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Frequency of local, regional, and long-distance dispersal of diploid and tetraploid *Saxifraga oppositifolia* (Saxifragaceae) to Arctic glacier forelands[1]

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- *Premise of the Study:* Climate change forces many species to migrate. Empirical small-scale data on migration and colonization in the Arctic are scarce. Retreating glaciers provide new territory for cold-adapted plant species, but the genetic consequences depend on dispersal distances and frequencies. We estimated local, regional, and long-distance dispersal frequencies, as well as their effect on levels of genetic diversity, in diploid and tetraploid individuals of *Saxifraga oppositifolia*.
- *Methods:* Samples were collected in four aged moraines in each of three glacier forelands, in surrounding areas and reference populations in the Arctic archipelago Svalbard. These samples were analyzed for neutral amplified fragment length polymorphisms (AFLPs, n = 707) and ploidy levels (n = 30).
- *Key Results:* Genetic clustering and ploidy analyses revealed two distinct genetic groups representing diploids and tetraploids, with few intermediate triploids. The groups were intermixed in most sampled populations. No differences in genetic diversity were found between tetraploids and diploids, or between established and glacier foreland populations. Seeds were dispersed over local, regional, and long distances, with the highest proportions of seeds originating from close sources. A minimum of 4–15 founding individuals from several source populations had initially established in each glacier foreland.
- *Conclusions:* Our data suggest that *S. oppositifolia* can rapidly colonize new deglaciated areas without losing genetic diversity. Thus, glacier forelands can be alternative habitats for cold-adapted vascular plants tracking their climatic niche. Our data show no difference in colonization success between diploid and tetraploid individuals.

Key words: AFLP; Arctic; colonization; dispersal; founder; genetic diversity; glacier forelands; migration; ploidy; *Saxifraga oppositifolia*.

During recent decades, several studies have focused on Arctic plant species and their large-scale responses to Pleistocene range shifts (Abbott et al., 1995, 2000; Eidesen et al., 2007a, b; Westergaard et al., 2010). These studies clearly show that historical range shifts have altered the genetic diversity within several Arctic plant species. Nearly all Arctic species will presumably experience range shifts, or range contractions, in response to ongoing climate warming (Parmesan, 2006). These future range shifts may lead to a considerable loss of genetic diversity over a relatively short period (i.e., centuries; Alsos et al., unpublished data). Nevertheless, in many Arctic areas, new territory for colonization is continuously provided in front of retreating glaciers. It has been suggested that some Arctic plants may endure future climate alterations by finding adequate habitats in such formerly glaciated areas (Crawford, 2008). Detailed studies of small-scale plant migration are therefore needed to evaluate the effect of ongoing climate change on dispersal and potential loss of genetic diversity during short-term range shifts in the Arctic.

Several colonization models (Ibrahim et al., 1996; Austerlitz et al., 1997; Bialozyt et al., 2006) and empirical studies about postglacial colonization (Hewitt, 1996; Schönswetter et al., 2003) suggest that range expansion into formerly glaciated areas can result in decreased genetic diversity owing to subsequent bottlenecks and founder effects. In addition, newly founded populations can diverge genetically from source populations because of a different selection pressure in the new habitat (McCauley and Wade, 1980). Reduced genetic diversity may have negative effects on factors like adaptation potential, resistance to disturbance, and stability of a species (Reusch et al., 2005; Hughes et al., 2008). Thus, persistence of a species is not guaranteed solely by moving; it also has to have sufficient genetic variation to endure in the long run. However, the modeling studies suggest that genetic diversity in newly founded populations can be high if there is a high proportion of long-distance dispersal coupled with local diffusion (stratified dispersal) (Ibrahim et al., 1996; Bialozyt et al., 2006). This phenomenon has been supported through several large-scale empirical studies (Alsos et al., 2005, 2009; Skrede et al., 2006). Similar patterns have also been shown in several small-scale colonization studies, with no founder effects and only minor differences in genetic diversity between newly founded populations and source populations (Von Flüe et al., 1999; Raffl et al., 2006; Yang et al., 2008).

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In Svalbard, a High Arctic archipelago, glaciers cover about 60% of the land mass; thus, deglaciated areas are continuously provided. Several well-documented colonization studies of glacier forelands in Svalbard are already available (Hodkinson et al., 2003; Moreau et al., 2005, 2008). In addition, this area provides several dated chronosequences (Dzierzek et al., 1990; Sletten et al., 2001; Rachlewicz et al., 2007). Ecosystems in Svalbard have comparatively simple interactions (Jónsdóttir, 2005) and little anthropogenic disturbance (Krzyszowska, 1985, 1989). Thus, glacier forelands in Svalbard provide an ideal model system for investigating genetic effects of ongoing plant colonization.

Saxifraga oppositifolia L. (Purple Saxifrage) is a long-lived, perennial, insect-pollinated, mainly outcrossing herb (Kevan, 1972; Gugerli, 1998). This species is an early colonizer, disperses via seeds and presumably also via propagules (Cooper, 2006), and is the first dominant vascular plant in young moraines after deglaciation (Hodkinson et al., 2003; Raffl et al., 2006). It has a circumpolar Arctic–Alpine distribution (Hultén, 1986) and displays considerable morphological and genetic variation throughout its range (Abbott et al., 2000; Abbott and Comes, 2004; Elven et al., 2011). Two subspecies are proposed in the Arctic: the amphi-Beringian subspecies *smalliana* and the more widely distributed subspecies *oppositifolia* (Elven et al., 2011). In addition, two distinct morphotypes were described, one cushion-like type growing mostly in dry, wind-exposed ridges, and one prostrate type with long branches and distant leaves mainly found in damp, less exposed habitats, though a range of intermediate types exists (Crawford et al., 1995; Elven and Elvebakk, 1996). Three ploidy levels have been observed, with mainly diploids (2n = 26) in Europe and northern Canada, tetraploids (2n = 52 and few deviating counts) in Beringia; and diploids, tetraploids and triploids (2n = 39) in Greenland (Elven et al., 2008). Surprisingly neither earlier suggested subspecies nor morphotypes or ploidy levels in *S. oppositifolia* are fully concordant with the registered genetic and morphological variation (Brysting et al., 1996; Rønning, 1996; Gabrielsen et al., 1997; Abbott and Comes, 2004). *Saxifraga oppositifolia* is also common throughout Svalbard (Alsos et al., 2011) and found in most plant communities (Elvebakk, 1994). Individuals from Svalbard have also shown high morphological variation (Rønning, 1996), and the two morphotypes and intermediates grow in Svalbard in spatial proximity (Brysting et al., 1996). Regarding genetic diversity in *S. oppositifolia*, Svalbard is no exception. High levels of genetic diversity were registered on the basis of RAPD data (Gabrielsen et al., 1997), and two chloroplast genotypes have been recorded on the basis of RFLP data (Abbott et al., 2000). There are only three chromosome counts registered for Svalbard, one diploid (Jørgensen et al., 1958) and two tetraploid counts (Flovik, 1940; Chapman, 1995). Theoretically, polyploids have three advantages compared with diploids: heterosis, improved self-compatibility, and gene redundancy (Comai, 2005), which are expected to be especially beneficial for vascular plants during colonization (Soltis and Soltis, 2000).

In the present study, we investigated populations of *S. oppositifolia*, which successively colonized new territory in Arctic periglacial habitats, and evaluated genetic diversity, dispersal distances, and minimum number of founders. We analyzed samples collected from populations of *S. oppositifolia* in three recently colonized glacier forelands and potential sources. We examined the genetic structure and then compared it with (1) the genetic diversity in recently founded populations versus older populations in the surroundings; (2) the proportion of local, regional, and long-distance dispersed immigrants; and (3) the minimum number of founding individuals contributing to the genetic patterns observed in the newly founded populations.

MATERIALS AND METHODS

Sampling—Leaves of *S. oppositifolia* were collected in three forelands of the glaciers Midtre Lovènbreen, Hørbyebreen, and Renardbreen in Svalbard. Chronosequences of these glacier moraines were reconstructed with aerial images from the Norwegian Polar Institute for the years 1936, 1960, and 1990. In addition, the chronosequences and the outermost moraine of the "Little Ice Age" were verified using earlier studies from each of the three moraines (Dzierzek et al., 1990; Moreau et al., 2005; Rachlewicz et al., 2007). In each glacier foreland, one population from each of the four moraine stages as well as four populations from surrounding potential sources were collected (Fig. 1). The four sources in each collection region were chosen with regard to the ease of access and to the distance to the glacier foreland. With each successive source, the distance to the glacier foreland was increased. Therefore, the closest sources were 0.43–1.99 km and the most distant sources 5.25–8.42 km away from the oldest moraine. Leaves were collected from 30 individuals that were ≥10 m apart. If fewer individuals were present, fewer samples were collected. To include reference populations, we additionally collected 10 individuals from seven other locations in Svalbard. Thus, in total, 780 samples from 31 populations were collected in silica gel (Table 1). One voucher specimen was collected from each collection site (Table 1) and deposited at Tromsø University Museum (TROM).

DNA isolation and AFLP fingerprinting—The DNA of ~20 mg of leaf material was extracted with the DNeasy Plant Mini Kit (Qiagen, 2006) following the manufacturer's protocol except that dried samples were crushed in a mixer mill (MM301; Retsch GmbH, Haan, Germany) at 25 Hz. From each population collected in Svalbard, one individual was extracted twice and functioned as repeatability control to calculate the error rate for the AFLP procedure (Bonin et al., 2004).

The AFLP analyses (Vos et al., 1995) were performed according to the protocol in Gaudeul et al. (2000) with the following modifications: DNA concentration in 102 randomly chosen samples was tested on agarose gel electrophoresis with a 1% gel. The adaptors (http://biomers.net, Ulm, Germany) were denatured at 95°C for 5 min, and annealing was achieved by slow cooling at room temperature. The 11-μL restriction-ligation mixture contained 1.1 μL ligase buffer and 1 unit T4 DNA ligase (Fermentas, St. Leon-Rot, Germany). Incubation time was 3 h at 37°C. The 25-μL preselective polymerase chain reaction (PCR) mixture contained 2.5 μL AmpliTaq buffer (Roche, Basel, Switzerland). All selective PCR mixtures had half the volume (12.5 μL) used by Gaudeul et al. (2000) and contained 2.5 μL AmpliTaqGold buffer (Roche) and 0.2 μM fluorescence-labeled *Eco*RI-AXX primer. Both preselective and selective PCRs were run on a GeneAmp PCR system 9700 thermocycler (Applied Biosystems, Foster City, California, USA). Twenty-seven primer combinations were tested on four individuals originating from different geographic locations in Svalbard. The following three primer combinations yielded the highest variation and were selected for the full analysis: 6-FAM-*Eco*RI-ATG – *Mse*I-CAT; NED- *Eco*RI-AGC – *Mse*I-CTA, and VIC-*Eco*RI-ATC – *Mse*I-CTG. For each sample, 1.5 μL of 6-FAM, 1.9 μL of NED, and 0.9 μL VIC-labeled selective PCR products were mixed with 13 μL purified water. One microliter of this mixture was combined with 0.3 μL GeneScan ROX 500 (Applied Biosystems) and 8.7 μL HiDi formamide (Applied Biosystems), and the fragment analysis was carried out on a capillary sequencer (ABI PRISM 3100; Applied Biosystems).

