markers (see e.g. Eidesen et al. 2007), and may possibly reflect genetic differentiation from before colonization of Svalbard. This and the clear genetic split between *S. procumbens* from Europe and the North-Atlantic area/North-America should however be further investigated.

For *C. capillaris* and *K. simpliciuscula*, the split between main genetic groups can be explained by the inclusion of different subspecies. The main genetic groups of *C. capillaris* are for instance accompanied by morphological differentiation and greatly correspond to the two subspecies *C. capillaris* ssp. *capillaris* and *C. capillaris* ssp. *fuscidula* (but see comment in the methods section; Elven et al. 2011). Overall, our results indicate that *C. capillaris* ssp. *fuscidula*, *S. procumbens* and *C. tenellum* populations from Svalbard belong to the same genetic groups as populations from Russia and/or Norway—information that is valuable both when managing the Svalbard populations and also for long-term conservation of genetic variation at species level.

**Conclusions**

In this study, we have shown that some of Svalbard’s most threatened plant species have experienced strong genetic founder- and/or bottleneck effects, likely due to climatic limitations. Their Svalbard occurrences should be considered as management units with importance for the long-term persistence of the species. At present, the species generally have small and/or few populations in Svalbard and the best management strategy would be further tracking of potential population size changes. This may also give valuable insights into plant responses to climate change.

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**Contributions by the Authors**

IGA conceived the idea, designed the project and served as project leader together with AKB. RE was the main taxonomic advisor. SB and IEBS contributed equally to this work and performed all laboratory and data analyses, as well as the drafting of the article. All authors conducted field collections and commented on the manuscript.

**Conflict of Interest Statement**

None declared.

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**Supporting Information**

The following additional information is available in the online version of this article —

Table S1. Red list categories for the study species in the Regional Red List for Svalbard in 2006, 2010 and 2015.

Table S2. Species traits for the seven study species: Pollination mode, breeding system, dispersal mode, life span, potential for clonal growth and ploidy level.

Table S3. AFLP details: Number of samples analysed in study species and outgroups, number of primer combinations tested, primer combination used, number of AFLP markers obtained, number and percentage of...
polymorphic AFLP markers obtained, and “mismatch error rate”.

Table S5. Details of the two separate principal component analyses summarising the measures of genetic founder/bottleneck effects and climatic adaptation.

Table S6. Results from multi-locus assignment tests performed in AFLPOP.

Figure S4. PCO (principal coordinates analysis) of AFLP multilocus phenotypes based on Dice similarity of 68 Carex capillaris individuals. The figure shows that Greenland (triangle) is separated from the other samples along the first PCO axis. Geographic regions are indicated by symbols: filled circle, Svalbard; filled square, Northern Norway; circle, Southern Norway; square, Iceland; filled triangle, Alps; triangle, Greenland.

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