Molecular analysis of the Pleistocene history of *Saxifraga oppositifolia* in the Alps

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**Abstract**

A recent circumpolar survey of chloroplast DNA (cpDNA) haplotypes identified Pleistocene glacial refugia for the Arctic-Alpine *Saxifraga oppositifolia* in the Arctic and, potentially, at more southern latitudes. However, evidence for glacial refugia within the ice sheet covering northern Europe during the last glacial period was not detected either with cpDNA or in another study of *S. oppositifolia* that surveyed random amplified polymorphic DNA (RAPD) variation. If any genotypes survived in such refugia, they must have been swamped by massive postglacial immigration of periglacial genotypes. The present study tested whether it is possible to reconstruct the Pleistocene history of *S. oppositifolia* in the European Alps using molecular methods. Restriction fragment length polymorphism (RFLP) analysis of cpDNA of *S. oppositifolia*, partly sampled from potential nunatak areas, detected two common European haplotypes throughout the Alps, while three populations harboured two additional, rare haplotypes. RAPD analysis confirmed the results of former studies on *S. oppositifolia*; high within, but low among population genetic variation and no particular geographical patterning. Some Alpine populations were not perfectly nested in this common gene pool and contained private RAPD markers, high molecular variance or rare cpDNA haplotypes, indicating that the species could possibly have survived on ice-free mountain tops (nunataks) in some parts of the Alps during the last glaciation. However, the overall lack of a geographical genetic pattern suggests that there was massive immigration of cpDNA and RAPD genotypes by seed and pollen flow during postglacial times. Thus, the glacial history of *S. oppositifolia* in the Alps appears to resemble closely that suggested previously for the species in northern Europe.

**Keywords**: Alpine species, cpDNA RFLPs, phylogeography, Pleistocene glacial survival, RAPDs, *Saxifraga oppositifolia*

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**Introduction**

Recent research on the phylogeography and evolution of Arctic and/or Alpine plant species has focused largely on locations of refugia during Pleistocene glaciations and migration routes during the postglacial period (Comes & Kadereit 1998; Gugerli & Holderegger 2001). Much of this research has centred on Arctic-Alpine plants with geographical distributions that often include the European Alps (e.g. Abbott et al. 1995; Brochmann et al. 1998; Tollefsrud et al. 1998); however, detailed analysis of populations from the Alps has often been lacking (but see Bauert et al. 1998). Only recently has this omission begun to be rectified (e.g. Stehlik et al. 2001a; Zhang et al. 2001; Kropf et al. 2002).

Several studies on the phylogeography of Arctic species have been conducted on the holarctic (Webb & Gornall 1989) purple saxifrage, *Saxifraga oppositifolia* (Abbott et al. 1995, 2000; Gabrielsen et al. 1997). An important question was whether this species survived glaciation on ice-free mountain tops (nunataks) within Pleistocene ice sheets or
whether it re-immigrated from periglacial refugia during the postglacial period. Gabrielsen et al. (1997) inferred high levels of migration from clinal variation in random amplified polymorphic DNA (RAPD) data of S. oppositifolia in Scandinavia and Svalbard. They concluded that potentially in situ surviving, resident genotypes were swamped by massive re-immigration of periglacial genotypes after glaciation and that glacial survival in refugia within this region did not matter. Subsequently, a restriction fragment length polymorphism (RFLP) analysis of chloroplast DNA (cpDNA) was conducted on individuals from almost the entire circumpolar distribution range of S. oppositifolia (Abbott et al. 2000). High cpDNA diversity was found in Arctic areas known to have remained ice-free during the last ice age (e.g. Alaska and the Taymyr region of north-central Siberia), indicating that these were major refugia for the species during this period. In contrast, areas that had been glaciated contained only a few common cpDNA haplotypes. Abbott et al. (2000) concluded that glacial refugia for S. oppositifolia most likely existed in ice-free areas to the north, south, east and west of the ice sheets that covered much of North America and a large part of Eurasia during the Pleistocene glaciations. However, their results, like those of Gabrielsen et al. (1997), indicated that the offspring of plants, which may have survived on nunataks within glaciated regions, have not contributed significantly to the presence of the species in these areas today.