Consistency of the raw data and size standard quality were checked with Peak Scanner version 1.0 (Applied Biosystems). Selected samples were aligned and scored with Genographer version 2.1.4 (Banks and Benham, 2008). File type option "ABI GeneMapper Filter" was applied and sizing algorithm "CubicSplineInterpolator" was chosen to size the fragments against the internal standard. Unambiguous fragments between 70 and 500 base pairs were scored using the semi-automatic scoring option. Each peak was controlled in the thumbnail option, which allows parallel comparison of all samples for each marker. In addition to the semi-automatic scoring, all AFLP profiles were scored automatically in GeneMapper version 4.0 (Applied Biosystems). Data from both scoring methods were exported as presence (1)/absence (0) matrix.

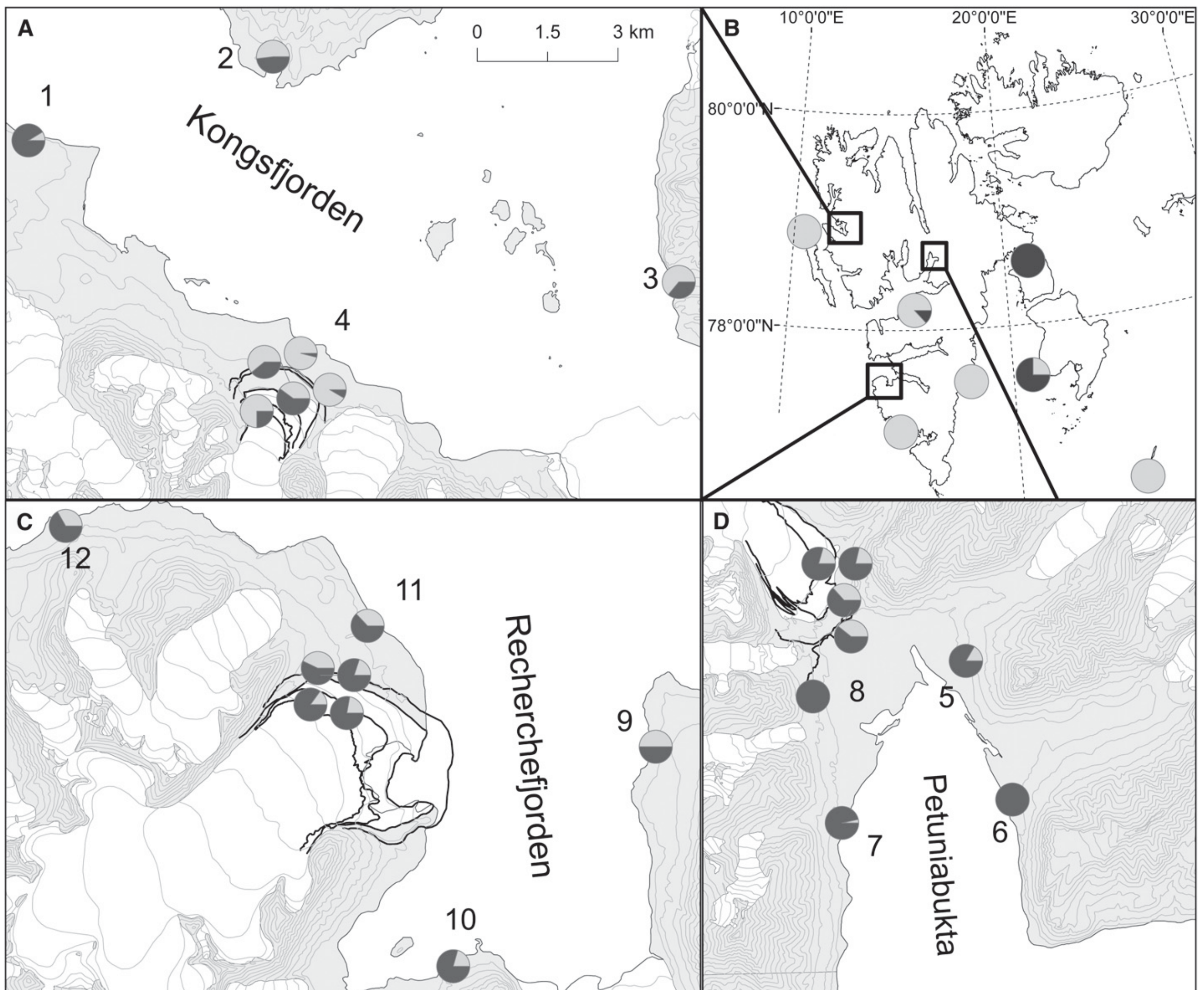

Fig. 1. Overview of the sampling sites of *Saxifraga oppositifolia*. Pie charts show population clusters identified by the program Structure: light gray diploid cluster and dark gray tetraploid cluster. (A) Kongsfjorden with the sample sites in the glacier foreland of Midtre Lovénbreen. (B) Svalbard, including all sampled reference populations. (C) Recherchefjorden sample sites in the glacier foreland of Renardbreen. (D) Petuniabukta with the sample sites in the glacier foreland of Hørbyebreen. Black lines at the glacier fronts indicate the retreat of the glacier: first and outermost line, glacier front at ~1880; second line, ~1936; third, ~1960; and fourth, ~1990. The fifth line at Midtre Lovénbreen indicates the approximate glacier front in 2008. For sources 1–12 see Table 1.

Analysis of ploidy levels—To analyze whether *S. oppositifolia* is truly present in two different ploidy levels on Svalbard, we recollected five samples in 2011 from source six (16.575798 E, 78.725797 N; Fig. 1) and germinated seeds from a reference population in Endalen (15.749509 E, 78.185874 N). Ten of the seedlings and leaves from the five samples were analyzed for their DNA content using flow cytometry. The samples were compared with a sample of expected diploid level collected in Telegrafbukta, Tromsø, Norway (18.907478 E, 69.631747 N), and to an internal standard (*Vinca minor*). In addition, 30 silica-dried samples from several collection sites of individuals analyzed with AFLP were analyzed by applying flow cytometry.

Samples were prepared by chopping 20–50 mg with a razor blade in an ice-cold buffer containing 5 mM hepes, 10 mM magnesium sulphate heptahydrate, 50 mM potassium chloride, 0.2% triton X-100, 0.1% dithiothreitol, 1% polyvinylpyrrolidone, and 2 µg mL^{-1} DAPI; buffer pH was 8.0. Approximately 2 mL of the solution containing cells and tissue was passed through a nylon filter of 50-µm mesh size. Fluorescence was measured with a CyFlow ML flowcytometer (Partec GmbH, Münster, Germany). The flowcytometer was equipped with a high-pressure mercury lamp (Osram HBO 100 long life), heat protection filter KG-1, excitation filters UG-1 and BG-38, dichroic mirrors TK 420 and TK 560, and an emission filter GG 435. In addition, Flowmax software (version 2.4; Partec) was used. All analyses were made by Plant Cytometry Services (AG Schijndel, The Netherlands).

Data evaluation and population structure—All individuals with poor AFLP profiles and/or size standards were excluded from further analyses. Conversions of the data matrix format for the different analyses of the AFLP data were carried out with the AFLPdat R-script (Ehrich, 2006). Linkage among markers was evaluated by using a neighbor-joining tree of the markers, which was calculated with Treecon version 1.3b (Van der Peer and De Wachter, 1994), based on Nei and Li's (1979) distance measure.

Principal coordinate analyses (PCO), including all samples from Svalbard (Table 1), were calculated using the Euclidean similarity index (Kosman and Leonard, 2005; Bonin et al., 2007) with the software Past version 2.03 (Hammer et al., 2001). We inferred models for the population structure using the Bayesian

TABLE 1. Geographic origin, collection data, genetic diversity (*D*) including the 95% confidence intervals, and rarity index (DW) for the 31 populations of *Saxifraga oppositifolia*; *n* = number of plants successfully analyzed with three AFLP primer combinations; all sampling sites in Svalbard, collectors Pernille Bronken Eidesen (PBE) and Eike Müller (EM).

