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Glacial survival does not matter: RAPD phylogeography of Nordic *Saxifraga oppositifolia*

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Abstract

The arctic–alpine *Saxifraga oppositifolia* has recently been suggested to have survived the last glaciation in high-arctic refugia, based on a finding of more genetic (RFLP) variation in Svalbard compared with more southern areas. To elucidate the migration history of this allogamous species, we analysed 18 populations from Norway, Svalbard and Novaya Zemlya using random amplified polymorphic DNAs (RAPDs). There was no more RAPD variation in the high Arctic than further south. In an analysis of molecular variance (AMOVA), most of the RAPD variation was found within populations (64%). There was less intrapopulational variation in Svalbard (65%) than in northern Norway (78%) and southern Norway (86%), suggesting that there is more inbreeding towards the north, probably because of lower pollinator activity. Twenty-eight per cent of the RAPD variation was found among populations within these geographical regions, and only 9% was found among the regions. In PCO and UPGMA analyses, plants and populations of different geographical origins were to a large extent intermingled. There was, however, a distinct, south–north clinal geographical structuring of the RAPD variation both in the PCO analysis and in a spatial autocorrelation (Mantel) analysis. These results suggest that there has been extensive gene flow among more or less continuously distributed populations of *S. oppositifolia* during the Weichselian, and that the extant Nordic populations were established after massive, centripetal immigration from these genetically variable, periglacial populations. The postglacial period may not have been sufficiently long for the subsequently isolated populations of this long-lived, allogamous perennial to diverge. Given the high levels of migration inferred from this study, genetic differentiation of glacial survivor populations, if any existed, would most likely have been swamped in the postglacial period. Thus, our molecular data support recent conclusions based on palaeobotanical and biogeographical data that the glacial survival hypothesis is superfluous.

Keywords: phylogeography, RAPDs, migration, glacial survival, *Saxifraga oppositifolia*

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Introduction

What is the origin of the Nordic arctic–alpine flora? Did all plants immigrate after the last glaciation, or did some of them survive *in situ* during the last or several of the Pleistocene glaciations? According to the 'nunatak hypothesis' or 'glacial survival hypothesis', it is necessary to invoke glacial refugia within the North European ice sheet to explain the geographical distributional patterns of many Nordic arctic–alpine plants (e.g. Sernander 1886; Nordhagen 1936, 1963; Knaben 1959a,b; Gjærevoll 1963; Löve & Löve 1963; Dahl 1963, 1987, 1990). The alternative 'tabula rasa hypothesis' states that all plants immigrated after the last glaciation (cf. Nordal 1987).

The level of migration of arctic–alpine plants is a fundamental issue in the discussion of the glacial survival hypothesis vs. the tabula rasa hypothesis. The North Atlantic Ocean has been considered a major barrier to plant dispersal by supporters of the glacial survival

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hypothesis, because special adaptations to long-distance dispersal are rarely found in arctic–alpine plants (Dahl 1963; Löve 1963). Thus, it has been claimed that arctic floras are ancient, and that adaptation rather than migration is the main factor ensuring their survival (Crawford *et al*. 1993). It has been suggested, however, that special adaptations are of less importance for long-distance dispersal than for short-distance dispersal (Berg 1983). More or less accidental long-distance dispersal via migrating birds, drifting ice and strong winds has been suggested to be particularly important for the establishment of new populations in the treeless arctic areas (Savile 1972).

The climatic changes of the late Pleistocene probably happened rapidly (e.g. Alley *et al*. 1993; Taylor *et al*. 1993). Conditions probably switched from glacial to near-interglacial in periods of less than a decade in central Greenland (Taylor *et al*. 1993). Recently collated palaeobotanical data suggest that many arctic–alpine plants grew beyond the ice sheet during the Weichselian glaciation, and migrated and colonized open habitats as rapidly as they became available following ice-melting (Birks 1994). The late Weichselian fossil record on Andøya, northern Norway, shows a dynamic picture of immigration, expansion, and disappearance of plants in periods of climatic change (Alm & Birks 1991). The fossil record of European insects also suggests that there has been frequent migration and recolonization episodes (Coope 1995). The dynamic patterns and rapidity of migration emerging from the fossil records suggest that the glacial survival hypothesis is superfluous (Birks 1994). In addition, recent statistical analyses of the present geographical distributions and species-richness patterns of mountain plants in Norway suggest that there is no need to invoke glacial refugia to explain these patterns when modern ecological factors are considered (Birks 1993, 1996).