Based on pollination experiments and high RAPD variation within local populations, S. oppositifolia is mainly outbred in the Alps (Gugerli 1997; Gugerli et al. 1999), where it is widely distributed, occurring commonly between 1800 and 3800 m above sea level (a.s.l.). It is also found far below the timberline in Alpine valleys and sometimes as low as 580 m a.s.l. (Kaplan 1995). The species occurs on siliceous and calcareous bedrock.

The wide distribution of S. oppositifolia, both in longitudinal and altitudinal terms, as well as its wide ecological amplitude designate this species as a candidate for in situ glacial survival within the Alps. This leads to the biogeographic question concerning where Alpine S. oppositifolia might have survived the ice ages. Did it persist on isolated northern Alpine or on high Alpine, ice-free nunataks, or did it re-immigrate from major southern refugia or even from the periglacial lowlands? Because S. oppositifolia is distributed almost throughout the Alpine arc and because fossils of the species are known from the adjacent lowlands from late glacial or early postglacial periods only (Kaplan 1995; Burga & Perret 1998), it has not been possible to state explicitly the putative locations of glacial refugia or nunatak areas of S. oppositifolia within the Alps. Thus, the principal question addressed in the present study was: is it possible using molecular methods to deduce the Pleistocene history of a widespread and common Alpine species such as S. oppositifolia, whose present-day ecology and distribution do not allow any exact hypotheses on its glacial history in the Alps?

Materials and methods
The species
Saxifraga oppositifolia L. (Saxifragaceae) is a long-lived, evergreen, runner-forming cushion plant (Webb & Cornall 1989). It occurs almost throughout the European mountains, where it is a member of a group of closely related taxa. In the Alps, this group comprises S. biflora, S. nephrophylla, S. oppositifolia, S. retusa and S. rudolphiana (Kaplan 1995). Saxifraga oppositifolia is diploid (2n = 26) within its Alpine distribution (Küpper & Rais 1983). Its stems usually bear single, outbred protandrous flowers, but selfing is possible (Gugerli 1997).

Sampling
Former molecular genetic studies on S. oppositifolia from the Alps either did not sample in potential nunatak areas (Abbott et al. 2000; Fig. 1) or covered only a limited geographical region (Canton of Grisons, Switzerland; Gugerli et al. 1999).

The middle part of the Alps is particularly suitable for studies on the phylogeography and glacial history of Alpine plants (Stehlik 2000). This part of the Alpine arc has potentially been colonized by (re-)immigration waves from western, southern and/or eastern major glacial refugia of Alpine plants, e.g. from the Grajc, Cottic, Bergamas, or eastern Austrian Alps. In addition, peripheral northern Alpine nunataks (e.g. the mountains Vanil Noir and Pilatus in Switzerland) and even high Alpine nunataks (e.g. in the Penninic or Rhaetic Alps) are known in this part of the Alps (Stehlik 2000). Therefore, we sampled leaf material (dried in silica gel) from 13 populations of S. oppositifolia from the middle, Swiss part of the Alps and from one additional population each from the French (Écrins) and Italian Alps (Passo Tonale; Fig. 1). Nine of these 15 populations were located in areas described in the literature either as major southern refugia, northern Alpine nunataks, or high Alpine nunataks (Stehlik 2000). The other six populations were sampled at high elevations between the above populations (Table 1, Fig. 1). No population included in this study had been part of any former investigations on Alpine S. oppositifolia.
DNA extraction

Genomic DNA was extracted from 80 mg of dried leaf material using the protocol of Whittemore & Schaal (1991) plus modifications and cleaning steps given in Milne et al. (1999). Total DNA was additionally cleaned with 7.5 M ammonium acetate and a Wizard DNA clean up column (Promega).

RFLP analysis of cpDNA

We followed strictly the protocol of Abbott et al. (2000), details of which are given in Milne et al. (1999). We used the same four principal probe × enzyme combinations, i.e. mung bean MB3 × EcoRI, MB3 × BfiI, MB7 × EcoRI and MB7 × BfiI. The haplotypes of some individuals were additionally verified using the combination MB3 × DraI.