		Coordinates							95% CI			*D*	
Site	Collection site	Longitude	Latitude	Type	Collector	Voucher	*n*	*D*	Lower bound	Upper bound	DW	Diploid cluster	Tetraploid cluster
Dickson Land	Hørbyebreen 1880	16.46632516	78.73598132	Glacier foreland	PBE, EM	COL-106	23	0.26	0.23	0.30	3.57	0.23	0.23
	Hørbyebreen 1936	16.45983918	78.73906939	Glacier foreland	PBE, EM	COL-100	30	0.26	0.22	0.29	3.72	0.22	0.22
	Hørbyebreen 1960	16.44400116	78.74481076	Glacier foreland	PBE, EM	COL-108	28	0.23	0.20	0.26	3.55	0.24	0.19
	Hørbyebreen 1990	16.43608975	78.74505501	Glacier foreland	PBE, EM	COL-109	29	0.28	0.24	0.31	3.98	0.23	0.26
Oscar II Landm	Midtre Lovénbreen 1880	12.07961995	78.90507101	Glacier foreland	PBE, EM	SO2-1880	24	0.25	0.21	0.28	3.18	0.24	0.28
	Midtre Lovénbreen 1936	12.07661320	78.90319396	Glacier foreland	PBE, EM	SO2-1936	23	0.28	0.24	0.32	3.63	0.25	0.23
	Midtre Lovénbreen 1960	12.06960315	78.89987862	Glacier foreland	PBE, EM	SO2-1960	25	0.27	0.23	0.30	3.38	0.24	0.23
	Midtre Lovénbreen 1990	12.04602957	78.89722687	Glacier foreland	PBE, EM	SO2-1990	28	0.26	0.23	0.30	3.38	0.25	0.21
Wedel Jarlsberg Land	Renardbreen 1880	14.47511557	77.54692927	Glacier foreland	PBE, EM	SO3-1880	30	0.25	0.22	0.29	3.91	0.27	0.22
	Renardbreen 1936	14.47271064	77.54582240	Glacier foreland	PBE, EM	SO3-1936	28	0.28	0.25	0.32	3.92	0.27	0.25
	Renardbreen 1960	14.49563404	77.53972749	Glacier foreland	PBE, EM	SO3-1960	27	0.28	0.24	0.31	3.91	0.25	0.26
	Renardbreen 1990	14.46637985	77.54129361	Glacier foreland	PBE, EM	SO3-1990	20	0.25	0.21	0.29	3.56	0.21	0.24
Dickson Land	Pyramiden	16.45227135	78.69547563	Source 7	PBE, EM	SO-S4	28	0.25	0.22	0.29	4.20	—	0.25
	Ragnardalen	16.57579775	78.72579741	Source 5	PBE, EM	COL-110	30	0.26	0.23	0.30	3.86	0.26	0.25
	Skottehytta	16.61693571	78.69909056	Source 6	PBE, EM	SO-S1	29	0.22	0.19	0.25	3.56	—	0.22
	Svenbrehøgda	16.42736724	78.71975042	Source 8	PBE, EM	SO-S3	28	0.22	0.19	0.26	4.00	—	0.22
Haakon VII Land	Blomstrandhalvøya	12.04423962	78.96493164	Source 2	PBE, EM	SO-S5	27	0.27	0.23	0.31	3.85	0.28	0.25
	Ossian Sarsfjellet	12.45851911	78.92563942	Source 3	PBE, EM	SO-S7	25	0.27	0.24	0.30	3.63	0.23	0.24
Oscar II Land	Brøggerhalvøya	11.80521936	78.94651246	Source 1	PBE, EM	SO-S6	23	0.29	0.25	0.33	3.81	0.25	0.28
	Nannestadelva	12.08665487	78.90867336	Source 4	PBE, EM	SO2-MAX	25	0.24	0.21	0.28	3.28	0.24	—
Wedel Jarlsberg Land	Calypsostranda	14.51642114	77.55640606	Source 11	PBE, EM	SO-S10	27	0.26	0.22	0.30	3.58	0.25	0.21
	Lyellstranda	14.24774626	77.57487512	Source 12	PBE, EM	SO-S8	24	0.29	0.25	0.32	3.95	0.24	0.27
	Reinodden	14.77221645	77.53366600	Source 9	PBE, EM	SO-S11	25	0.27	0.24	0.31	3.95	0.23	0.25
	Vestervågøyra	14.59441445	77.49155690	Source 10	PBE, EM	SO-S9	29	0.26	0.22	0.29	3.64	0.24	0.24
Barentzøya	Frankenhalvøya	21.11797114	78.58623227	Reference	EM	SO-R-BØ	8	0.25	0.21	0.29	3.22	—	0.25
Edgeøya	Årdalen	20.82160141	77.52882137	Reference	EM	SO-R-EØ	8	0.22	0.18	0.26	2.90	0.23	0.17
Nordenskiöld Land,	Endalen	15.74950905	78.18587364	Reference	EM	SO-S15	23	0.22	0.19	0.25	3.38	0.19	0.20
Hopen	Iversenfjellet	24.97319789	76.45519366	Reference	EM	SO-R-H	10	0.19	0.15	0.23	2.66	0.19	—
Heer Land	Kvalhovddalen	18.16178255	77.50834461	Reference	EM	Ref-2-coll	7	0.22	0.18	0.26	2.56	0.22	—
Wedel Jarlsberg Land	Kvartsittsletta	15.12696559	77.05923705	Reference	EM	Ref-1-coll	8	0.28	0.24	0.32	3.02	0.28	—
Prins-Karls-Foreland	Fuglehukfjellet	10.48275037	78.88433515	Reference	EM	SO-R-PKF	8	0.20	0.16	0.24	2.89	0.20	—

clustering method implemented in Structure version 2.2.3 (Pritchard et al., 2000). All analyses with Structure were run at the Bioportal at the University of Oslo. Every individual collected in Svalbard was included in the analysis, which was run assuming an admixture model with a burning-in period of 20^5 iterations followed by 10^6 iterations. Each run was replicated 10 times with a *K* from 1 to 10. The major clusters inferred were analyzed for further substructure with the same parameters and *K* from 1 to 15. A model for the population structure was selected with the aid of the Structure-sum R script (Ehrich et al., 2007) according to Pritchard et al. (2000) and Evanno et al. (2005).

Further, to unravel the partitioning of the molecular variance between groups and among populations, we ran several analyses of molecular variance (AMOVA) for different group and population clustering, applying the software Arlequin version 3.5.1.2 (Excoffier and Lischer, 2010). To test for isolation-by-distance, Mantel tests were calculated with 10^4 iterations using the R package ncf 1.1-3 (Bjornstad, 2009). For genetic distances, we applied only significant population-based pairwise F_{ST} values, which were estimated with Arlequin. Geographic distances were inferred from latitude and longitude coordinates.

Founder events—To identify differences in diversity between source and newly founded populations, genetic diversity was calculated after Nei (1987). To quantify the occurrence of rare markers, the frequency down-weighed marker value (DW; also called "rarity index") was calculated (Schönswetter and Tribsch, 2005). Both indices were calculated with the aid of the AFLPdat R-script (Ehrich, 2006). To find the most likely source of the individuals in the glacier foreland populations, multilocus assignment tests were performed as described in Skrede et al. (2006) using AFLPop version 1.0 (Duchesne and Bernatchez, 2002). Individuals were assigned to populations when their likelihood of having originated from a particular population was 10 or more times higher than the likelihood to originate from any other population. When differences in likelihood were less, we identified whether an individual had a 10 or more times higher likelihood of having originated from a particular collection region (Kongsfjorden, Recherchefjorden, or Petuniabukta). Individuals were classified as unassigned if the difference in likelihood between populations as well as between regions was less. The minimum number of founders necessary to bring all markers observed in the founded population was estimated as described in Alsos et al. (2007). Individuals were sampled at random from the main source until all markers observed in the founded population were observed in the sample. This procedure was repeated 10^5 times to find the minimum number of founders. If some markers were not found in the main source population, individuals were added from other sources that carried these markers. The AFLP profiles were inspected to find individuals, which carried combinations of markers. This calculation was run for each Structure cluster separately, and minimum numbers of founders for each of the four sampled moraine stages in each glacier foreland were estimated. The minimum numbers of founders for each Structure cluster were also estimated for each glacier foreland in total.

Differences in number of markers between the Structure clusters were tested with a *t*-test. To evaluate further differences in markers, a linear mixed-effects model with Structure clusters (cluster 1 and cluster 2) and sample origin (glacier foreland or source) as fixed factors and collection region (Kongsfjorden, Petuniabukta, and Recherchefjorden) as random factor was fitted to marker numbers. For this model the R package nlme version 3.1-101 (R Development Core Team, 2011) was used. To evaluate whether the sample origin and the Structure clusters had an effect on genetic diversities, we applied a generalized linear model (GLM, normal errors) with sample origin (source or glacier foreland population) and Structure cluster as factorial variables (cluster 1 and cluster 2). The reference populations were excluded. We tested whether DW values were related to sample size with a linear model. A *t*-test was applied to test whether the populations belonging to the Structure clusters had different DW values. Another GLM (normal errors), with sample origin as factorial variable (source or glacier foreland populations), Structure cluster as factorial variable (cluster 1 and cluster 2), and individuals per sampled population as continuous variable, was fitted to the DW values of the sampled populations (reference population excluded). With this model, we assessed whether DW values were different in glacier forelands and source populations, and whether there was an interaction between Structure cluster and sample origin (source or glacier foreland populations). Differences in Structure cluster ratios in glacier forelands and their four sources were tested with *F*-tests. Whether the minimum number of founders was influenced by Structure cluster was tested with a *t*-test and by sample size with a linear model. All Mantel tests, *t*-tests, *F*-tests, and statistical models were calculated using the statistical software package R version 2.12.0 (R Development Core Team, 2010).

RESULTS

Semi-automatic vs. automatic scoring—For the three selected primer combinations including replicates, 802 individuals of *S. oppositifolia* were successfully semi-automatically scored, resulting in 152 markers. The error rate calculated on the basis of 83 replicates was initially 2.81%. Five markers with >10 scoring mismatches, five individuals with >15 scoring mismatches, and seven individuals with exceptionally low numbers of scored markers were excluded, resulting in a mean scoring error of 1.62%. Another 32 markers, which were polymorphic in <1.62% of the total numbers of individuals, were removed from the data set. Thus, the final matrix consisted of 115 markers and 707 individuals, in which 67% of the markers occurred in 10–90% of the individuals.

Applying automatic scoring including replicates, 810 individuals could be scored, resulting in 1039 markers with an initial error rate based on 89 replicates of 4.53%. The difference in analyzed individuals between semi-automatic scoring and automatic scoring is based on the programs' different abilities to read the length standard. Cleaning the data set following the same procedure as described above resulted in a mean scoring error of 1.21%. Subsequently, 606 markers polymorphic in <1.21% of the total number of individuals were removed. The final data set consisted of 712 individuals and 266 markers, but only 26% of the markers occurred in 10–90% of the individuals. In addition, two PCO axes explained only 6.69% of the variation and three axes only 9.03%. This is much less than the PCO of the data when semi-automatic scoring is applied. Considering the marker distribution, the less separated data in the PCO, and that markers with intermediate frequencies contain most information about population structure, the semi-automatic scored data set was used in all further analyses (Appendix S1; see Supplemental Data with the online version of this article).

Main genetic structure—The PCO of the data set with all samples collected in Svalbard showed a weak separation into two clusters (Fig. 2A). Two PCO axes explained 13.45% of the variation, and three axes explained 17.07% of the variation. The Structure analysis including all samples collected on Svalbard identified the same clusters (Fig. 2A) (Appendix S2). The results of the flow cytometry from the fresh material confirmed the existence of DNA-diploid and DNA-tetraploid individuals of *S. oppositifolia* in Svalbard. Furthermore, the ploidy analysis of 30 silica-dried samples using AFLP-analyzed individuals indicated that the two observed clusters represented two ploidy levels. Nine of 13 individuals from the first cluster were diploids. Only one individual of the first cluster was tetraploid, another one was triploid, and for two individuals the ploidy level could not be determined. All 13 individuals from the second cluster were tetraploid. In addition, of four individuals placed between the clusters, one was triploid, one diploid, one tetraploid, and for one individual the ploidy level could not be determined (Fig. 2B; Table 2).