Isozyme and restriction fragment length polymorphism (RFLP) analyses have recently been used to elucidate the phylogeography (cf. Avise *et al*. 1987) of some arctic–alpine plants. The degree of geographical structuring of their genetic variation may reflect the importance of migration in response to past climatic changes. Whereas long-term barriers to migration promote differentiation of populations because of genetic drift, mutation, and/or selection, migration constrains differentiation by increasing the genetic similarity between geographically separated populations (cf. Slatkin 1987). Several Nordic species of *Draba* lack consistently geographically structured variation at isozyme loci, suggesting that these species have experienced frequent migrations (Brochmann *et al*. 1992a,b). Other studies of isozyme variation (e.g. Haraldsen *et al*. 1991; Haraldsen & Wesenberg 1993) support such a dynamic view of the arctic–alpine flora, whereas isozyme variation in a single species (*Pedicularis dasyantha*; Odasz *et al*. 1991) has been claimed to support the more static view consistent with the glacial survival hypothesis. In a study of RFLP variation in chloroplast DNA (cpDNA) in *Saxifraga oppositifolia* L., Abbott *et al*. (1995) suggested that this hardy species survived the last glaciation in high-arctic refugia, based on the observation of more cpDNA variation in a population from Svalbard than in populations from more southerly latitudes. Glacial survival of *S. oppositifolia* has also been proposed based on more morphological variation, a wider ecological amplitude, and higher anoxia tolerance in this species in Svalbard compared with more southerly latitudes (Crawford *et al*. 1994, 1995).

Saxifraga oppositifolia is an arctic–alpine, early-flowering perennial. This diploid or rarely tetraploid species (Flovik 1940; Löve & Löve 1956) is self-compatible, but mainly outcrossing (Kevan 1972; Tikhmenev 1984; Stenström & Molau 1992). It occurs almost ubiquitously in the Arctic, with extensions southwards into mountain ranges of Asia, North America and Europe. The species is morphologically and ecologically variable, and two morphs which can be distinguished mainly by growth form are found; a prostrate form grows in snow-protected, damp habitats, and a cushion-like form grows on dry, wind-exposed ridges. The two morphs were recently recognized as different subspecies in the new Flora of Svalbard (Rønning 1996), but we have shown that they merely represent endpoints of local ecoclines without taxonomic significance (Brysting *et al*. 1996). It has been suggested that *S. oppositifolia* may adapt to changing climates and survive *in situ* through glaciations by altering frequencies of the two morphs (Crawford *et al*. 1995).

In this study, we use random amplified polymorphic DNAs (RAPDs, Williams *et al*. 1990), a molecular technique that amplifies random segments of mainly nuclear DNA, to examine the genetic variation and phylogeography of *S. oppositifolia*. RAPD analysis proved to be a useful tool in a recent study assessing the polyploid origin of the Scandinavian endemic *Saxifraga osloensis* (Brochmann *et al*. 1996b). Our main objective in the present study is to test if molecular data support the suggestion based on biogeographical and palaeobotanical data that the glacial survival hypothesis is superfluous (Birks 1993; Birks 1994). We also re-examine the observation based on RFLP data that *S. oppositifolia* is more variable in the Arctic than in more southern areas.

The most recent geological evidence suggests that at the maximum extent of the last glaciation, coalescent ice sheets covered Scandinavia, Svalbard, the Barents Sea and Novaya Zemlya (Andersen & Borns 1994; J. Y. Landvik personal communication). However, if some plants survived the last glaciation in glacial refugia, and experienced the long-term isolation and restricted migration consistent with the glacial survival hypothesis, a consistent geographical pattern of the genetic diversity can be expected.

A similar pattern could also be expected under the tabula rasa hypothesis, however, assuming limited amounts of migration and immigration from disjunct and divergent periglacially surviving populations. On the contrary, if there are or have been high levels of migration, less distinct geographical structuring of the genetic variation can be expected both under the glacial survival hypothesis and the tabula rasa hypothesis. Genetic differentiation among putative glacial survivor populations may nevertheless have been swamped by immigrants in the postglacial period. Thus, it may not be possible to differentiate between the tabula rasa and glacial survival hypotheses. The geographical structuring of the genetic variation may nevertheless reflect the levels of migration and, thus, the necessity of invoking glacial survival to explain present-day patterns of distribution and variation of Nordic arctic–alpine plants.

Materials and methods

Materials

Leaf material from 18 populations (88 plants) of the prostrate form of *Saxifraga oppositifolia* (Table 1) was collected from Svalbard (seven populations), Norway (10 populations) and Novaya Zemlya (one pooled population). From most populations, five plants separated by at least 10 m were sampled within an area of about 150 × 150 m. The populations were sampled as distantly as possible. Because our aim was to investigate large-scale geographical variation, we decided to analyse a small number of plants from a large number of populations rather than a large number of plants from a small number of populations. The plants from Novaya Zemlya were obtained as a mixed sample and analysed as one pooled population. Leaf material was dried in fine-grained silica gel.

Table 1 Collection data for the 18 populations of *Saxifraga oppositifolia* analysed for RAPD variation. Five plants were analysed from each population except SB7, from which three plants were analysed. The five plants from Novaya Zemlya were analysed as one pooled sample