Table 1 Abbreviation and location of the investigated populations of *Saxifraga oppositifolia* from the Alps and designation of the sampling sites in the biogeographic literature (Stehlik 2000)

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Location</th>
<th>Elevation (m a.s.l.)</th>
<th>Co-ordinates</th>
<th>Designation</th>
<th>N</th>
</tr>
</thead>
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<td>EV</td>
<td>Ecrins, France</td>
<td>2680</td>
<td>06°11'00&quot;E/44°49'15&quot;N</td>
<td>major southern refugia</td>
<td>3/1/3</td>
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<td>Rochers de Naye, Switzerland</td>
<td>1720</td>
<td>06°58'45&quot;E/46°27'19&quot;N</td>
<td>northern Alpine nunatak</td>
<td>5/1/10</td>
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<tr>
<td>VV</td>
<td>Vanil Noir, Switzerland</td>
<td>1680</td>
<td>07°08'03&quot;E/46°32'36&quot;N</td>
<td>northern Alpine nunatak</td>
<td>5/1/10</td>
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<tr>
<td>TA</td>
<td>Augstbordpass, Switzerland</td>
<td>2890</td>
<td>07°45'10&quot;E/46°12'32&quot;N</td>
<td>no particular designation</td>
<td>5/1/10</td>
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<td>2640</td>
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<td>high Alpine nunatak</td>
<td>6/1/10</td>
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<td>5/1/10</td>
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<tr>
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<td>5/1/10</td>
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<td>6/1/10</td>
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<td>CT</td>
<td>Piz Tom, Switzerland</td>
<td>2360</td>
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<td>no particular designation</td>
<td>6/1/10</td>
</tr>
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<td>FF</td>
<td>Fuorcla Faller, Switzerland</td>
<td>2900</td>
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<td>high Alpine nunatak</td>
<td>6/1/10</td>
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<tr>
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<td>5/1/10</td>
</tr>
<tr>
<td>BL</td>
<td>Piz Lagalp, Switzerland</td>
<td>2950</td>
<td>10°01'25&quot;E/46°25'37&quot;N</td>
<td>high Alpine nunatak</td>
<td>6/1/10</td>
</tr>
<tr>
<td>PP</td>
<td>Passo Tonale, Italy</td>
<td>2750</td>
<td>10°38'15&quot;E/46°18'35&quot;N</td>
<td>major southern refugia</td>
<td>5/1/10</td>
</tr>
</tbody>
</table>

N, number of investigated individuals per population in the cpDNA RFLP analysis using Southern hybridization, in PCR-RFLP of cpDNA and with RAPDs.
Haplotype designation was the same as in Abbott et al. (2000). Five to six individuals per population were investigated (except for population Ecrins; Table 1).

Since only four different haplotypes were detected in the above analysis (see Results section), we examined the two common haplotypes A and B further using polymerase chain reaction (PCR)-RFLP. We selected one individual per population (Table 1), comprising eight individuals with haplotype A and seven individuals with haplotype B. PCR reactions followed the protocol of Demesure et al. (1995) with only minor modifications. Noncoding regions of cpDNA were amplified with (1995) with only minor modifications. Noncoding regions (Promega) using the primer pairs

\[ \text{trn}\text{psb}C/\text{trn}\text{S} \]

and

\[ \text{psa}A/\text{trn}\text{S} \]

and

\[ \text{trn}\text{M}rbcL \]

(Demesure et al. 1995). Each PCR product was digested with each of the five restriction enzymes (Alu, Cfo, HaeII, HpaII and RsaI) according to the manufacturer’s instructions (Promega). RFLP profiles were analysed on 8% polyacrylamide gels stained with ethidium bromide.