Individuals from both clusters appeared in most sampled populations. The proportion of the individuals belonging to the diploid cluster and to the tetraploid cluster was 39.60–60.40% (n = 707; Fig. 1). When comparing each region, the tetraploid cluster also had a higher presence in Petuniabukta and in Recherchefjorden, whereas the opposite was observed in Kongsfjorden (Fig. 1). There were no differences in frequencies of the clusters between source populations and glacier foreland

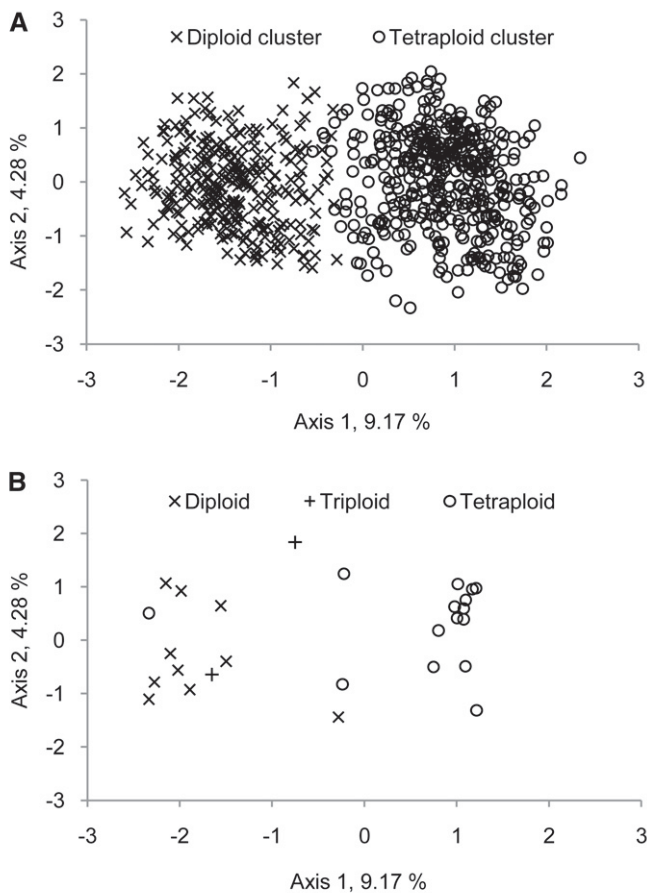

Fig. 2. (A) Principal coordinate analysis (PCO) of 707 individuals from 31 collection sites in Svalbard based on Euclidian similarity among amplified fragment length polymorphism (AFLP) multilocus phenotypes. The two clusters identified with the program Structure are shown. (B) Only 27 individuals analyzed with flow cytometry are shown.

TABLE 2. Analysis results of the flow cytometry and Structure clustering for 27 from 30 silica dried individuals and one fresh reference sample of *Saxifraga oppositifolia*; 4x = DNA-tetraploid individuals, 3x = DNA-triploid individuals, and 2x = DNA-diploid individuals; three samples were excluded because measurements could not be interpreted.

Sample	Structure cluster 1	Structure cluster 2	Flow cytometry
Source 6	0.005	0.995	4x
Midtre Lovénbreen 1880	0.005	0.995	4x
Source 8	0.006	0.994	4x
Source 6	0.007	0.993	4x
Source 7	0.008	0.992	4x
Source 10	0.008	0.992	4x
Source 7	0.01	0.99	4x
Hørbyebreen 1990	0.01	0.99	4x
Hørbyebreen 1990	0.011	0.989	4x
Barentzøya	0.011	0.989	4x
Source 3	0.011	0.989	4x
Source 1	0.017	0.983	4x
Source 7	0.241	0.759	4x
Source 9	0.5	0.5	4x
Source 9	0.582	0.418	2x
Source 5	0.595	0.405	3x
Renardbreen 1990	0.981	0.019	2x
Hopen	0.994	0.006	2x
Prins-Karls-Foreland	0.995	0.005	2x
Hopen	0.995	0.005	3x
Source 4	0.995	0.005	2x
Source 12	0.996	0.004	2x
Midtre Lovénbreen 1880	0.997	0.003	2x
Prins-Karls-Foreland	0.997	0.003	2x
Midtre Lovénbreen 1960	0.997	0.003	4x
Source 4	0.997	0.003	2x
Source 12	0.997	0.003	2x
Source 6	—	—	4x

populations in Recherchefjorden (P = 0.1770) and Kongsfjorden (P = 0.1105). In Petuniabukta, however, a higher proportion of individuals from the glacier foreland of Hørbyebreen was assigned to the diploid cluster (diploids = 29.09%, tetraploids = 70.91%) than in the source populations (diploids = 6.03%, tetraploids = 93.97%, P = 5.511e-06). The diploid cluster had no exclusive markers, whereas the tetraploid cluster had three exclusive markers and two of them were comparatively common. Based on the individual number of markers, individuals sorted into the tetraploid cluster had, on average, 5.4 more markers than individuals of the diploid cluster (t = −11.24, df = 635, P = 2.2e-16). Of individuals with measured ploidy levels, the 15 tetraploid individuals had, on average, 2.3 markers more than the 10 diploid individuals, but this difference was not significant (t = −0.84, df = 20, P = 0.413).

According to the AMOVA analyses, 14.05% of the molecular variation occurred among the two clusters and 11.00% among sampling populations (all P < 0.0001, n = 707; Table 3). With the individuals split into groups of glacier foreland populations and source populations, within each collection region, an AMOVA explained some of the variance for the tetraploid cluster between these two groups (Kongsfjorden: 6.90%, P = 0.04, n = 77; Petuniabukta: 5.99%, P = 0.03, n = 187; Recherchefjorden: 3.09%, P = 0.04, n = 146). No such pattern was observed for the diploid cluster (Kongsfjorden and Recherchefjorden: <1% and not significant; Petuniabukta was excluded owing to the small sample size).

Isolation-by-distance—There was a positive relationship between pairwise genetic and geographic distances when all samples from Svalbard were included (Mantel test, r = 0.53, P = 0.0002, n = 457). Similarly, a Mantel test of each cluster separately on the scale of Svalbard showed that pairwise genetic distances were correlated with geographic distances (diploid cluster: r = 0.54, P = 0.0002, n = 308; tetraploid cluster: r = 0.42, P = 0.0002, n = 301). The average increase of F_{ST} was 0.04 for every 100 km for all samples. Analyzing each cluster separately gave the same result for the tetraploid cluster (0.04) and a very similar result for the diploid cluster (increase of F_{ST} 0.03 every 100 km). When the dominant cluster for each glacier foreland was analyzed separately, a significant positive correlation between pairwise genetic and geographic distance was found for the diploid cluster in Kongsfjorden (r = 0.64, P = 0.0172, n = 24). The same was found in Petuniabukta for the tetraploid cluster (r = 0.68, P = 0.0014, n = 27). In Recherchefjorden, however, no isolation-by-distance was found for the dominant tetraploid cluster (r = −0.22, P = 0.3187, n = 25). The increase of F_{ST} was almost 3× larger in Petuniabukta (0.18 on 10 km) than in Kongsfjorden (0.06 on 10 km). No test for isolation-by-distance was made for the subdominant cluster owing to small sample sizes.

TABLE 3. Results of four different AMOVAs (n = 707) with different group and population definitions.

Group definition	Population definition	Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	*P*
None	Collection sites	Among populations	30	1876.50	2.10	12.48	<0.00001
		Within populations	676	9975.62	14.76	87.52	<0.00001
None	Diploid and tetraploid cluster	Among populations	1	908.85	2.64	14.54	<0.00001
		Within populations	705	10943.26	15.52	85.46	<0.00001
Glacier foreland vs. source and reference populations	Collection sites	Among groups	1	104.25	0.11	0.63	0.10456
		Among populations	29	1772.24	2.05	12.12	<0.00001
		Within populations	676	9975.62	14.76	87.26	<0.00001
Diploid and tetraploid cluster	Collection sites	Among groups	1	908.85	2.55	14.05	<0.00001
		Among populations	53	2067.10	2.00	11.00	<0.00001
		Within populations	652	8876.16	13.61	74.95	<0.00001

Genetic diversity, rarity, and number of markers— Nei's genetic diversities for the sampled populations split into the diploid and tetraploid cluster ranged from 0.17 to 0.28 (Table 1). The average genetic diversity of all source and glacier foreland populations was 0.24, and a GLM fitted to the diversity data (n = 44) showed neither a difference in genetic diversities between the two clusters (t = −0.874, df = 40, P = 0.387) nor a difference between source and glacier foreland populations (t = 0.589, df = 40, P = 0.559). Also, no interaction between clusters and sample origin (source or glacier foreland) could be found (t = 0.188, df = 40, P = 0.852). Comparing the genetic diversities of each collection region separately, genetic diversities were also similar, with overlapping confidence intervals indicating no significant losses of genetic diversities in the glacier forelands (Fig. 3). Interestingly, genetic diversities remained constant over time in the diploid and the tetraploid cluster, as indicated by similar genetic diversities in the different moraine stages. In the youngest stages, calculated genetic diversities were already high (Fig. 4).

The DW values for sampled populations, split into the diploid and tetraploid clusters, ranged from 1.30 to 3.11 (Table 1). The DW values for populations with small sample sizes were usually low, and sample size had a significant influence on DW values (r^2 = 0.64, df = 51, P = 5.756e-13). The diploid cluster (mean = 1.94 ± 0.23) had a lower DW value (with SD) than the tetraploid cluster (mean = 2.41 ± 0.41, t = −5.11, df = 39, P = 9.703e-06). A GLM fitted to the DW values of the 44 glacier foreland and source populations revealed that the DW values of populations collected in glacier forelands and sources within both ploidy levels were not significantly different (t = 0.461, df = 39, P = 0.6477) and that there was no interaction between di- or tetraploid clusters and sources or glacier forelands (t = 1.105, df = 39, P = 0.2757).

In the glacier foreland populations belonging to the diploid cluster, we found two rare markers that were not present in other diploid populations sampled elsewhere. By migrating into the glacier foreland, all populations lost rare markers that were present in the surrounding populations. However, in most glacier foreland populations, markers also appeared that were not present in the surrounding populations (Table 4). The linear mixed-effects model fitted to marker numbers (635 individuals from sources and glacier forelands) revealed that individuals collected in glacier foreland populations independent of the diploid or tetraploid cluster had, on average, 1.88 ± 0.84 (t = 2.254, df = 629, P = 0.0245) fewer markers than individuals collected in source populations.