Pop'n no.	Pop'n name	Main geographical region	Collection data
94-518	SN1	SN Vest-Agder, Farsund	Lista, Sigersvoll, 58°10′30′′N, 6°41′42′′E, alt. 10 m, 16.07.94. TGG & TMG.
94-521	SN2	SN Hordaland, Ulvik	SSE faced hill on the mountain Sandalsnut, 60°36′48′′N, 7°31′6′′E, alt. 1450 m, 19.07.94. TMG & MMT.
94-527	SN3	SN Oppland, Vang	SW side of the mountain Smådalsfjellet, 61°3′54′′N, 8°33′48′′E, alt. 1280–1300 m, 21.07.94. CB, TMG & MMT.
94-529	SN4	SN Oppland, Vågå	Gjendehalsen N of Gjendesheim, 61°30′6′′N, 8°49′0′′E, alt. 1160–1180 m, 22.07.94. CB, TMG & MMT.
94-539	SN5	SN Sør-Trøndelag, Oppdal	NW slope of Nordre Knutshø, 62°20′30′′N, 9°38′6′′E, alt. 1100 m, 26.07.94. TMG & MMT.
94-576	SN6	SN Nord-Trøndelag, Grong	SW of Medjås NE of Grong station, 64°27′42′′N, 12°20′48′′E, alt. 180 m, 19.08.94. AO.
94-552	NN1	NN Nordland, Fauske	Sulitjelma, E-NE of Storelvvatnan, 67°9′54′′N, 16°12′42′′E, alt. 810–830 m, 15.08.94. AH, TMG & MMT.
94-544	NN2	NN Troms, Nordreisa	E of top 759 of the mountain Pihkahistama, 69°30′24′′N, 21°16′12′′E, alt. 700–750 m, 08.07.94. TMG & AH.
94-574	NN3	NN Finnmark, Sør-Varanger	Grense Jacobselv, the cliffs over Heggedalsmoen at 69°44′24′′N, 30°51′54′′E, alt. 80–120 m, 26.07.94. AO.
94-575	NN4	NN Finnmark, Nordkapp	The Nordkapp plateau, 71°9′54′′N, 25°47′36′′E, alt. 300 m, 01.08.94. AO.
94-571	SB1	SB Bear Island	Bjørnøya Radio, 74°30′N, 19°0′E, alt. 10 m, 16.07.94. AH.
93-318	SB2	SB Edgeøya	Tusenøyene S of Edgeøya, Lurøya, 77°N, 22°E, 15.08.93. IG.
93-91	SB3	SB Spitsbergen	Sørkappland: Sørkapphytta, Sørflya, SSW of Kistefjellet, 76°30′N, 16°30′E, alt. 10 m, 04.07.93. CB.
93-105	SB4	SB Spitsbergen	Wedel Jarlsberg Land: Kapp Borthen W of Torellbreen, near the lighthouse, 77°10′N, 14°30′E, alt. 5 m, 05.07.93. CB.
93-145	SB5	SB Spitsbergen	Dickson Land: Kapp Thordsen, near the lighthouse, 78°20′N, 15°50′E, alt. 20 m, 08.07.93. CB.
93-138	SB6	SB Spitsbergen	Prins Karls Forland: Fuglehuken, near the lighthouse, 78°50′N, 10°30′E, alt. 15–100 m, 07.07.93. CB.
93-193	SB7	SB Spitsbergen	Andree Land: Mushamna, near the cabin, 79°30′N, 14°0′ E, alt. 10 m, 14.07.93. CB
94-345	NZ	Novaya Zemlya	Guba Bezymyannaya, near the coast, alt. 70 m, 05.08.94. HS.

Abbreviation of collectors: CB, Christian Brochmann; TGG, Thor G. Gabrielsen; TMG, Tove M. Gabrielsen; IG, Ian Gjertz; AH, Aslaug Hagen; AO, Anders Often; HS, Hallvard Strøm; MMT, Mari Mette Tollefsrud. SN, southern Norway; NN, northern Norway; SB, Svalbard; NZ, Novaya Zemlya.

RAPD analysis

DNA was extracted from 25 mg of dried leaf material using a CTAB miniprep method modified after Hombergen & Bachmann (1995). PCR reactions were modified after Williams *et al*. (1990) and Roelofs & Bachmann (1995). Reactions were performed in volumes of 25 μL containing 1 × PCR buffer (HT Biotechnology), 100 μM of each dNTP, 0.2 μM primer (Operon Technologies), 0.25 U Super *Taq* polymerase (HT Biotechnology) and 0.5 ng DNA. The reaction mixtures were overlaid with one drop of mineral oil and run in a MJ Research PTC-100/96 thermocycler programmed for 3 min at 94 °C, followed by 35 cycles of 15 s at 94 °C, 30 s at 40 °C and 60 s at 72 °C. These cycles were followed by 5 min at 72 °C for extra elongation. PCR products were separated on 1.5% agarose gels and visualized by ethidium bromide staining.

Five plants representing the geographical regions of southern Norway, northern Norway and Svalbard were prescreened using 125 primers (Operon kits A, B, C, D, F, H and K). Thirty-eight primers that produced scorable polymorphic bands were selected for further tests of the level of intrapopulational variation. Because we were mainly interested in the interpopulational level of variation, we selected 12 of the primers (A01, A10, A11, A15, A19, B11, C02, C08, C13, F01, F05, F12) that showed at least some variation among populations for analyses of all plants. Polymorphic, reproducible bands were scored as present or absent.

Statistics and multivariate analyses

The RAPD phenotype of each individual plant was expressed as a vector of zeros and ones. Euclidean squared distances between each pair of plants were calculated using NTSYS-pc (Rohlf 1990). All analyses were performed based on Euclidean squared distances. Some analyses were repeated using Dice's similarity coefficient, which excludes shared absence of bands, and the simple matching similarity coefficient, which includes shared absence. The various coefficients produced similar results, and only the analyses based on Euclidean squared distances are shown.

The analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) procedure converts a squared distance matrix into an analysis of variance format to extract variance components at different hierarchical levels. AMOVA was originally developed for RFLP haplotypes, but it is appropriate also for RAPD phenotypes (Huff *et al*. 1993; Peakall *et al*. 1995). Based on a matrix of Euclidean squared distances between RAPD phenotypes, a total sum-of-squares of the distances was calculated. Following classical analysis of variance, the total sum-of-squares was apportioned into sum-of-squares at different hierarchical levels; among individuals within populations, among populations within regions, and among regions. The variance component of each level was estimated by the expected mean squared deviation. For each analysis, 1000 permutations were performed to obtain significance levels of the variances.

The population from Novaya Zemlya was excluded from the AMOVA analyses, because only one pooled sample was investigated from this area. The other populations were divided into three groups according to main geographical region; southern Norway, northern Norway, and Svalbard. To investigate possible differences between the main geographical regions in the hierarchical apportioning of the RAPD variation, subsets containing populations from each region were analysed separately. In one analysis of the south-Norwegian subset, a geographically isolated coastal population (SN1; cf. Table 1) was excluded.

AMOVA analyses were performed using the WINAMOVA 1.55 program provided by L. Excoffier (http://anthropologie.unige.ch/ftp/comp; see also Excoffier *et al*. 1992). Principal coordinate analysis (PCO) and UPGMA clustering were performed using NTSYS-pc (Rohlf 1990).

Spatial autocorrelation statistics quantify the genetic similarity between pairs of individuals as a function of their spatial distance. The correlogram is a plot of similarity (correlation) as a function of geographical distance classes. To investigate the relationship between genetic and geographical distance between plants of *S. oppositifolia*, Mantel correlograms (the multivariate analogue of the spatial correlogram) were computed (Oden & Sokal 1986; Legendre & Fortin 1989; Bjørnstad *et al*. 1995). A matrix based on distances (measured on a map) between each

Geographical distance class	Mean distance (km)
Within populations	< 0.15
Within Svalbard	271
Within northern Norway	385
Within southern Norway	232
Between Svalbard and northern Norway	945
Between northern Norway and southern Norway	704
Between Svalbard and southern Norway	1048

Table 2 Classes of geographical distances used to compute Mantel correlogram values for the relationship between genetic (RAPD) and spatial distances among plants within and between the main regions. The south-Norwegian, isolated coastal population (SN1; cf. Table 1) was excluded