**RAPD analysis**

Ten individuals per population (except for population Ecrins; Table 1) were used in the RAPD analysis. We followed the methods of Gabrielsen et al. (1997) as closely as possible, using the same *Taq* polymerase and the same make of thermocycler (MJ Research PTC 100/96). Nevertheless, some modifications were necessary. We used 0.75 U of Super *Taq* polymerase (HT Biotechnology) with 1 ng genomic DNA per reaction volume, and the thermocycler was programmed for 180 s at 94 °C, followed by 40 cycles of 60 s at 94 °C, 60 s at 40 °C and 90 s at 72 °C. Final extension lasted for 300 s at 72 °C. PCR products were separated on 1.4% agarose gels and visualized with ethidium bromide. We considered six of the 12 Operon enzymes (Alu, Cfo, HaeII, HpaII and RsaI) according to the manufacturer’s instructions (Promega). RFLP profiles were analysed on 8% polyacrylamide gels stained with ethidium bromide.

**Statistical analysis**

For each of the two data sets, the cpDNA RFLP analysis using Southern hybridization and the RAPDs, the presence or absence of fragments was recorded in a binary matrix. We applied a set of diversity measurements to the RAPD data often used to determine potential glaciated refugia (Stehlik et al. 2001a; Widmer & Lexer 2001a): i.e. diversity of multi-locus phenotypes per population, *M*, number of private (restricted to a single population) and rare (occurring in no more than 10% of all individuals) RAPD markers per population and, as a measure of genetic diversity, the molecular variance [(SS/N − 1), where *N* is the number of individuals investigated per population, and SS is the sum of squares obtained in AMOVA; see below; Landergott et al. 2001]. Additionally, we applied the concept of nestedness used in biodiversity research (Ganzhorn & Eisenbeiss 2001). The concept of nestedness represents a null model that measures the order in presence–absence matrices, i.e. randomness of deviations. The model assumes that there existed a single source of species assemblage, that habitat islands are uniform with no significant clinal ecological gradients and that islands are geographically equally isolated. For instance, species distribution patterns in fragmented habitats are often exhibited to a high degree of nestedness (Atmar & Patterson 1993). Nestedness analysis tests whether assemblages of species (marker bands within populations in the present case) represent nested subsets of a common species pool (gene pool). Species assemblages (populations) not fitting the hypothesis of nestedness, hence, by definition, differing from randomness, are referred to as ‘idiosyncratic’. Therefore, genetic idiosyncrasy points to divergent biogeographic histories of particular populations. The corresponding analysis based on the above binary RAPD matrix was performed using NESTEDNESS TEMPERATURE CALCULATOR (Atmar & Patterson 1993). Significance was tested with 100 Monte Carlo permutations.

A correspondence analysis using Euclidean distances among individual multiband phenotypes and a neighbour joining clustering of pairwise *F*sex values among populations taken from AMOVA (see below) were calculated for the RAPD data set with NTSYS-pc 2.02 (Rohlf 1997). The significance of clusters in the neighbour-joining tree was tested by 100 bootstrap replicates in PAUP 4.0b3a (Swofford 1998) and by a co-phenetic correlation coefficient calculated with NTSYS-pc 2.02 (Rohlf 1997). As an estimate of isolation by distance, a Mantel test was used to investigate the relationship between genetic Euclidean distance and geographic distance among samples with R package 4.0 (Cagström & Legendre 1999) and 999 permutations applied for significance testing.

Conventional AMOVAs were performed on both the cpDNA RFLP and the RAPD data sets. AMOVAs were based on pairwise squared Euclidean distances between multiband phenotypes and were performed using AMOVA PREP (Miller 1998) and AMOVA 1.55 (Excoffier 1993). For hierarchical variance partitioning, populations were grouped according to the two main clusters found in the neighbour-joining tree (see above). Significance tests were based on 999 permutations. To quantify the relative proportions of pollen and seed migration, we calculated measurements of overall genetic differentiation among populations, *F*ST, for both the maternally inherited cpDNA markers and the mainly nuclear, biparentally inherited RAPD markers using corresponding variation components from AMOVA. The relative rates of gene flow through pollen or seed were then determined according to Ennos (1994).
Results

