Population genetics of purple saxifrage (Saxifraga oppositifolia) in the high Arctic archipelago of Svalbard

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Received: 20 February 2013; Accepted: 18 March 2013; Published: 8 April 2013

Citation: Pietiläinen M, Korpelainen H. 2013. Population genetics of purple saxifrage (Saxifraga oppositifolia) in the high Arctic archipelago of Svalbard. AoB PLANTS 5: plt024; doi:10.1093/aobpla/plt024

Abstract. We investigated patterns of genetic variability in Saxifraga oppositifolia in the isolated Arctic Svalbard archipelago. The genetic analysis included genotyping using nine polymorphic microsatellite markers and sequencing of the nuclear internal transcribed spacer region. Among populations, mean allele numbers per microsatellite locus ranged from 2.0 to 2.6, and 9% of alleles were unique. Observed (H_0) and expected (H_E) heterozygosities averaged 0.522 and 0.445, respectively. Typically negative but non-significant F_IS values (mean −0.173) were found in S. oppositifolia populations. F_ST values were relatively low (mean 0.123). The Bayesian structure analysis provided additional information on population genetic structures. Seven out of 11 studied populations, including populations located both near each other and far apart (distances 5–210 km), showed relatively homogeneous clustering patterns, while one population located on a slope in the main settlement of Longyearbyen possessed a unique genetic structure. The Mantel test proved that there is no significant correlation between genetic and geographical distances. Different growth habits (compact, trailing and intermediate) did not possess distinct genetic compositions based on microsatellite variation. Internal transcribed spacer sequencing revealed 12 polymorphic sites. Among 24 sequenced Svalbard samples, eight haplotypes were detected, none shared by the mainland samples. Population genetic structures of S. oppositifolia in Svalbard show that both genetic variation and differentiation levels are modest, outcrossing is the main mating system, and dispersal and gene flow are important, probably attributable to strong winds and human and animal vectors.

Keywords: Arctic; ITS sequencing; microsatellites; population genetic structure; Saxifraga oppositifolia.

Introduction

In natural environments, organisms are typically exposed to several stress factors simultaneously, and the stresses are often most pronounced in extreme habitats, such as those at high latitudes and altitudes. For instance, Arctic plants may encounter many unique environmental factors, such as drought, permafrost, nutrient leaching, cryoturbation, extreme temperatures and short growing seasons (Körner 2003). The extent to which an organism is able to deal with stress determines the limits of its ecological amplitude. It is well established that biological systems are dynamic: genetic variation enables adaptation through selection, while in small and isolated populations random evolutionary processes, such as genetic drift, may become strong. The consequences of evolutionary actions will be visible in the pattern of genetic diversity and differentiation of populations.

Purple saxifrage, Saxifraga oppositifolia, is an Arctic-Alpine early flowering perennial herb. It is a circumpolar, ecologically and morphologically variable species with a wide range of habitats. It probably has the widest global
distribution in the family Saxifragaceae (Webb and Gornall 1989). Saxifraga oppositifolia grows in dry to moist soil and from sea level up to 4500 m in the Alps, including the coldest known places with angiosperm plant life (Körner 2011). It is also mentioned as the northernmost vascular plant species, having been found up in northern Greenland at 83°15’ (Gjaervoll and Rönning 1999). Saxifraga oppositifolia is not endangered at the moment, but climate change, the potential warming or drying of northern areas, and increased UV radiation could become a threat in the future (Körner 2003). It is self-compatible but mainly outcrossing and depends on its pollinators, which are mainly bumblebees (Bombus sp.) (Stenström and Molau 1992; Stenström and Bergman 1998). However, there are no bumblebees in some Arctic regions, such as Svalbard, where the pollinating insects are probably mainly small insects of the order Diptera (Coulson et al. 2003).

Previous population genetic and phylogeographic analyses on S. oppositifolia include random amplification of polymorphic DNA studies by Gabrielsen et al. (1997) and Gugerli et al. (1999), amplified fragment length polymorphism (AFLP) studies by Alsos et al. (2007), Kropf et al. (2008), Müller et al. (2012) and Winkler et al. (2012), restriction fragment length polymorphism investigations (Abbott et al. 1995; Abbott and Comes 2004) and studies based on cpDNA and nuclear internal transcribed spacer (ITS) sequences (Holderegger and Abbott 2003; Winkler et al. 2012). All these DNA studies have used universal binary markers or sequence data. On the other hand, our study utilizes microsatellite markers that we recently developed for S. oppositifolia (Pietiläinen and Korpelainen 2010), which, besides allowing precise genetic analyses, make it possible to draw conclusions on the effects of mating systems on population genetic structures.