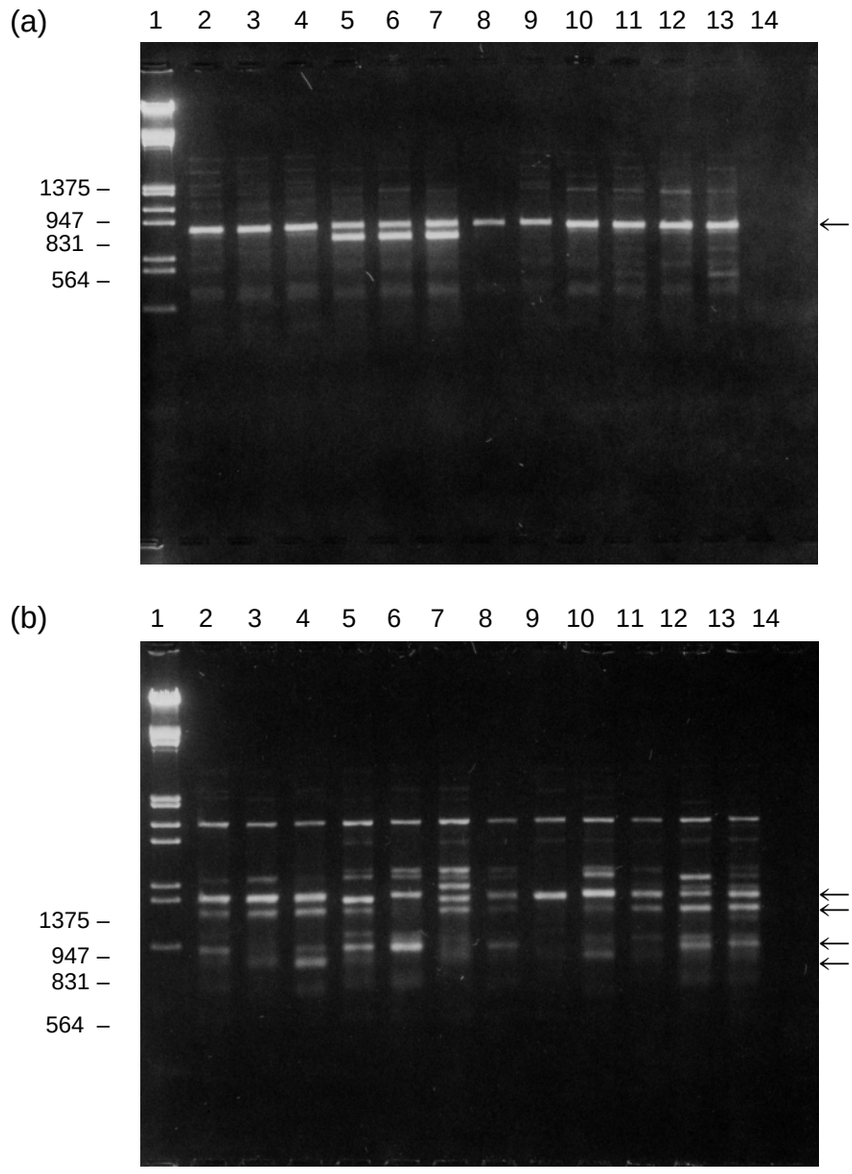

Fig. 1 (a) RAPD gel for primer OPA01, mainly revealing variation among populations. Lane 1, λ marker with band sizes in bp; lanes 2–4, population SN1; lanes 5–7, population SN3; lanes 8–10, population NN3; lanes 11–13, population SB5; lane 14, negative control.
(b) RAPD gel for primer OPA11 revealing intrapopulational variation. Lane 1, λ marker with band sizes in bp; lanes 2–4, population SN1; lanes 5–7, population SN3; lanes 8–10, population NN3; lanes 11–13, population SB5; lane 14, negative control. Population names refer to Table 1.

pair of plants was constructed. Based on this matrix, 14 classes of geographical distances were constructed for use in the Mantel analysis. The first distance class was set to less than 150 m to include all plants within the same population. The limits of the other distance classes were chosen to contain approximately the same number of distances.

To examine possible differences in genetic similarity between plants within and between the main geographical regions, distance classes containing pairs of distances among plants within and between the main geographical regions were constructed (Table 2). The isolated, coastal population from southern Norway (SN1; cf. Table 1) was excluded from this analysis. The population from Novaya Zemlya was excluded from all the Mantel analyses.

For each geographical distance class, the standardized Mantel statistic was computed (Mantel 1967). Each value of the Mantel statistic (Mantel *R*) was tested for significance by Mantel's normal approximation. To adjust for multiple testing, Bonferroni corrected *P*-values were calculated (Oden 1984; Rice 1989). The analyses were carried out using the '*R* package' (Legendre & Vaudor 1991).

Results

Saxifraga oppositifolia is not more polymorphic in the high Arctic

Thirty-five polymorphic RAPD bands were scored, identifying 79 different phenotypes of *Saxifraga oppositifolia*. All

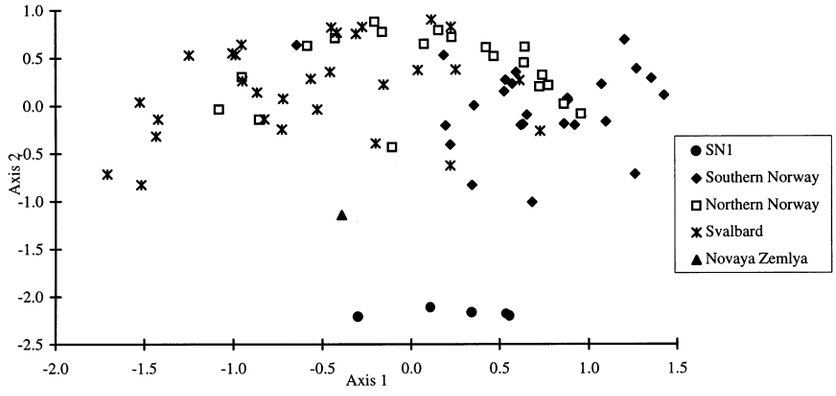

Fig. 2 PCO analysis of 88 plants (79 RAPD phenotypes) of *Saxifraga oppositifolia*. Axis 1 extracted 13% of the variance, and axis 2 extracted 11% of the variance. SN1 is the south-Norwegian, isolated coastal population (cf. Table 1)

markers except one varied to some extent within populations (Fig. 1). The substantial amount of intrapopulational variation was evident in the AMOVA analysis (Table 3), in which 64% of the total variation was found among individuals within populations. Twenty-eight per cent of the total variation was found among populations within regions, and 9% of the variation was found among the main geographical regions of southern Norway, northern Norway and Svalbard. There was a lower amount of intrapopulational variation in Svalbard (65%) than in northern Norway (78%) and southern Norway (86%; the south-Norwegian coastal population excluded). When the isolated, coastal population (SN1; cf. Table 1) was included in the south-Norwegian subset, 70% of the variation was found among individuals within populations (Table 3). The total genetic diversity, estimated as (no of polymorphic bands within a region)/(no of plants within the region) was also higher within southern Norway (91%) than within northern Norway (72%) and Svalbard (77%). The estimate from northern Norway may be biased due to the fewer plants analysed from this region (20) compared with southern Norway (30) and Svalbard (33).

PCO and Mantel analyses revealed an overall, south–north cline

The first three axes in the PCO analysis extracted 33% of the total variation. This analysis revealed no distinct groups except the south-Norwegian coastal population, which was clearly separated along the second axis (Fig. 2). However, an overall, south–north clinal geographical pattern could be recognized. Most plants from Svalbard had low coordinates along the first axis, whereas most plants from southern Norway had higher coordinates. Plants from northern Norway had coordinates more or less intermediate between those of the Svalbard plants and the south-Norwegian plants (Fig. 2). There was no distinct geographical structure of the genetic variation within the different regions. The pooled population from Novaya Zemlya had intermediate coordinates along both axes.