RFLP analysis of cpDNA

RFLP analysis of cpDNA using the Southern hybridization method revealed four different haplotypes in *Saxifraga oppositifolia* from the middle part of the Alps (Fig. 1). Haplotype A was most common, occurring in all except two populations and having an overall frequency of 0.71. Haplotype B, differing from haplotype A in an MB7 × EcoRI site mutation, was less abundant with a frequency of 0.25 but still widespread (Fig. 1). There was no obvious geographical pattern in the distribution of haplotypes A and B. Two rare haplotypes were also found: (i) haplotype D (frequency 0.03), characterized by a MB7 × BclI site mutation from haplotype A, occurred in the Seehorn and Gemsstock populations, and (ii) haplotype R (frequency 0.01) was detected in the Flüelapass population only (Fig. 1). Haplotype R represented a ≈ 80 base-pair (bp) MB3 length mutation of haplotype A and was not found in the circumpolar study on *S. oppositifolia* by Abbott et al. (2000). Two populations sampled from potential major southern refugia (Table 1), Ecrins and Passo Tonale, were monomorphic either for haplotype A or B. Populations monomorphic for haplotype A also occurred in the central Alps. One of the two most diverse populations, each harbouring three different haplotypes, stemmed from a well known high Alpine nunatak area (Seehorn population) in the Valais; the other represented an Alpine population not specifically designated in the biogeographic literature (Flüelapass; Table 1). In the potential northern Alpine nunataks, the Rochers de Nayes, Vanil Noir and Pilatus populations (Table 1), both common haplotypes A and B were found (Fig. 1).

PCR-RFLP analysis of cpDNA using 25 primer pair × restriction enzyme combinations did not generate further haplotypic variation. It did not even distinguish haplotype A from haplotype B. The low level of haplotype diversity resolved in the Southern hybridization analysis was thus fairly robust.

RAPD fingerprinting

The 10 RAPD primers yielded 154 scorable marker bands of which only three were monomorphic in the whole data set. Genetic diversity in Alpine *S. oppositifolia* was high; each of the 143 individuals sampled exhibited a unique RAPD multiband phenotype (*M* = 100%).

No consistent pattern among the investigated populations was found with respect to the different statistical measurements used to detect glacial refugia or nunatak areas. Populations that were characterized by the occurrence of private markers or a high number of rare markers often had low molecular variances or were perfectly nested within the whole sample set. Thus, they did not show specific peculiarities in their RAPD profiles (Table 2). Qualitatively, none of the three populations that had private markers (Seehorn, Passo Cristallina and Piz Tom) was characterized by a particularly high number of rare markers, and only one of them (Passo Cristallina) was defined as idiosyncratic (overall nestedness: *T* = 44.6, *P* ≤ 0.001; Table 2). Quantitatively, molecular variance measurements ranged within narrow limits (14.33–20.30; Table 2). Only the Augstbordpass population harboured both a high number of rare markers and high molecular variance. Nevertheless, genetic diversity in this population was a common subsample of the overall gene pool, i.e. the population was not idiosyncratic (Table 2). In more detail, the four populations

![Fig. 2 Scatter plot showing the first two axes of a correspondence analysis using Euclidean distances based on RAPD profiles between individuals of *Saxifraga oppositifolia* from the Alps (for population abbreviations see Table 1). Western populations are indicated by solid symbols, eastern populations by open symbols.](image-url)
sampled from high Alpine nunatak areas (Saas Fee, Seehorn, Fuorcla Faller and Piz Lagalp; Table 1) did not stand out from the whole data set, although the Seehorn population exhibited two private fragments and relatively high molecular variance (Table 2).

Correspondence analysis revealed a genetic transgression from the western towards the eastern populations along the first axis, while separation along the second axis was low (Fig. 2). The first two axes explained only a small amount of the total variation in the data set (8.68% and 7.50%). Concordantly, the overall Mantel test value of $r_m = 0.137 \ (P \leq 0.001)$ indicated a significant, though low increase of genetic distance with increasing geographic distance among samples. The shift along the first axis of the correspondence analysis could nevertheless not be interpreted in a strictly clinal way, as individuals from the most western populations (Table 1) were situated near to the centre of the scatter plot. In particular, we could not find any clearly separated entities or groups in the correspondence analysis (Fig. 2). Rather, the correspondence analysis gave the picture of a large common nuclear gene pool, only slightly changing from the west to the east.