In this study we aimed to reveal patterns of genetic variability in S. oppositifolia in the isolated Arctic Svalbard archipelago. We hypothesized that (i) populations possess low levels of genetic variation and are genetically differentiated due to the colonization history and small population sizes and consequent effects of genetic drift, (ii) inbreeding and heterozygote deficiency are negligible due to supposedly prevalent outcrossing, and (iii) founder dynamics combined with gene flow from various sources has generated an admixed genetic structure in populations exposed to considerable human interference when compared with pristine populations. Since S. oppositifolia is morphologically variable and presents different growth habits (compact, trailing and intermediate; Aiken et al. 2005), even within populations, we also investigated whether such morphological features have any relationship with the pattern of genetic variability.

In addition, we compared nuclear ITS sequence variation of plants from Svalbard and more southern mainland regions of distribution in order to reveal relationships among plants across a wider geographic area.

### Methods

#### Sampling, morphological observations and DNA analyses

We sampled fresh leaf material from 11 populations in late June 2005 (except population NYB in late June 2010) in the Arctic Svalbard archipelago in locations between latitudes 78 and 80°N (Table 1; Fig. 1). The growth form of the sampled plants was classified as compact, trailing (creeping) or intermediate. Pairwise distances between populations varied from 5 to 210 km. The collected leaf samples were desiccated in silica gel and stored at –80°C until DNA extraction, which was conducted using commercial kits (DNeasy Plant Mini Kit, Qiagen Inc., and E.Z.N.A. Plant DNA Mini-prep Kit, Omega Bio-tek, Inc.) following the manufacturers’ instructions.

We determined the population genetic characteristics of the populations using the following nine polymorphic microsatellite markers developed for S. oppositifolia (Pietiläinen and Korpelainen 2010): SaxJC, SaxS1C, SaxS2C, SaxT9C, SaxT10C, S1_2, S4_2, S8_2 and S21_2. In genotyping, one of the primers in each primer pair was fluorescently (FAM, HEX or TET) labelled. Amplifications were performed in 5-µL reactions, containing 0.5 µL of DNA (about 5 ng), 2.75 µL of ddH2O, 0.5 µL of 10× buffer, 0.1 µL of dNTP mix (10 mM), 0.15 µL of DyNAzyme II DNA polymerase (Finzymes) (2 U µL⁻¹) and 0.5 µL of both primers (5 pmol µL⁻¹). The polymerase chain reactions (PCR) were carried out as follows: DNA denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 46-60°C (depending on the primer pair) for 45 s, and elongation at 72°C for 1 min, with a final elongation at 72°C for 10 min. For genotyping, PCR products were first diluted with ddH2O. Following this, 2 µL of each sample were pipetted into a 96-well plate, and 8 µL of standard (MegaBACE ET400-R, GE Healthcare) diluted with ddH2O (1/45) were added. The plates were then run with the MegaBACE 1000 DNA Analysis System (Amersham Biosciences). Genotyping results were then analysed with the MegaBACE Fragment Profiler 1.2 (Amersham Biosciences).

To complement microsatellite analyses, we sequenced 24 samples from Svalbard (Table 1) and five samples from mainland Norway for the nuclear ITS region using primers ITS1 (5’-TCC GAT GGT GAA CCT GCG G-3’) and ITS4 (5’-TCC TCC GCT TAT TGA TAT GC-3’) (White et al. 1990).
Sample sizes of populations LYR and KIN were larger (5) because LYR was of special interest due to the expected great anthropogenic influence, while KIN was an opposite example. The amplified region covered the whole distance from the end of the 18S rRNA gene to the beginning of the 26S rRNA gene. Polymerase chain reaction amplifications were performed in a total volume of 20 μL containing 2 μL of DNA (about 20 ng), 13 μL of ddH₂O, 2 μL of 10× buffer, 0.4 μL of dNTP mix (10 mM), 0.6 μL of Dynazyme II DNA polymerase (2 U μL⁻¹) and 1 μL of both primers (5 pmol μL⁻¹). The PCR cycle was similar to that in microsatellite genotyping, but the annealing temperature was 50 °C. Amplification products were run on a 1% agarose gel, and the DNA fragments were excised and purified prior to sequencing using the E.Z.N.A. Gel Extraction Kit (Omega Bio-Tek). Purified DNA samples were sequenced at Macrogen Inc. using PCR primers. Internal transcribed spacer sequences were aligned with CLUSTAL W (Thompson et al. 1994), and any variable sites were recorded.