The Mantel correlogram calculated for 14 classes of geographical distances between all plants also revealed a clinal structure of the RAPD variation. In general, plants in close spatial proximity were genetically more similar than more distant plants (Fig. 3). Positive autocorrelations were

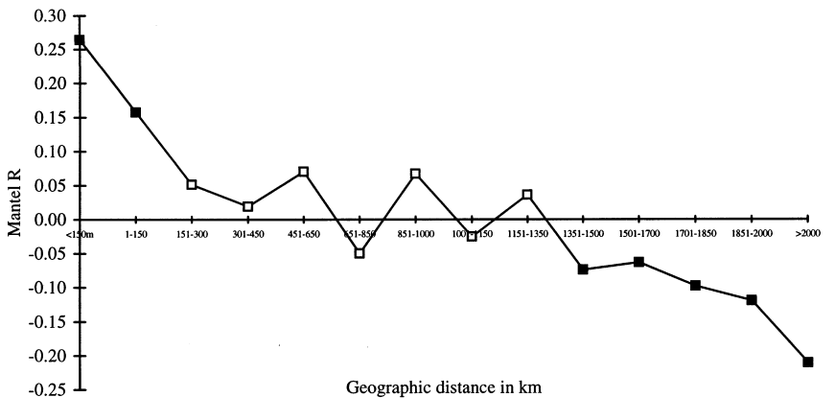

Fig. 3 Mantel correlogram showing the relationship between genetic (RAPD) distances and spatial distances (14 classes) between pairs of plants of *Saxifraga oppositifolia*. ■, autocorrelations significant at Bonferroni-corrected level (0.05/14 = 0.0036), □, autocorrelations not significant

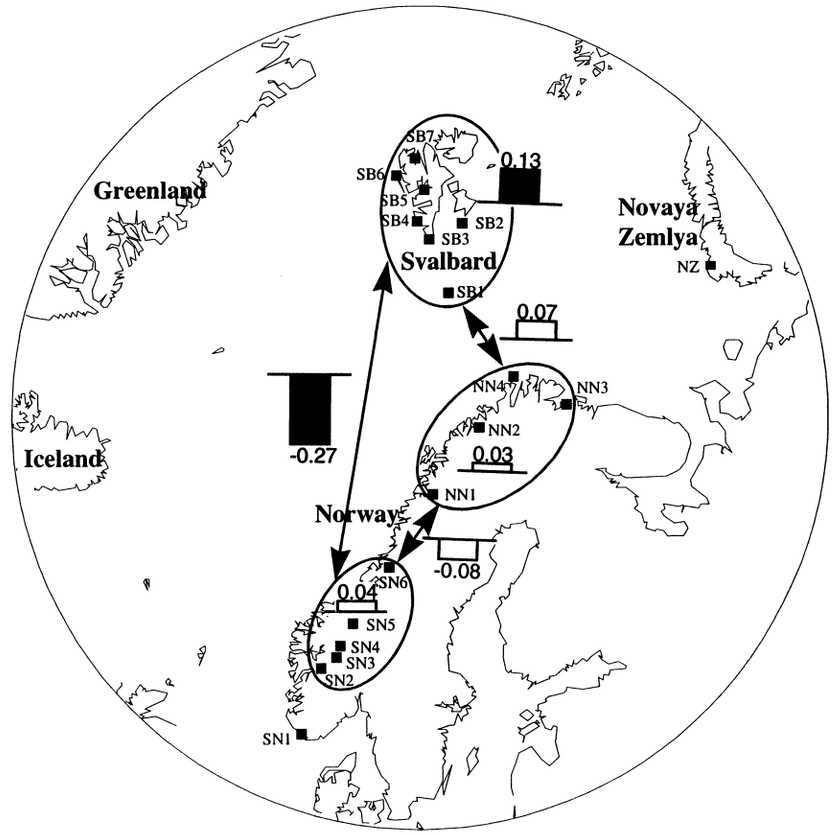

Fig. 4 Map showing sample sites for the 18 populations analysed of *Saxifraga oppositifolia*, and Mantel correlogram values for the relationship between genetic (RAPD) and spatial distances between pairs of plants within and between the main geographical regions. The geographically isolated coastal population from southern Norway (SN1; cf. Table 1) was highly divergent (cf. Fig. 2), and therefore excluded from this analysis. Filled bars, autocorrelations significant at Bonferroni-corrected levels (0.05/7 = 0.007), open bars, autocorrelations not significant.

found in the lowest distance classes (up to 150 km), and negative autocorrelations were found in the highest distance classes (>1350 km). Plants separated by distances of more than 2000 km were most strongly negatively correlated. Autocorrelation values in the intermediate distance classes were slightly positive or negative, but not significant at Bonferroni corrected levels (0.05/14 = 0.0036).