The neighbour-joining clustering based on pairwise $F_{ST}$ values between populations revealed two major clusters (Fig. 3). One cluster comprised four populations (Seehorn, Ritterpass, Augsporbordpass and Saas Fee) from a well known high Alpine nunatak area in the Valais (Table 1), and the adjacent population of Passo Cristallina in the Ticino (Figs 1, 3). All other populations were placed in a second cluster with eastern, western and northern Alpine populations (Fig. 3). However, there was no bootstrap support above 50% for any of the branchings, and the low co-

Table 2 Statistical measurements of genetic diversity based on RAPD data in populations of Saxifraga oppositifolia from the Alps (for population abbreviations see Table 1), i.e. the number of private markers (only occurring in a single population), the number of rare markers (occurring in up to 10% of all individuals), molecular variance (from AMOVA) and idiosyncratic (i.e. not perfectly nested) populations based on nestedness analysis.

<table>
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<th>Pop.</th>
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<th>Rare markers</th>
<th>Molecular variance</th>
<th>Idiosyncratic population</th>
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</tr>
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<td>16.93</td>
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</table>

* Only three individuals were investigated in population EV, while 10 individuals were analysed in all other populations.

Fig. 3 Neighbour-joining tree based on pairwise $F_{ST}$ values between populations (taken from AMOVA) of RAPD data on Saxifraga oppositifolia from the Alps (for population abbreviations see Table 1; western populations are indicated by solid symbols, eastern populations by open symbols; see Fig. 2). Bootstrap support was < 50% for all bifurcations; co-phenetic correlation coefficient: $r = 0.50$.

Comparison of RAPD and RFLP data

AMOVAS on RAPD and on cpDNA data led to a generally similar partitioning of genetic variance components (Table 3). Very low, and in the case of cpDNA non-significant, genetic variation was detected among groups of populations (4.4% for RAPDs and 4.14% for cpDNA RFLPs; Table 3). This pointed to either no or, at best, weak regional differentiation in the two data sets (groups of populations were defined according to the two main
clusters in the neighbour-joining tree (Fig. 3). This was also
evident from the rather low, similar measurements of
overall genetic differentiation among populations, although
the latter was somewhat higher in cpDNA ($F_{ST} = 0.213$, $P \leq 0.001$) than in the RAPD data ($F_{ST} = 0.152$, $P \leq 0.001$).
Most genetic variation was found within populations
(84.98% in RAPDs and 78.58% in cpDNA RFLPs), while the
genic variation among populations within groups
differed somewhat more between the two marker types
(10.82% in RAPDs and 17.20% in cpDNA RFLPs, Table 3).

The absolute estimate of the relative proportion of pollen
and seed migration, using the above $F_{ST}$ measurements,
equaled 0.49. This indicates that gene flow by the two dis-
persal agents were of similar magnitude (Whitlock &
McCauley 1999).

### Discussion

Despite the application of a whole set of statistical tools
including nestedness analysis, neither the cpDNA RFLP
nor the RAPD data of *Saxifraga oppositifolia* from the middle
part of the Alps gave unequivocal evidence on where
representatives of the species in this region survived the
Pleistocene glaciations, i.e. whether plants re-immigrated
from periglacial refugia or whether populations survived
in situ within the Alps. Moreover, it was not possible to
deduce potential re-colonization routes.

### Evidence from cpDNA data

Two widespread, common cpDNA RFLP haplotypes, A
and B, were found in Alpine populations of *S. oppositifolia*.
Two additional, rare haplotypes also occurred in just three
populations (Fig. 1). In their circumpolar study on *S.
oppositifolia*, Abbott et al. (2000) found only haplotypes
A and B among the 67 individuals surveyed from the
Alps (Fig. 1). The frequencies of these haplotypes given
in Abbott et al. (2000), i.e. A, 0.87 and B, 0.13, were not
significantly different from those recorded in the present
investigation ($\chi^2 = 6.079$, $P = 0.108$). In both studies, haplo-
types A and B were found to occur throughout the Alps
without showing any particular spatial pattern (Fig. 1).
Abbott et al. (2000) did not find any haplotypes other
than A and B in Europe including northwestern Russia,
Svalbard and Iceland.