Immigration routes and minimum number of founders— All allocation tests indicated that the close source populations contributed most to the colonization of new habitats (Table 5). At Midtre Lovénbreen, the glacier foreland samples from the oldest stages were predominantly allocated to the closest source populations (29%). The oldest stage in the forelands of Renardbreen and Hørbyebreen, however, had a higher percentage of individuals allocated to the second or third closest source population (30% and 17%, respectively) than to the closest source population. In all three glacier forelands, the highest numbers of individuals from younger stages (1936–1990) were allocated to the preceding older stages (Fig. 5). A high number of individuals, however, was still allocated to the four different surrounding source populations (Fig. 5). Compared with the other two glacier forelands, at the glacier foreland of Hørbyebreen in Petuniabukta the allocation of individuals to the older stages was higher; compared with the surrounding source populations, it was lower; and compared with populations outside Petuniabukta, it was higher.

Overall, the calculation of the minimum number of founders indicates that all sampled glacier foreland populations were established by several individuals (Table 4). Further, the majority of the glacier foreland populations were not exclusively founded by the closest or major source, since more distant sources must have contributed to 20 of the 24 glacier foreland populations. In these 20 glacier foreland populations, markers occurred that were not present in the sampled main source. Only four of these populations could theoretically have been founded by one source, because for these populations all observed markers occurred in a single source. There was no evidence that the diploid or tetraploid clusters influenced the estimated minimum

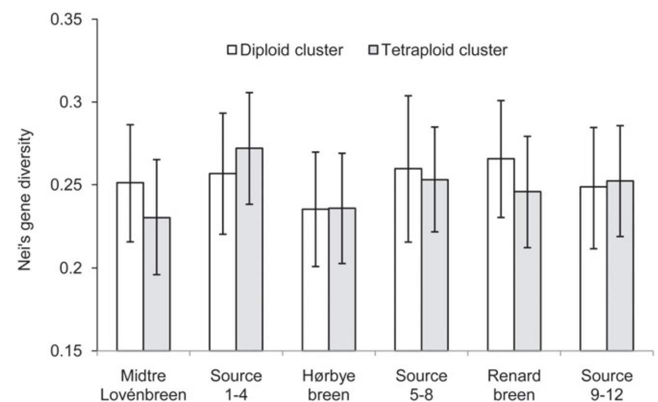

Fig. 3. Nei's genetic diversities for the diploid and tetraploid clusters, including 95% confidence intervals calculated for each glacier foreland and source region.

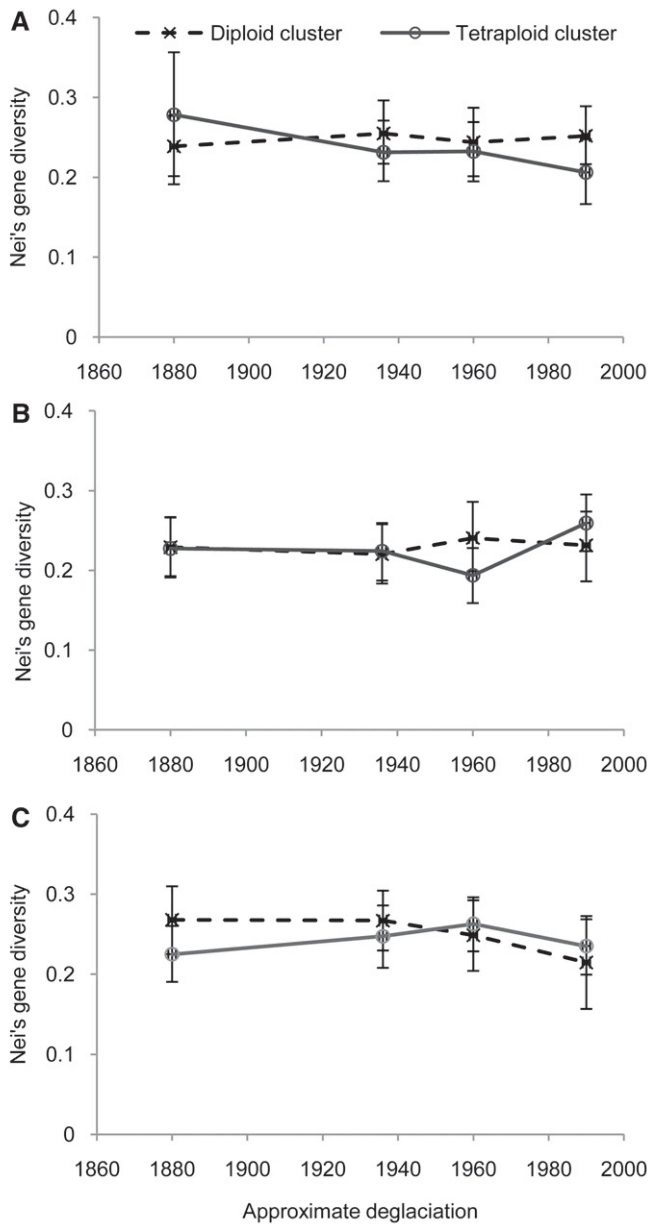

Fig. 4. Nei's genetic diversities for the diploid and tetraploid clusters, including 95% confidence intervals in the different moraine stages of (A) Midtre Lovénbreen, (B) Hørbyebreen, and (C) Renardbreen.

number of founders ($t = -0.2485$, df = 22, $P = 0.8069$). The minimum number of founders was also weakly related to the size of the sampled glacier foreland population; small populations required fewer minimum founders than larger populations ($r^2 = 0.21$, df = 22, $P = 0.0147$). Compared with the other two glacier forelands, both for the diploid and the tetraploid cluster, the glacier foreland of Hørbyebreen required the most founders (Fig. 6).

DISCUSSION

Overall, no difference in genetic diversity could be found between newly founded glacier forelands and potential source populations, or between the clusters representing the two main ploidy levels of *S. oppositifolia* in Svalbard. Further, the chosen rarity measure (DW) did not differ between glacier foreland populations and source populations. Hence, this species is able to track its ecological niche by migrating into new habitats in a comparatively short time (approximately 10–20 yr) without losing a significant amount of genetic diversity. Although the potential source contributing most to the colonization of the glacier foreland was usually close, and the dispersal within each region was high, compelling evidence for long-distance dispersal was found. The minimum numbers of founders appeared to be low, but considering seed loss during migration and establishment processes, the real numbers of seeds that arrived in the glacier forelands must be orders of magnitude greater than the minimum numbers.

Genetic Structure—The occurrence of two ploidy levels was confirmed by fresh material that was recollected within two collection sites, which were earlier included in the AFLP analysis. The result was clearly interpretable (Appendix S3). Further, the silica-dried ploidy and AFLP-analyzed samples provide strong evidence that the two main genetic groups identified within *S. oppositifolia* in Svalbard corresponded to DNA-diploid and DNA-tetraploid individuals. That dried plant material and, especially, silica-dried samples can be reliably analyzed with flow cytometry has been demonstrated recently (Suda and Trávníček, 2006).

Several earlier molecular-based studies on *S. oppositifolia* included samples from Svalbard (Abbott and Comes, 2004; Abbott et al., 1995, 2000; Gabrielsen et al., 1997) but did not investigate whether the genetic structure was influenced by ploidy. A study based on pollen size also did not find evidence of multiple ploidy levels on Svalbard (Brysting et al., 1996). Hence, the existence of diploid and tetraploid individuals at close proximity was unexpected. However, the occurrence of different chromosome numbers (e.g., Schönswetter et al., 2007) and ploidy levels within the same species complex seems to be common in the Arctic (Brochmann et al., 2004; Eidesen et al., 2007a) and can be a result of a single polyploidization event, as in *S. rivularis* L. (Jørgensen et al., 2006), or a recurrent process, as in *Empetrum nigrum* L. (Popp et al., 2011). Recurrent polyploidization should result in stronger geographic structure independent of ploidy (Soltis and Soltis, 1999; Levin, 2002). Thus, recurrent polyploidization of *S. oppositifolia* in Svalbard seems unlikely, as there was stronger dissimilarity between ploidy levels than geographic structure (Table 3), and two exclusive markers in the tetraploid cluster were frequent in nearly all sampled populations. The occurrence of triploids and the indistinct transition between the two clusters might indicate some gene flow between ploidy levels. However, because of the exclusive markers in the tetraploid cluster, we expect that gene flow does mainly occur from diploids to tetraploids. Another possibility for the formation of triploids can be unreduced gametes (Bretagnolle and Thompson, 1995). Whether the different ploidy levels are truly unrelated to taxonomical differences (Abbott and Comes, 2004) or the two cpDNA haplotypes observed in Svalbard (Abbott et al., 2000) represent diploid and tetraploid clusters needs to be investigated separately.

Focusing on the overall ratio of individuals belonging to the diploid or tetraploid cluster in Svalbard, the tetraploid cluster showed no advantage over the diploid cluster, as might be assumed for polyploid vascular plants (Sitte et al., 1999; Brochmann et al., 2004). Considering the detailed ratios at each collection site

TABLE 4. Lost and gained markers, theoretical minimum number of founding individuals, and number of markers for the 12 populations of *Saxifraga oppositifolia* sampled in the three glacier forelands. Numbers were calculated separately for each cluster defined by the program Structure.

	Lost markers		Gained markers			Minimum number of founders		Number of markers	
Glacier foreland	Diploid cluster	Tetraploid cluster	Diploid cluster	Tetraploid cluster	Ice-free since approximately	Diploid cluster	Tetraploid cluster	Diploid cluster	Tetraploid cluster
Renardbreen	2	3	4	3	1880	9	9	89	104
					1936	10	8	97	100
					1960	8	10	89	105
					1990	5	5	74	93
Hørbyebreen	7	4	7	0	1880	11	15	85	98
					1936	9	10	90	102
					1960	10	11	85	100
					1990	7	12	83	106
Midtre Lovénbreen	5	10	2	0	1880	9	4	91	69
					1936	9	7	91	91
					1960	8	10	83	91
					1990	9	6	96	81

(Fig. 1), there is no evidence that polyploids were more successful in colonizing new territory as implied by Soltis and Soltis (2000). If the tetraploid cluster is at an advantage because of higher ploidy, it may be canceled out by the disadvantages of polyploids (Comai, 2005), or unimportant on the studied time and spatial scale. Given that the ratio between diploids and polyploids varies locally from exclusively polyploid populations through co-occurrence of diploid and tetraploid populations to exclusively diploid populations, it can be reasoned that the ratio of diploids and polyploids in Svalbard is influenced by the local habitat. Ecological differentiation commonly occurs between diploids and their autopolyploids (Parisod et al., 2010). Thus, we hypothesize that diploids and tetraploids have largely overlapping, but different, ecological amplitudes.