The Mantel correlogram values computed for pairs of plants within and between the main geographical regions also revealed the clinal variation, but indicated that genetic similarity does not only depend on spatial distance (Fig. 4). Plants from Svalbard, which were separated by a mean distance of 271 km, were significantly positively correlated ($r = 0.13$, $P = 0.002$), whereas plants from northern Norway (mean distance 385 km) and southern Norway (mean distance 232 km) were not significantly correlated ($r = 0.03$, $P = 0.21$; $r = 0.04$, $P = 0.18$, respectively). Between pairs of plants from different regions, the only significant correlation was found between plants from Svalbard and southern Norway, which were strongly negatively correlated ($r = -0.27$, $P < 0.001$). Plants from Svalbard and northern Norway were, however, more genetically similar ($r = 0.07$, $P > 0.007$) than plants from northern Norway and southern Norway ($r = -0.08$, $P > 0.007$), although not significant at Bonferroni corrected levels (0.05/7 = 0.007). The autocorrelation coefficient within populations was 0.26 ($P < 0.001$).

Populations and plants were intermingled in the UPGMA *analysis*

In the UPGMA analysis (Fig. 5), the south-Norwegian, isolated coastal population (SN1; cf. Table 1) and the population from Novaya Zemlya were separated at a fairly high level. Plants from the remaining populations did often not cluster together. Two main clusters could be recognized. One cluster contained mainly plants from southern Norway, whereas another cluster contained mainly plants from northern Norway and Svalbard (Fig. 5). Within the two main clusters, there were no clear geographical patterns.

Discussion

Although the intrapopulational variation values obtained are underestimates because of the few plants analysed from each population and the criteria we used for primer selection, all following conclusions based on these values could only have been strengthened by higher values and

Fig. 5 UPGMA analysis of 88 plants (79 RAPD phenotypes) of *Saxifraga oppositifolia*. Population names refer to Table 1.

by more precise estimates. This concerns the relative proportioning of the genetic variation as well as the comparisons of the intrapopulational variation among regions.

Breeding system

The breeding system is one of the main factors determining the genetic structuring of plant populations (Hamrick & Godt 1989). The considerable proportion (64%) of intrapopulational RAPD variation in *S. oppositifolia* clearly reflects its allogamous mode of reproduction. Isozyme studies have shown that allogamous plants usually maintain more genetic variation within populations and show less variation between populations than autogamous plants (Loveless & Hamrick 1984; Hamrick & Godt 1989). In the autogamous, arctic–alpine polyploids *Cerastium arcticum* Lange and *Saxifraga cespitosa* L., much less intrapopulational RAPD variation and more interpopulational and interregional RAPD differentiation have recently been found compared with *S. oppositifolia* (Brochmann *et al*. 1996a).

Phylogeography: molecular and palaeobotanical evidence

There was no consistent geographical structuring of the RAPD variation in *S. oppositifolia* compatible with restricted amounts of migration and possible long-term isolation in glacial refugia. The overall clinal geographical structuring of the RAPD variation, and the many exceptions to this overall structure evident in the PCO and UPGMA analyses, rather suggest high levels of migration. Present gene flow among the more or less disjunctly distributed populations in the Nordic area is not very likely. In the late part of the Weichselian, however, large, continuous areas along the margin of the North European ice sheet were polar desert or tundra (Andersen & Borns 1994). Its present habitat preferences, which include recently deglaciated ground, as well as the fossil record suggest that *S. oppositifolia* was abundant in this periglacial area. Macrofossils of *S. oppositifolia*, which are easily recognizable, and pollen of *S. oppositifolia*-type (which also includes *S. aizoides* L.; cf. Prentice 1981) have been recorded in several full- and late-glacial sediments. Macrofossils

have been recorded in north-west Scotland (Allerød & Younger Dryas), and PreBoreal Birks 1984), south-east Scotland (Allerød & Younger Dryas, Webb & Moore 1982), Denmark (deglaciation and Younger Dryas, Jensen 1985), western south Norway (late-glacial and PreBoreal, H.H. Birks and M. van Dinter personal communication), western Norway (deglaciation, Younger Dryas, and PreBoreal, H. H. Birks personal communication), and Svalbard (*c.* 8000–0 BP, Birks 1991). Thus, the fossil record shows that *S. oppositifolia* was distributed around the ice sheet margin and rapidly colonized recently deglaciated terrain as the ice retreated. As denser vegetation developed in mainland Scandinavia, *S. oppositifolia* was probably excluded by competition, and retreated to the mountains where it is presently most abundant.

In a late-glacial scenario with large, more or less continuously distributed periglacial populations of *S. oppositifolia*, gene flow among populations may have been frequent due to strong glacial winds, migrating birds, and floating icebergs. These dispersal agents may have been efficient between the European mainland and the arctic archipelagos. Winter ice and icebergs were present in the Norwegian Sea until 11000–10000 BP (Andersen & Borns 1994), and may have enhanced dispersal between Svalbard and northern Norway. *Saxifraga oppositifolia* has been collected from a floating iceberg moving from Alaska towards Russia, and the plant flowered after a week in a greenhouse (Hultén 1962). Thus, the closer genetic relationship among the populations from Svalbard and northern Norway compared with the populations from northern Norway and southern Norway (see Figs 4 and 5) may be caused by dispersal via floating icebergs and/or strong winds across the winter sea ice of the Norwegian Sea.