It was not possible to differentiate further the two
cpDNA haplotypes A and B using PCR-RFLPs. Since these
two haplotypes are common throughout Europe, they can-
not be used to deduce the existence or locations of potential
glacial refugia in the Alps or neighbouring regions. Both
haplotypes could have survived on northern Alpine,
almost periglacial nunataks and subsequently re-colonized
the Central Alps. Alternatively, they could have resisted
the ice ages on high Alpine nunataks in the central chains
and expanded their distribution after the retreat of the gla-
ciers. Equally, they might have re-immigrated after glacia-
tion from major southern refugia. Even the lowlands north
of the Alps could have served as refugia, because late gla-
cial or early postglacial fossils of *S. oppositifolia* have been
found there (Burga & Perret 1998).

Do the two rare cpDNA haplotypes D and R from the
potential nunatak Seehorn population and from the two
Gemsstock and Flüelapass populations allow more specific
conclusions? Haplotype D, together with A and B, belongs
to a Eurasian clade (Abbott et al. 2000). Prior to this
study, haplotype D had only been recorded in the Siberian
Taymyr region. Disregarding potential homoplasy, the
occurrence of this haplotype in the Alps could point to a
glacial refugium there. However, the question remains
why this haplotype did not disperse further after glacia-
tion and is nowadays so rare and geographically restricted
in the Alps. The conclusions of Gabrielsen et al. (1997) for
nor-dic *S. oppositifolia*, that the genetic traces of genotypes
potentially having survived in situ were erased by massive
immigration of periglacial genotypes after glaciation,
appear to also fit the patterns of the present cpDNA
haplotype distribution in the Alps. Haplotype R was only
found once in the Alps, and it is not known from any
other population (Abbott et al. 2000). Assuming an average
mutation rate of cpDNA (Harrl & Clark 1997), haplotype
R might simply represent a local, perhaps even postglu-
cial, length mutation of haplotype A, thus, not being

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Table 3 Amova of RAPD and cpDNA RFLP data on *Saxifraga oppositifolia* from the Alps

<table>
<thead>
<tr>
<th>Marker</th>
<th>Source of variation†</th>
<th>d.f.</th>
<th>SS</th>
<th>Percentage of variance component</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAPDs</td>
<td>among groups</td>
<td>1</td>
<td>103.96</td>
<td>4.40***</td>
</tr>
<tr>
<td></td>
<td>among populations within groups</td>
<td>13</td>
<td>524.62</td>
<td>10.82***</td>
</tr>
<tr>
<td>cpDNA RFLP</td>
<td>among groups</td>
<td>1</td>
<td>1.82</td>
<td>4.14***</td>
</tr>
<tr>
<td></td>
<td>among populations within groups</td>
<td>13</td>
<td>12.38</td>
<td>17.20***</td>
</tr>
<tr>
<td></td>
<td>within populations</td>
<td>65</td>
<td>28.57</td>
<td>78.58***</td>
</tr>
</tbody>
</table>

† For grouping of populations see Figs 1 and 3.
patterns, which is in line with the interpretation of a
differences, we interpret the low genetic variation among
morphic markers per primer. Despite these methodical
markers per primer, whereas we recorded 15.1 poly-
populations of
et al
individual investigated in the present study. Gabrielsen
a unique multiband genotype was assigned to every
variation in
Gugerli
which is almost identical to the corresponding 85% found
area comparable in size with that of the present study,
geographically restricted Alpine data set on
in our Alpine data set (Table 3). Gugerli
found high amounts of RAPD variation among populations in a
geographically restricted Alpine data set on S. oppositifolia, which they attributed to recurring seed migration at the landscape level and the mainly outcrossing breeding sys-
tem of the species (Gugerli 1997).