The genetic separation between the diploid and the tetraploid cluster, as well as former data from phylogeographic analyses (Abbott and Comes, 2004), suggests that Svalbard may have been colonized from two sources. A potential source for the tetraploid cluster could be northern Siberia, because tetraploid counts have been reported from that area (Elven et al., 2011). The occurrence of *S. oppositifolia* subsp. *glandulisepala* in the sampled material could, however, be excluded by a voucher examination (R. Elven and I. G. Alsos, personal observation). Several potential sources are possible for the diploid cluster, given that diploids occur in Greenland and northern Europe (Elven et al., 2011). Our results, in congruence with Alsos et al. (2007), imply that Svalbard is a mixing zone for different genetic lineages of circumpolar distributed species, and may link northern Siberia, northern Europe, and Greenland.

Colonization of glacier forelands—Glacier foreland populations were not genetically differentiated from established populations. In addition, there were no significant differences in minimum numbers of founders between the clusters representing the ploidy levels. On average, the glacier foreland populations had lost only 1.9 out of 115 makers. Hence, there is no evidence for a bottleneck or founder effect during colonization in *S. oppositifolia*. The rather constant genetic diversity in the different moraine stages is similar to that found in other small-scale studies on glacier forelands (Raffl et al., 2006; Raffl et al., 2008) and in concordance with the results of other colonization studies (Von Flüe et al., 1999; Yang et al., 2008). Further, in our study, the populations of the youngest sampled glacier foreland stages (1990) had a similar diversity as the older stages, indicating a rapid build-up of genetic diversity in these young populations. That similar diversities are due to current gene flow between populations via pollen is rather unlikely, in that Svalbard lacks bees and bumblebees (Coulson, 2007). In addition, the ability to carry pollen and vitality of other insects in Svalbard is unclear. On the basis of our data, we can conclude that seed dispersal is high in the Arctic.

The Mantel tests showed isolation-by-distance on a larger geographic scale (within Svalbard), and a significant amount of molecular variation was explained by geographic separation (among sampled populations: 11%). However, the increase of genetic distance with geographic distance was small and almost identical in both ploidy clusters, and the molecular variation explained among populations collected throughout Svalbard was smaller than in studies of neighboring valleys conducted in the Alps (Raffl et al., 2006, 2008). Thus, although not unlimited, dispersal abilities of both ploidy levels were high within Svalbard. Further, our allocation tests show that although close sources contributed most, all sampled glacier foreland populations of *S. oppositifolia* received migrants from several distant sources. Because not all subpopulations could be sampled in this almost continuously distributed species, long-distance dispersal may have been somewhat overestimated. Nevertheless, on the basis of theoretical dispersal studies, the combined forms of migration (high long-distance dispersal combined with local dispersal) are necessary to create high genetic diversity in newly founded populations (Ibrahim et al., 1996; Bialozyt et al., 2006, Excoffier et al., 2009). This combination of both dispersal processes, described as stratified dispersal, implies that seeds are often moved by more than one process or dispersal mode (Higgins and Richardson, 1999; Cain et al., 2000).

Higher seed dispersal of *S. oppositifolia* in Svalbard than in mainland Norway was already suggested by Gabrielsen et al. (1997). Seeds of this species have no particular dispersal adaptation, but they are small and light (e.g., Pluess et al., 2005). In High Arctic areas like Svalbard, seed dispersal may be facilitated by thin snow layers, leaving the apical opening of the fruit above the snow, and creating smooth surfaces facilitating wind dispersal (Savile, 1972). *Saxifraga oppositifolia* is present in seed banks in Svalbard (Cooper et al., 2004) and may remain viable while buried for several years (Schwienbacher et al., 2010). Thus, seeds may accumulate long before they meet appropriate

Table 5. Allocation to possible source populations of 315 individuals of *Saxifraga oppositifolia* collected in glacier forelands. Allocation to underlined collection sites indicates long-distance dispersal to the respective glacier foreland.

Sampling origin	Distance to sources (km)	Allocated to sampled population	Individuals	Allocated to collection region	Individuals
Midtre Lovénbreen 1880	0.43	Source 4	7	Kongsfjorden	5
(*n* = 24)	8.42	Source 3	4		
	6.70	Source 2	2		
	7.46	Source 1	1		
Midtre Lovénbreen 1936	0.22	Midtre Lovénbreen 1880	5	Kongsfjorden	5
(*n* = 23)	0.65	Source 4	1		
	8.54	Source 3	1		
	7.54	Source 1	1		
	99.01	Source 5	1		
Midtre Lovénbreen 1960	0.51	Midtre Lovénbreen 1880,1936	9	Kongsfjorden	11
(*n* = 25)	1.04	Source 4	1		
Midtre Lovénbreen 1990	0.88	Midtre Lovénbreen 1880-1960	15	Kongsfjorden	4
(*n* = 28)	1.54	Source 4	2		
Hørbyebreen 1880	2.63	Source 5	7	Petuniabukta	3
(*n* = 23)	1.99	Source 8	1		
	88.84	Source 3	3		
	63.21	Endalen	2		
Hørbyebreen 1936	0.37	Hørbyebreen 1880	13	Petuniabukta	5
(*n* = 30)	2.26	Source 8	1	Recherchefjorden	2
	96.10	Source 4	1		
Hørbyebreen 1960	0.91	Hørbyebreen 1880,1936	16	Petuniabukta	3
(*n* = 28)	3.56	Source 5	1		
	2.81	Source 8	1		
	139.97	Source 9	1		
Hørbyebreen 1990	1.11	Hørbyebreen 1880-1960	19	Petuniabukta	2
(*n* = 29)	3.71	Source 5	1	Kongsfjorden	1
Renardbreen 1880	6.79	Source 10	5	Recherchefjorden	14
(*n* = 30)	7.28	Source 9	3		
	1.45	Source 11	2		
	6.27	Source 12	1		
Renardbreen 1936	0.14	Renardbreen 1880	7	Recherchefjorden	7
(*n* = 28)	6.28	Source 12	3		
	1.58	Source 11	1		
	7.31	Source 9	1		
	56.42	Kvartsittsletta	1		
	160.06	Source 3	1		
Renardbreen 1960	0.91	Renardbreen 1880,1936	11	Recherchefjorden	9
(*n* = 27)	140.18	Source 5	1		
	160.86	Source 3	1		
Renardbreen 1960	0.63	Renardbreen 1880-1960	12	Recherchefjorden	3
(*n* = 20)	2.06	Source 11	1		
	161.32	Source 4	1		

germination conditions at the glacier front. Because several years of soil development were necessary (approximately 10–16) until a quantifiable number of vascular plants occurred in a glacier foreland in Svalbard (Hodkinson et al., 2003), it can be assumed that even before a new population is founded, seeds that have dispersed locally, regionally, and over long distances accumulate. Our study indicates that the dispersal of seeds was high enough to establish populations with genetic diversity similar to the older populations in the surroundings within 20 yr. The calculated minimum numbers of founders necessary to bring the observed markers into the different glacier foreland stages were rather small in both diploid and tetraploid populations, but they are theoretical minimums. Germination experiments in two different substrates found that 20% of the seeds of *S. oppositifolia* germinated on field plots within 2 yr (Müller et al., 2011). Given the low proportion of seedling survival in marginal habitats (Bell and Bliss, 1980; Svoboda and Henry, 1987; Cooper, 2011), the number of immigrating seeds necessary to obtain the calculated minimum number of seeds established is probably much higher.

***Regional variation in glacier foreland systems*—**In some cases, overall data for Svalbard and data from regional subsets for each glacial foreland did not fully match. This was most obvious from the regional AMOVAs and Mantel tests. One reason for these mismatches may be seed influx to the glacier forelands from unsampled sources. Apart from higher gene flow over short distances, newly founded populations can be closely related because of their shared origin (Whitlock and McCauley, 1999). The differences among subsets were most noticeable in the glacier foreland of Hørbyebreen. Different proportions of diploids and tetraploids in the glacier forelands than at the sources (Fig. 1), different allocation patterns (Table 5; Fig. 5), and higher minimum numbers of founders (Table 4; Fig. 6) hint at some regional and local specificity. These dissimilarities can most likely be attributed to different seed-influx patterns. In particular, it can be assumed that local topography has an influence on seed dispersal. For example, several small valleys with glaciers connected to a larger icecap (Lomonosov Fonna) ending in Petuniabukta, and locally strong katabatic winds (Bednorz and Kolendowicz, 2010), may disperse seeds from more distant

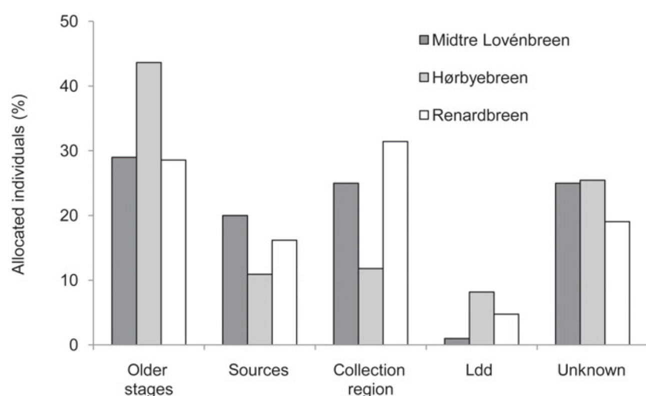

Fig. 5. Percentages of allocated individuals from the three sampled glacier forelands. "Older stages" include the individuals allocated to older stages than the sampled stage; "sources" represent the individuals allocated to one of the four collection sites in the surroundings of each glacier foreland; "collection region" are the individuals that were allocated to a certain collection region (Kongsfjorden: sources 1–4; Petuniabukta: sources 5–8; or Recherchefjorden: sources 9–12) but could not be uniquely assigned to one of the four sources; "ldd" represents the individuals allocated to a collection site outside the immediate glacier foreland and the surrounding four sources; and "unknown" are individuals that could not be allocated to a source population or a region.

unsampled or inaccessible sources, thus disconnecting the sampled source populations from the glacier foreland.

Conclusion—Local plant dispersal and establishment capacity can be high in the Arctic. All newly established populations of the study species showed high gene flow among one another and from potential source populations and had achieved similar levels of diversity as older populations within 20 yr. Thus, genetic diversity can be retained during local range expansion when both regional and local dispersal are high. Regional differences in topography influenced the proportions by which local, regional, and long-distance dispersal contributed to the colonization of the sampled glacier forelands.