Data analyses

Based on microsatellite data, we calculated mean numbers of alleles per locus, mean observed and expected heterozygosities (H₀ and Hₑ), F-statistics estimators Fₛₚ and inbreeding coefficients Fᵢₛ, and conducted Hardy–Weinberg equilibrium (HWE) testing for all loci with χ² tests, and performed a Mantel test to discover possible relationships between genetic and geographical distances, all using Arlequin v3.5.1.2 (Excoffier and Lischer 2010). The Bonferroni correction was applied for the tests. In addition, we performed a Bayesian analysis of population structure using the software Structure 2.3 (Pritchard et al. 2000) to determine the number and the distribution of genetic clusters among all samples. The correlated allele frequency model was used, which assumes that at each locus allele frequencies are correlated. With this assumption, the model infers a population structure with K number of clusters based on the genotype data. An admixture model was applied. At each stage, the analysis was repeated with different values of K (range 1–15) to

Table 1. Sampling sites, coordinates and numbers of samples collected from each S. oppositifolia population in Svalbard, studied using microsatellite markers (MS) and ITS sequencing (ITS).

| Population | Locality     | Latitude   | Longitude   | Sample size MS | Sample size ITS |
|------------|--------------|------------|-------------|----------------|-----------------|
| BIS        | Biscayerhuk   | N79° 50' 18.9" | E12° 23' 30.1" | 20             | 3               |
| FOR        | Forkdalen    | N79° 31' 49.8" | E15° 12' 33.3" | 14             | 2               |
| ENG        | Engelsbukta 1| N78° 51' 16.8" | E11° 43' 36.7" | 24             | 2               |
| ED         | Engelsbukta 2| N78° 51' 16.0" | E11° 43' 42.0" | 17             | 1               |
| BOH        | Bohemanflya  | N78° 24' 22.6" | E14° 41' 25.7" | 29             | 1               |
| LYR        | Longyearbyen | N78° 14' 46.0" | E15° 27' 56.0" | 28             | 5               |
| NYB        | Nybyen       | N78° 12' 05.1" | E15° 35' 26.9" | 22             | 0               |
| END        | Endalen      | N78° 11' 12.2" | E15° 43' 07.1" | 20             | 1               |
| BRE        | Breinnosa    | N78° 11' 01.5" | E16° 43' 23.4" | 21             | 3               |
| KIN        | Kinnvika     | N80° 03' 15.2" | E18° 15' 10.5" | 22             | 5               |
| FLO        | Florabukta   | N80° 01' 48.5" | E18° 41' 33.7" | 14             | 1               |

![Figure 1](Figure 1. The study area of S. oppositifolia in Svalbard. Sampling details are given in Table 1.)
discover the value with the highest estimate of log-likelihood probability (ln Pr(X|K)) of the data. For each value of K, 10 independent runs were conducted with a burn-in length of 100,000 iterations, followed by a data collection period of 10^6 iterations. Furthermore, to identify the correct number of clusters (K) that best explains the data, ΔK values were calculated according to Evanno et al. (2005).

Since vegetative propagation is a potential mode of reproduction in *S. oppositifolia*, we used the Excel Microsatellite Toolkit (http://www.animalgenomics.ucd.ie/sdepark/ms-toolkit/) in each population to distinguish plants with a common clonal ancestry from plants sharing the same microsatellite-based multilocus genotype (MLG) by chance. When MLGs were found more than once in a population, the software MLGsim (Stenberg et al. 2003) was used to calculate the \( P_{\text{SEX}} \) values (probability that two individuals would share the same MLG by chance) and to simulate the corresponding critical values for the significance level \( P < 0.05 \). To obtain the critical values for the test statistic, \( P_{\text{SEX}} \), the number of simulations was increased until stable critical values were reached.