Evidence from RAPD markers
In accordance with the studies of Gabrielsen et al. (1997) and Gugerli et al. (1999), we found high amounts of RAPD variation in S. oppositifolia. By using 10 RAPD primers, a unique multiband genotype was assigned to every individual investigated in the present study. Gabrielsen et al. (1997) detected 86% of the genetic variation within populations of S. oppositifolia from southern Norway (an area comparable in size with that of the present study), which is almost identical to the corresponding 85% found in our Alpine data set (Table 3). Gugerli et al. (1999) observed 95% RAPD variation among populations in a geographically restricted Alpine data set on S. oppositifolia. It is possible that the differences within the three studies were partly caused by the well-known laboratory dependence of RAPD markers (Nybom & Bartish 2000). While Gabrielsen et al. (1997) only considered a mean of 2.9 polymorphic markers per primer, a rather conserva-
tive approach, Gugerli et al. (1999) used 21 polymorphic
markers per primer, whereas we recorded 15.1 poly-
morphic markers per primer. Despite these methodical
differences, we interpret the low genetic variation among
regions of 4.4% (Table 3) as a lack of distinct geographical patterns, which is in line with the interpretation of a corresponding value of 8.58% by Gabrielsen et al. (1997).

RAPD studies on S. oppositifolia all failed to find distinct geographical patterns. While Gabrielsen et al. (1997) found weak upcma clustering of individuals into geographical regions, our study showed that individuals could be assigned to a continuous Alpine gene pool because of (i) a rather low among-population component of genetic vari-
ation as compared with other species (Nybom & Bartish 2000), (ii) nestedness of most populations, (iii) an only slight transgression of genotypes from the west to the east as shown in the correspondence analysis (Fig. 2) and (iv) a low, though significant, Mantel correlation between geo-
graphical and genetic distances pointing to only weak iso-
lolation by distance.

Nevertheless, a relatively distinct cluster of five populations, four from the central Alpine Valais and one from an adjacent high Alpine area, was detected in our survey (Fig. 3). Several regions of the Penninic Valais represent
populations from the central Alpine Valais exhibited some peculiarities, which might be interpreted as weak evidence for in situ glacial survival of the species within the Alps.

Conclusions
In the present study, both cpDNA RFLP and RAPD data on Alpine S. oppositifolia showed low variation among populations and regional groups of populations, but high within-population variance components (Table 3). Both data sets provided, at best, weak evidence for in situ glacial survival of S. oppositifolia within the Alps. The strongest point in favour of survival on high Alpine nunatak was the occurrence of two rare cpDNA haplotypes, one of them hitherto only known from Siberia (Abbott et al. 2000). The present study thus failed to re-construct the Alpine Pleistocene history of this common and widespread Arctic-Alpine plant species; an which is important result with regard to the growing body of phylogeographic studies on mountain plants (Gugerli & Holderegger 2001).

In general, the phylogeographic signal detected in haplo-
typic cytoplasmic DNA in plants, as revealed by RFLPs or sequence analysis, has been found to be much stronger than that detected in nuclear DNA using various tech-
niques such as isozymes, RAPDs, amplified fragment

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length polymorphisms (AFLPs) or inter-simple sequence repeats (ISSRs) for nuclear sequences see Hare (2001). This is because the former does not recombine and is usually uniparentally inherited, while the latter is recombinant and distributed both by seed and pollen (Schaal et al. 1998; Newton et al. 1999). Since gene flow by pollen is usually much higher than by seed (Ennos 1994), historical geographical patterns tend to be erased more rapidly for nuclear markers than for cytological markers.

In contrast to the present investigation, some recent studies on Alpine plants using both cpDNA analysis and nuclear DNA markers such as isozymes or AFLPs have succeeded in proving the long-standing hypothesis of high Alpine nunatak species (e.g. Draba rupicola: R. Füchtet & A. Widmer, ETH Zurich, unpublished data; Eriachne ramosum: Stehlik et al. 2001a; Stehlik et al. unpublished data; also see Stehlik et al. 2001b). It is feasible that such proof is more difficult to obtain for a widespread, common and non-specialist Alpine species such as Saxifraga oppositifolia.

In conclusion, we could neither prove nor falsify the hypothesis of in situ glacial survival of Saxifraga oppositifolia on Alpine nunataks. It is possible that what happened in the Alps was similar to what may have occurred in northern Europe (Gabrielsen et al. 1997), i.e. resident genotypes surviving glaciation in situ were swamped by massive immigration of periglacial genotypes after glaciation.

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