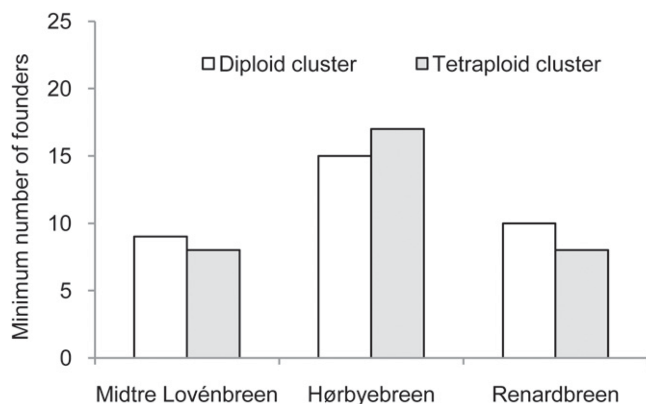

Fig. 6. Number of minimum founders necessary to deliver the observed markers to the glacier forelands, calculated for the diploid and tetraploid cluster for each glacier foreland.

REFERENCES

ABBOTT, R. J., H. M. CHAPMAN, R. M. M. CRAWFORD, AND D. G. FORBES. 1995. Molecular diversity and derivations of populations of *Silene acaulis* and *Saxifraga oppositifolia* from the High Arctic and more southerly latitudes. *Molecular Ecology* 4: 199–208.

ABBOTT, R. J., AND H. P. COMES. 2004. Evolution in the Arctic: A phylogeographic analysis of the circumarctic plant, *Saxifraga oppositifolia* (Purple saxifrage). *New Phytologist* 161: 211–224.

ABBOTT, R. J., L. C. SMITH, R. I. MILNE, R. M. M. CRAWFORD, K. WOLFF, AND J. BALFOUR. 2000. Molecular analysis of plant migration and refugia in the Arctic. *Science* 289: 1343–1346.

ALSOS, I. G., T. ALM, S. NORMAND, AND C. BROCHMANN. 2009. Past and future range shifts and loss of diversity in dwarf willow (*Salix herbacea* L.) inferred from genetics, fossils and modelling. *Global Ecology and Biogeography* 18: 223–239.

ALSOS, I. G., P. B. EIDESEN, D. EHRICH, I. SKREDE, K. B. WESTERGAARD, G. H. JACOBSEN, J. Y. LANDVIK, ET AL. 2007. Frequent long-distance plant colonization in the changing Arctic. *Science* 316: 1606–1609.

ALSOS, I. G., R. ELVEN, B. E. SANDBAKK, AND G. ARNESEN. 2011. Svalbardflora.net. Website: http://svalbardflora.net.

ALSOS, I. G., T. ENGELSKJØN, L. GIELLY, P. TABERLET, AND C. BROCHMANN. 2005. Impact of ice ages on circumpolar molecular diversity: Insights from an ecological key species. *Molecular Ecology* 14: 2739–2753.

AUSTERLITZ, F., B. JUNG-MULLER, B. GODELLE, AND P.-H. GOUYON. 1997. Evolution of coalescence times, genetic diversity and structure during colonization. *Theoretical Population Biology* 51: 148–164.

BANKS, T. W., AND J. J. BENHAM. 2008. Genographer, version 2.1.4. Website: http://sourceforge.net/projects/genographer.

BEDNORZ, E., AND L. KOLENDOWICZ. 2010. Summer 2009 thermal and bioclimatic conditions in Ebba Valley, central Spitsbergen. *Polish Polar Research* 31: 327–348.

BELL, K. L., AND L. C. BLISS. 1980. Plant reproduction in a High Arctic environment. *Arctic and Alpine Research* 12: 1–10.

BIALOZYT, R., B. ZIEGENHAGEN, AND R. J. PETIT. 2006. Contrasting effects of long distance seed dispersal on genetic diversity during range expansion. *Journal of Evolutionary Biology* 19: 12–20.

BJORNSTAD, O. N. 2009. Ncf: spatial nonparametric covariance functions, version 1.1-3. Website: http://cran.r-project.org/web/packages/ncf/index.html. [accessed 15.04. 2011].

BONIN, A., E. BELLEMAIN, P. BRONKEN EIDESEN, F. POMPANON, C. BROCHMANN, AND P. TABERLET. 2004. How to track and assess genotyping errors in population genetics studies. *Molecular Ecology* 13: 3261–3273.

BONIN, A., D. EHRICH, AND S. MANEL. 2007. Statistical analysis of amplified fragment length polymorphism data: A toolbox for molecular ecologists and evolutionists. *Molecular Ecology* 16: 3737–3758.

BRETAGNOLLE, F., AND J. D. THOMPSON. 1995. Tansley Review No. 78. Gametes with the stomatic chromosome number: Mechanisms of their formation and role in the evolution of autopolypoid plants. *New Phytologist* 129: 1–22.

BROCHMANN, C., A. K. BRYSTING, I. G. ALSOS, L. BORGEN, H. H. GRUNDT, A.-C. SCHEEN, AND R. ELVEN. 2004. Polyploidy in Arctic plants. *Biological Journal of the Linnean Society* 82: 512–536.

BRYSTING, A. K., T. M. GABRIELSEN, O. SØRLIBRÅTEN, O. YTREHORN, AND C. BROCHMANN. 1996. The purple saxifrage, *Saxifraga oppositifolia*, in Svalbard: Two taxa or one? *Polar Research* 15: 93–105.

CAIN, M. L., B. G. MILLIGAN, AND A. E. STRAND. 2000. Long-distance seed dispersal in plant populations. *American Journal of Botany* 87: 1217–1227.

CHAPMAN, H. M. 1995. IOPB Chromosome data 9. *IOPB Newsletter* 24: 10.

COMAI, L. 2005. The advantages and disadvantages of being polyploid. *Nature Reviews Genetics* 6: 836–846.

COOPER, E. J. 2006. Reindeer grazing reduces seed and propagule bank in the High Arctic. *Canadian Journal of Botany* 84: 1740–1752.

COOPER, E. J. 2011. Polar desert vegetation and plant recruitment in Murchisonfjord, Nordauslandet, Svalbard. *Geografiska Annaler, Series A* 93: 243–252.

COOPER, E. J., I. G. ALSOS, D. HAGEN, F. M. SMITH, S. J. COULSON, AND I. D. HODKINSON. 2004. Plant recruitment in the High Arctic: Seed

bank and seedling emergence on Svalbard. *Journal of Vegetation Science* 15: 115–124.

COULSON, S. J. 2007. Terrestrial and freshwater invertebrate fauna of the High Arctic archipelago of Svalbard. *Zootaxa* 1448: 41–58.

CRAWFORD, R. M. M. 2008. Cold climate plants in a warmer world. *Plant Ecology & Diversity* 1: 285–297.

CRAWFORD, R. M. M., H. M. CHAPMAN, AND L. C. SMITH. 1995. Adaptation to variation in growing season length in Arctic populations of *Saxifraga oppositifolia* L. *Botanical Journal of Scotland* 47: 177–192.

DUCHESNE, P., AND L. BERNATCHEZ. 2002. AFLPOP: A computer program for simulated and real population allocation, based on AFLP data. *Molecular Ecology Notes* 2: 380–383.

DZIERZEK, J., J. NITYCHORUK, AND A. RZETKOWSKA. 1990. Geological-geomorphological analysis and ^{14}C dating of submoraine organogenic deposits within the Renardbreen outer margin, Wedel Jarlsberg Land, Spitsbergen. *Polar Research* 8: 275–281.

EHRICH, D. 2006. AFLPdat: A collection of r functions for convenient handling of AFLP data. *Molecular Ecology Notes* 6: 603–604.

EHRICH, D., M. GAUDEUL, A. ASSEFA, M. A. KOCH, K. MUMMENHOFF, S. NEMOMISSA, I. CONSORTIUM, ET AL. 2007. Genetic consequences of Pleistocene range shifts: Contrast between the Arctic, the Alps and the East African mountains. *Molecular Ecology* 16: 2542–2559.

EIDESEN, P. B., I. G. ALSOS, M. POPP, Ø. STERNSRUD, J. SUDA, AND C. BROCHMANN. 2007a. Nuclear vs. plastid data: Complex Pleistocene history of a circumpolar key species. *Molecular Ecology* 16: 3902–3925.

EIDESEN, P. B., T. CARLSEN, U. MOLAU, AND C. BROCHMANN. 2007b. Repeatedly out of Beringia: *Cassiope tetragona* embraces the Arctic. *Journal of Biogeography* 34: 1559–1574.

ELVEBAKK, A. 1994. A survey of plant associations and alliances from Svalbard. *Journal of Vegetation Science* 5: 791–802.

ELVEN, R., AND A. ELVEBAKK. 1996. A catalogue of Svalbard plants, fungi, algae, and cyanobacteria. Part 1. Vascular plants. *Skrifter - Norsk Polar-institutt* 198: 9–55.

ELVEN, R., D. F. MURRAY, V. Y. RAZZHIVIN, AND B. A. YURTSEV. 2008. Checklist of the panarctic flora (PAF) vascular plants. Website: http://www.binran.ru/infsys/paflist/index.htm.

ELVEN, R., D. F. MURRAY, V. Y. RAZZHIVIN, AND B. A. YURTSEV. 2011. Annotated checklist of the panarctic flora (PAF) vascular plants. Website: http://gbif.no/paf/.

EVANNO, G., S. REGNAUT, AND J. GOUDET. 2005. Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology* 14: 2611–2620.

EXCOFFIER, L., M. FOLL, AND R. J. PETIT. 2009. Genetic consequences of range expansions. *Annual Review of Ecology Evolution and Systematics* 40: 481–501.

EXCOFFIER, L., AND H. E. L. LISCHER. 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.

FLOVIK, K. 1940. Chromosome numbers and polyploidy within the flora of Spitzbergen. *Hereditas* 26: 430–440.

GABRIELSEN, T. M., K. BACHMANN, K. S. JAKOBSEN, AND C. BROCHMANN. 1997. Glacial survival does not matter: RAPD phylogeography of Nordic *Saxifraga oppositifolia. Molecular Ecology* 6: 831–842.

GAUDEUL, M., P. TABERLET, AND I. TILL-BOTTRAUD. 2000. Genetic diversity in an endangered Alpine plant, *Eryngium alpinum* L. (Apiaceae), inferred from amplified fragment length polymorphism markers. *Molecular Ecology* 9: 1625–1637.