We constructed haplotype networks for the ITS sequence data, including all our own data and comparable sequences of the same species available in GenBank, using the network building software TCS 1.2.1 (Clement et al. 2000), which uses statistical parsimony (95 %) and the genealogical reconstruction algorithms of Templeton et al. (1992). Indels were treated as a fifth state and coded as a single mutational event.

### Results

Identical MLGs were found in all populations, except in BIS and FLO. The total percentage of MLGs was 21 % of all genotyped individuals. In most cases the number of plants sharing the same MLG was two. However, according to the simulated critical values of \( P_{\text{SEX}} \), obtained using the program MLGsim for each population with MLGs, none of the MLGs were likely to represent clones (\( P > 0.05 \)). Therefore, no further adjustments due to MLGs were made for the data set.

Among populations, mean allele numbers per locus were relatively even, ranging from 2.0 (NYB and FLO) to 2.6 (BOH) (Table 2). Only 3 out of 32 alleles (9 %) were unique, i.e. found in only one population. Two of them were present in LYR and one in ENG. Observed (\( H_O \)) and expected (\( H_E \)) heterozygosities varied between 0.367–0.695 and 0.375–0.525, and averaged 0.522 and 0.445, respectively. Hardy–Weinberg equilibrium testing conducted for populations indicated significant heterozygote deficiencies and excesses at 5 and 23 % of loci, respectively. Inbreeding coefficients (\( F_{IS} \)) were commonly negative (mean \(-0.173\)), indicating slight heterozygote excesses, but the values were statistically non-significant in all populations.

The mean \( F_{ST} \) among all populations equalled 0.123, showing significant differentiation (\( P < 0.001 \)). The Mantel test revealed that there is no significant association between genetic and geographic distances. Population structures were also assessed using the Bayesian structure analysis by determining the log-likelihood probability of the data (ln Pr(X|K)) as well as the rate of

| Population | A | \( H_O \) | \( H_E \) | \( F_{IS} \) |
|------------|---|--------|--------|--------|
| BIS        | 2.4 | 0.375 | 0.450 | 0.167 |
| FOR        | 2.4 | 0.575 | 0.509 | -0.130 |
| ENG        | 2.2 | 0.499 | 0.423 | -0.180 |
| ED         | 2.2 | 0.534 | 0.458 | -0.166 |
| BOH        | 2.6 | 0.488 | 0.391 | -0.248 |
| LYR        | 2.3 | 0.460 | 0.375 | -0.227 |
| NYB        | 2.0 | 0.550 | 0.403 | -0.365 |
| END        | 2.1 | 0.566 | 0.435 | -0.301 |
| BRE        | 2.1 | 0.632 | 0.515 | -0.227 |
| KIN        | 2.3 | 0.695 | 0.525 | -0.324 |
| FLO        | 2.0 | 0.367 | 0.413 | 0.111 |
| Mean       | 2.2 ± 0.2 | 0.522 ± 0.099 | 0.445 ± 0.052 | -0.173 |
change values (ΔK). The highest ΔK value was detected at K = 5 (Fig. 2a). Seven populations (FOR, ENG, ED, NYB, END, KIN and FLO) showed relatively similar clustering patterns and their pairwise FST values ranged from 0 to 0.083. Populations BOH, BRE and also BIS resembled each other (pairwise FST equalling 0.024–0.070), while LYR possessed a more unique clustering pattern (pairwise FST compared with all other populations varying between 0.287 and 0.383). No evident genetic difference was detected between different growth habits (compact, trailing and intermediate) within populations (Fig. 2a) or across populations (Fig. 2b).

The ITS region was sequenced for 24 samples from Svalbard (GenBank accession numbers EF687783–EF687806) and five samples from mainland Norway (accession numbers EF687807–EF687811). The clear, recorded region included most of the ITS1 region, complete 5.8S rRNA and most of ITS2. Additionally, 11 ITS sequences previously available for S. oppositifolia in GenBank (accessions AF502089, AF504544, AY354296–AY354303 and AY357945) were included in the network analysis. In all, nine single-nucleotide polymorphisms and three 1-bp indels were detected. The 24 Svalbard sequences resulted in eight haplotypes, among which one haplotype with 11 samples was dominant (Fig. 3). The 14 sequences from mainland Europe included nine haplotypes, and the two sequences from the USA possessed a similar unique haplotype. Svalbard samples and mainland samples possessed no shared haplotypes. The five sequenced LYR samples resulted in four different haplotypes, two representing the dominant Svalbard haplotype but three sequences being unique.