Frequent gene flow among large, more or less continuously distributed and genetically variable populations of *S. oppositifolia* beyond the Weichselian ice sheet may have prevented divergence of these populations during the glaciation. The Nordic area was probably colonized by centripetal waves of immigrants of *S. oppositifolia* from the periglacial area. The postglacial period may not have been sufficiently long for the extant, more or less disjunctly distributed populations of this long-lived, allogamous species to diverge. The only populations that were clearly separated were the small, geographically isolated coastal population from southern Norway (SN1; cf. Table 1) and the population from Novaya Zemlya (see Figs 2 and 5). The distinct genetic divergence of the small, south-Norwegian coastal population is most likely due to genetic drift following a bottleneck in the postglacial warm period. Another explanation is long-distance dispersal from source populations in the Norwegian mountain

Table 3 Analysis of molecular variance (AMOVA) based on 79 RAPD phenotypes (88 plants, 18 populations) of *Saxifraga oppositifolia*. Significance levels were not obtained for the intrapopulational level of variation when each region was analysed separately. SN1 is the south-Norwegian, isolated coastal population (cf. Table 1)

Source of variation	Sum of squared deviations (SSD)	d.f.	Mean squared deviations (MSD)	Variance components	% of the total variance	*P*-value
Among main geographical regions	44.43	2	22.22	0.44	8.58	< 0.001
Among populations within regions	142.44	14	10.17	1.42	27.62	< 0.001
Among individuals within populations	216.07	66	3.27	3.27	63.80	< 0.001
Among populations in southern Norway	57.50	5	11.50	1.58	30.43	< 0.001
Among individuals within populations in southern Norway	86.60	24	3.61	3.61	69.57	–
Among populations in southern Norway (SN1 excluded)	28.36	4	7.09	0.64	13.99	< 0.001
Among individuals within populations in southern Norway (SN1 excluded)	78.20	20	3.91	3.91	86.01	–
Among populations in northern Norway	25.50	3	8.50	0.99	21.70	< 0.001
Among individuals within populations in northern Norway	57.00	16	3.56	3.56	78.30	–
Among populations in Svalbard	59.44	6	9.91	1.52	35.23	< 0.001
Among individuals within populations in Svalbard	72.47	26	2.79	2.79	64.77	–

range or from populations situated outside Scandinavia, for example in Scotland. The differentiation of the population from Novaya Zemlya may be due to separate immigration, possibly from more eastern refugia.

Regional differences in RAPD diversity: no evidence of high-arctic refugia

The present study does not confirm the results of Abbott *et al*. (1995), in which more genetic (RFLP) diversity was found in a high-arctic population of *S. oppositifolia* than in populations from more southerly latitudes. There was no more RAPD variation in the high Arctic than in Norway. Rather, the total RAPD diversity was slightly lower within Svalbard (77%) than within southern Norway (91%). There was also less variation within populations from Svalbard than within populations from northern Norway and southern Norway (Table 3). This result can most easily be interpreted in terms of present gene flow via pollen. Fewer pollinators, lower pollinator activity, and shorter pollinator flight distances may increase the number of sibling-matings and thereby reduce the genetic diversity within populations in Svalbard compared to populations in Norway. The different results obtained for *S. oppositifolia* based on RAPD data (this study) vs. RFLP data (Abbott *et al*. 1995) is most likely due to the very limited material and markers that were analysed in the RFLP study. It is also possible, however, that the different modes of inheritance and dispersal of the two marker types may have influenced the results. The mainly nuclear RAPDs are biparentally inherited (Williams *et al*. 1990), whereas most cpDNA RFLPs are maternally inherited and only dispersed via seeds (Mitton 1994). Other studies have revealed discordant geographical patterns when comparing nuclear and organellar markers (e.g. Soltis *et al*. 1992; Mitton 1994).

The populations of *S. oppositifolia* from Svalbard were genetically more similar to each other than the populations from Norway (see Fig. 4), a result that may indicate higher levels of seed dispersal in Svalbard, possibly because of dispersal across snow and ice. Although the regional differences in RAPD variation can easily be explained by present gene flow, the lower amount of diversity in Svalbard compared to Norway may indicate loss of genetic variation due to founder events and bottlenecks in periods of immigration.

Concluding remarks

There is no need to invoke high-arctic (cf. Abbott *et al*. 1995) or other glacial refugia within the north European ice sheet to explain the geographical distribution of the RAPD variation among Nordic populations of *S. oppositifolia*. This allogamous species probably migrated rather undramatically into the Nordic area from large, more or less continuously distributed and genetically variable periglacial populations. It cannot be entirely excluded, however, that some populations of *S. oppositifolia* may have survived *in situ* during the last or several of the Pleistocene glaciations. The high levels of migration inferred from this study suggest that any genetic differentiation of putative glacial survivor populations nevertheless would have been swamped in the postglacial period. Thus, the results of this study support the suggestion based on phytogeography (Birks 1993, 1996) and palaeodata (Birks 1994) that the glacial survival hypothesis is superfluous.

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