GUGERLI, F. 1998. Effect of elevation on sexual reproduction in Alpine populations of *Saxifraga oppositifolia* (Saxifragaceae). *Oecologia* 114: 60–66.

HAMMER, Ø., D. A. T. HARPER, AND P. D. RYAN. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4: 1–9.

HEWITT, G. M. 1996. Some genetic consequences of ice ages, and their role, in divergence and speciation. *Biological Journal of the Linnean Society* 58: 247–276.

HIGGINS, S. I., AND D. M. RICHARDSON. 1999. Predicting plant migration rates in a changing world: The role of long-distance dispersal. *American Naturalist* 153: 464–475.

HODKINSON, I. D., S. J. COULSON, AND N. R. WEBB. 2003. Community assembly along proglacial chronosequences in the High Arctic: Vegetation and soil development in north-west Svalbard. *Journal of Ecology* 91: 651–663.

HUGHES, A. R., B. D. INOUYE, M. T. J. JOHNSON, N. UNDERWOOD, AND M. VELLEND. 2008. Ecological consequences of genetic diversity. *Ecology Letters* 11: 609–623.

HULTÉN, E., AND M. FRIES. 1986. Atlas of North European Vascular Plants (North of the Tropic of Cancer), vols. I–III. Koeltz Scientific Books, Königstein.

IBRAHIM, K. M., R. A. NICHOLS, AND G. M. HEWITT. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity* 77: 282–291.

JÓNSDÓTTIR, I. S. 2005. Terrestrial ecosystems on Svalbard: Heterogenity, complexity and fragility from an Arctic island perspective. *Biology and Environment* 105: 155–165.

JØRGENSEN, C. A., T. SØRENSEN, AND M. WESTERGAARD. 1958. The flowering plants of Greenland: A taxonomical and cytological survey. *Biologiske Skrifter* 9: 1–172.

JØRGENSEN, M. H., R. ELVEN, A. TRIBSCH, GABRIELSEN, B. STEDJE, AND C. BROCHMANN. 2006. Taxonomy and evolutionary relationships in the *Saxifraga rivularis* complex. *Systematic Botany* 31: 702–729.

KEVAN, P. G. 1972. Insect pollination of High Arctic flowers. *Journal of Ecology* 60: 831–847.

KOSMAN, E., AND K. J. LEONARD. 2005. Similarity coefficients for molecular markers in studies of genetic relationships between individuals for haploid, diploid, and polyploid species. *Molecular Ecology* 14: 415–424.

KRZYSZOWSKA, A. J. 1985. Tundra degradation in the vicinity of the Polish Polar Station, Hornsund, Svalbard. *Polar Research* 3: 247–252.

KRZYSZOWSKA, A. J. 1989. Human impact on tundra environment at the Ny-Ålesund Station, Svalbard. *Polar Research* 7: 119–131.

LEVIN, D. A. 2002. The Role of Chromosomal Change in Plant Evolution. Oxford University Press, New York, New York, USA.

MCCAULEY, D. E., AND M. J. WADE. 1980. Group selection: The genetic and demographic basis for the phenotypic differentiation of small populations of *Tribolium castaneum. Evolution* 34: 813–821.

MOREAU, M., D. LAFFLY, D. JOLY, AND T. BROSSARD. 2005. Analysis of plant colonisaton on an Arctic moraine since the end of the Little Ice Age using remotely sensed data and a Bayesian approach. *Remote Sensing of Environment* 99: 244–253.

MOREAU, M., D. MERCIER, D. LAFFLY, AND E. ROUSSEL. 2008. Impacts of recent paraglacial dynamics on plant colonization: A case study on Midtre Lovénbreen foreland, Spitsbergen (79°N). *Geomorphology* 95: 48–60.

MÜLLER, E., E. J. COOPER, AND I. G. ALSOS. 2011. Germinability of Arctic plants is high in perceived optimal conditions but low in the field. *Botany* 89: 337–348.

NEI, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, New York, USA.

NEI, M., AND W. H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences,USA* 76: 5269–5273.

PARISOD, C., R. HOLDEREGGER, AND C. BROCHMANN. 2010. Evolutionary consequences of autopolyploidy. *New Phytologist* 186: 5–17.

PARMESAN, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution and Systematics* 37: 637–669.

PLUESS, A. R., W. SCHÜTZ, AND J. STÖCKLIN. 2005. Seed weight increases with altitude in the Swiss Alps between related species but not among populations of individual species. *Oecologia* 144: 55–61.

POPP, M., V. MIRRÉ, AND C. BROCHMANN. 2011. A single Mid-Pleistocene long-distance dispersal by a bird can explain the extreme bipolar disjunction in crowberries (*Empetrum*). *Proceedings of the National Academy of Sciences, USA* 108: 6520–6525.

PRITCHARD, J. K., M. STEPHENS, AND P. DONNELLY. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.

QIAGEN. 2006. DNeasy plant handbook. Qiagen GmbH, Hilden, Germany.

RACHLEWICZ, G., W. SZCZUCINSKI, AND M. EWERTOWSKI. 2007. Post-"Little Ice Age" retreat rates of glaciers around Billefjorden in central Spitzbergen, Svalbard. *Polish Polar Research* 28: 159–186.

RAFFL, C., R. HOLDEREGGER, W. PARSON, AND B. ERSCHBAMER. 2008. Patterns in genetic diversity of *Trifolium pallescens* populations do not reflect chronosequence on Alpine glacier forelands. *Heredity* 100: 526–532.

RAFFL, C., M. MALLAUN, R. MAYER, AND B. ERSCHBAMER. 2006. Vegetation succession pattern and diversity changes in a glacier valley, Central Alps, Austria. *Arctic, Antarctic, and Alpine Research* 38: 421–428.

RAFFL, C., P. SCHÖNSWETTER, AND B. ERSCHBAMER. 2006. 'Sax-sess' — genetics of primary succession in a pioneer species on two parallel glacier forelands. *Molecular Ecology* 15: 2433–2440.

R DEVELOPMENT CORE TEAM. 2010. The R project for statistical computing, version 2.12.0. Website: http://www.r-project.org/.

R DEVELOPMENT CORE TEAM. 2011. Nlme: linear and nonlinear mixed effects models, version 3.1-101. Website: http://cran.r-project.org/web/packages/nlme/index.html.

REUSCH, T. B. H., A. EHLERS, A. HÄMMERLI, AND B. WORM. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences, USA* 102: 2826–2831.

RØNNING, O. I. 1996. The flora of Svalbard. Norwegian Polar Institute, Oslo, Norway.

SAVILE, D. B. 1972. Arctic adaptations in plants. Research Branch, Canada Department of Agriculture, Ottawa, Ontario.

SCHÖNSWETTER, P., O. PAUN, A. TRIBSCH, AND H. NIKLFELD. 2003. Out of the Alps: Colonization of northern Europe by east Alpine populations of the Glacier Buttercup *Ranunculus glacialis* L. (Ranunculaceae). *Molecular Ecology* 12: 3373–3381.

SCHÖNSWETTER, P., J. SUDA, M. POPP, H. WEISS-SCHNEEWEISS, AND C. BROCHMANN. 2007. Circumpolar phylogeography of *Juncus biglumis* (Juncaceae) inferred from AFLP fingerprints, cpDNA sequences, nuclear DNA content and chromosome numbers. *Molecular Phylogenetics and Evolution* 42: 92–103.

SCHÖNSWETTER, P., AND A. TRIBSCH. 2005. Vicariance and dispersal in the Alpine perennial *Bupleurum stellatum* L. (Apiaceae). *Taxon* 54: 725–732.

SCHWIENBACHER, E., S. MARCANTE, AND B. ERSCHBAMER. 2010. Alpine species seed longevity in the soil in relation to seed size and shape—A 5-year burial experiment in the Central Alps. *Flora* 205: 19–25.

SITTE, P., H. ZIEGLER, F. EHRENDORFER, AND A. BRESINSKY. 1999. Lehrbuch der Botanik für Hochschulen. 34 ed. Spektrum Akademischer Verlag, Heidelberg.

SKREDE, I., P. B. EIDESEN, R. P. PORTELA, AND C. BROCHMANN. 2006. Refugia, differentiation and postglacial migration in Arctic–Alpine Eurasia, exemplified by the mountain avens (*Dryas octopetala* L.). *Molecular Ecology* 15: 1827–1840.

SLETTEN, K., A. LYSÅ, AND I. LØNNE. 2001. Formation and disinegration of a High-Arctic ice-cored moraine complex, Scott Turnerbreen, Svalbard. *Boreas* 30: 272–284.

SOLTIS, D. E., AND P. S. SOLTIS. 1999. Polyploidy: Recurrent formation and genome evolution. *Trends in Ecology & Evolution* 14: 348–352.

SOLTIS, P. S., AND D. E. SOLTIS. 2000. The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences, USA* 97: 7051–7057.

SUDA, J., AND P. TRÁVNÍČEK. 2006. Reliable DNA ploidy determination in dehydrated tissues of vascular plants by DAPI flow cytometry-new prospects for plant research. *Cytometry A* 69: 273–280.

SVOBODA, J., AND G. H. R. HENRY. 1987. Succession in marginal Arctic environments. *Arctic and Alpine Research* 19: 373–384.

VAN DER PEER, Y., AND R. DE WACHTER. 1994. TREECON for Windows: A software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Computer Applications in the Biosciences* 10: 569–570.

VON FLÜE, I., F. GUGERLI, R. HOLDEREGGER, AND J. J. SCHNELLER. 1999. Genetic and morphological variability in Alpine *Saxifraga oppositifolia* L. after colonisation. *Feddes Repertorium* 110: 555–559.

VOS, P., R. HOGERS, M. BLEEKER, M. REIJANS, T. VAN DE LEE, M. HORNES, A. FRITERS, ET AL. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407–4414.

WESTERGAARD, K. B., M. H. JØRGENSEN, T. M. GABRIELSEN, I. G. ALSOS, AND C. BROCHMANN. 2010. The extreme Beringian/Atlantic disjunction in *Saxifraga rivularis* (Saxifragaceae) has formed at least twice. *Journal of Biogeography* 37: 1262–1276.

WHITLOCK, M. C., AND D. E. MCCAULEY. 1999. Indirect measures of gene flow and migration: $F_{ST}1/(4Nm+1)$. *Heredity* 82: 117–125.

YANG, S., J. G. BISHOP, AND M. S. WEBSTER. 2008. Colonization genetics of an animal-dispersed plant (*Vaccinium membranaceum*) at Mount St Helens, Washington. *Molecular Ecology* 17: 731–740.