Discussion

Genetic characteristics of species impact their capacity to maintain their populations and colonize new areas. The presence of genetic diversity is especially important for plant populations in highly stochastic environments like the Arctic. The Arctic climate has been warming and the surface air temperature has increased, especially in the Atlantic region around the Svalbard archipelago (Przybylak 2007). More new areas for colonization are made bare by the retreating glaciers, and early successional species like S. oppositifolia may benefit from this. Müller et al. (2012) have actually shown that S. oppositifolia is a plant that can rapidly colonize new deglaciated areas without losing genetic diversity. In addition, Alsos et al. (2007) have suggested that past recurrent glacial cycles during the geological history have probably selected for a highly mobile Arctic flora in places like the Svalbard archipelago. The high levels of genetic diversity found in several species previously studied in Svalbard are consistent with the view that multiple long-distance dispersals have occurred (Alsos et al. 2007).

In the present study, although 21% of the genotyped samples possessed MLGs that were identical to another MLG in the same population, none of the MLGs were likely to represent clones (P > 0.05) based on the
testing of the $P_{SEX}$ values obtained. However, potential smaller clones were excluded on the grounds of the random sampling strategy. Among populations, mean allele numbers per locus ranged from 2.0 to 2.6, and only 3 out of 32 alleles (9%) were unique. Observed allele numbers per locus ranged from 2.0 to 2.6, and random sampling strategy. Among populations, mean smaller clones were excluded on the grounds of the same haplotype. Small squares on connecting spans indicate minimum numbers of individual mutations.

Figure 3. Haplotype network showing the relationships among 24 ITS sequences from Svalbard (Sv), five ITS sequences from mainland Norway (No) produced in this study and a total of 11 ITS sequences from mainland Norway (No), Austria (Au), Italy (It), Spain (Sp) and the USA (US) available in GenBank. The sizes of the circles and squares are proportional to the numbers of sequences with the same haplotype. Small squares on connecting spans indicate minimum numbers of individual mutations.

to reduced cross-pollination (Stenström and Bergman 1998). However, the population genetic structures we detected do not indicate that crosspollination would be rare.

Relatively low $F_{ST}$ values (mean 0.123) indicated a fair level of gene flow between populations occurring in different parts of Svalbard. Relatively low levels of differentiation have also been found in S. oppositifolia by Kropf et al. (2008), who in an AFLP-based study reported Nei’s genetic distances of 0.022–0.030 between regions in mountain areas in Central and Southern Europe. Previous genetic work on other Saxifraga species utilizing AFLP markers has revealed high levels of differentiation. Saxifraga callosa was studied in the Alps and showed extensive differentiation ($G_{ST} = 0.899$) (Grassi et al. 2009), which indicated limited gene flow among refugial areas. Saxifraga cernua and S. sibirica were investigated with AFLP markers in the Ural Mountains and were found to possess considerable levels of differentiation, $F_{ST}$ values equaling 0.452 and 0.362, respectively (Kapralov et al. 2006).

Besides $F_{ST}$-based differentiation measurements, the Bayesian structure analysis provided additional information on the population genetic structures. Seven out of 11 studied S. oppositifolia populations (FOR, ENG, ED, NYB, END, KIN and FLO), including populations located both near each other and far apart (distance variation 5–210 km), showed relatively homogeneous clustering patterns. Another set of three populations (BIS, BOH and BRE, pairwise geographic distances 52–206 km) resembled each other, while population LYR, located on a slope next to a new housing area and building sites in the main settlement of Longyearbyen, possessed a unique genetic structure. It is likely that the human-mediated colonization history and dispersal have been extensive, and that is reflected in the present genetic composition of S. oppositifolia in Longyearbyen. As expected from the Bayesian structure results, the Mantel test results proved that there is no significant correlation between genetic and geographical distances.

Different growth habits (compact, trailing and intermediate) of S. oppositifolia did not possess distinct genetic compositions based on microsatellite variation. For instance, population BOH containing mainly compact individuals and population BRE containing only trailing plants were genetically quite similar ($F_{ST} = 0.070$). Yet, earlier research on growth and reproduction patterns in S. oppositifolia has indicated that morphological variability is adaptive (Brysting et al. 1996; Kume et al. 1999). As the present work is based on the use of putatively neutral microsatellite markers, genetic differentiation at adaptive loci remains unobserved if not linked with the studied loci.
Sequencing of the nuclear ITS region revealed 12 polymorphic sites. Among 24 sequenced Svalbard samples, a total of eight haplotypes were detected, none shared by the mainland samples. One major Svalbard haplotype (46% of samples) was found in seven populations (BIS, FOR, BOH, LYR, BRE, KIN and FLO), which are located at distances 9–198 km from each other. Among the five samples sequenced for LYR, four haplotypes were found, two of which were unique among the study material. Based on the network analysis conducted, one of the unique ITS sequences of LYR appeared to descend from a haplotype discovered among samples from mainland Norway. These observations also support the view of the distinct colonization history of the LYR population. Among the nine mainland haplotypes, the Norwegian ones were related to each other or Svalbard haplotypes, except for a sample from Hordaland in southern Norway, which was identical to a haplotype also found in mountainous areas of Italy and Spain. Otherwise, the haplotypes from Austria, Italy and Spain were relatively closely related, while the haplotype revealed for the two samples from the USA was distinct. An increased sample size in mainland Europe, especially in Norway, could have provided a more precise insight into the relationship between Svalbard and other populations. Recently Winkler et al. (2012) discovered in their plastid sequence study that the haplotype of the single Svalbard sample of *S. oppositifolia* was also found in mainland Norway, Greenland, Slovakia and Switzerland.

In a related species, *Saxifraga paniculata*, Reisch (2008) discovered that Arctic populations contained less AFLP variation and fewer chloroplast haplotypes than populations from other regions of the distribution. The present study on *S. oppositifolia* indicates a fair level of haplotype diversity in the Arctic Svalbard populations. The ITS region as such is not supposed to be especially useful when studying larger-scale phylogeography of *S. oppositifolia* because of its largely unresolved topology with low statistical support (Holderegger and Abbott 2003). Yet, the moderate variability of the ITS region detected in our study, eight haplotypes just within Svalbard and nine additional haplotypes among the small set of samples from mainland Europe, provides interesting implications for phylogeographic studies on different geographic scales. For comparison, Winkler et al. (2012) discovered 27 haplotypes in a global study on 114 *S. oppositifolia* individuals based on plastid sequence data (psbA–trnH and trnTF intragenic spacers).

Strong winds in the open landscape, sea currents, drift ice and dispersal by vectors, such as humans and birds, may explain the present phylogeographic and population genetic patterns of *S. oppositifolia*. Small seeds are dispersed by the winds across the glaciers and whole plants have been known to float great distances on ice (Hultén and Fries 1986; Holderegger and Abbott 2003). It has also been reported that local reindeer forage on *S. oppositifolia*, particularly on its nutritious reproductive shoots (Cooper and Wookey 2003), especially in the northern parts of the archipelago where moss and lichen are scarce (Staaland and Punsvik 1980). Although Svalbard reindeer tend to remain in the local home range area, individual animals migrate regularly and plant dispersal may be more common than expected (Hansen et al. 2009). Previously, Alsos et al. (2007) proved by AFLP fingerprinting that long-distance colonization of Svalbard by *S. oppositifolia* has occurred repeatedly and from several source regions, probably by wind and drifting sea ice. Evidently, establishment limits distribution more than dispersal.

**Conclusions**

Population genetic structures detected in *S. oppositifolia* in Svalbard revealed that differentiation levels are modest, and that dispersal and gene flow are important phenomena within Svalbard, probably attributable to strong winds and human and animal vectors. The nine polymorphic microsatellite markers used and ITS sequencing are useful tools when examining population genetics and phylogeography of *S. oppositifolia* on different geographic scales.

**Sources of Funding**

Financial support was obtained from Kuopion Luonnon Ystävät Foundation and NordForsk.

**Contributions by the Authors**

M.P. collected most samples, conducted all laboratory work and part of the data analysis and manuscript preparation. H.K. collected some samples and conducted part of the data analysis and manuscript preparation.

**Conflicts of Interest Statement**

None declared.

**Acknowledgements**

We thank Dr Tove Gabrielsen, who provided plant samples from mainland Norway.
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