



## Same parental species, but different taxa: molecular evidence for hybrid origins of the rare endemics *Saxifraga opdalensis* and *S. svalbardensis* (Saxifragaceae)

SNORRE W. STEEN<sup>1</sup>, LUDOVIC GIELLY<sup>2</sup>, PIERRE TABERLET<sup>2</sup> AND CHRISTIAN BROCHMANN<sup>1\*</sup>

<sup>1</sup> Botanical Garden and Museum, University of Oslo, Trondheimsveien 23 B, N-0562 Oslo, Norway and <sup>2</sup> Laboratoire de Biologie des Populations d'Altitude, CNRS UMR 5553, Université Joseph Fourier, BP 53, F-38041 Grenoble Cedex 9, France

Received May 1999; accepted for publication August 1999

*Saxifraga opdalensis* was described from Oppdal in southern Norway and hypothesized to have originated as the hybrid *S. cernua* × *rivularis* or to have been derived from a *S. cernua*-like progenitor. We tested these alternative hypotheses using uni- and biparentally inherited molecular markers observed in *S. opdalensis* and its putative parental species at the type locality: PCR-RFLPs (restriction fragment length polymorphisms in amplified fragments of chloroplast DNA; cpDNA), sequences of the cpDNA intron *trnL* and the spacer *trnL-trnF*, and RAPDs (random amplified polymorphic DNAs). The data provided unambiguous support for the hybrid hypothesis. The cpDNA analyses distinguished two well-differentiated chloroplast genomes, one in *S. opdalensis* and *S. rivularis*, and another in *S. cernua*. The majority of the RAPD markers showed distinct additivity in *S. opdalensis* relative to its postulated parental species. Thus, *S. opdalensis* has probably originated from a hybrid with *S. rivularis* as the maternal parent and *S. cernua* as the paternal parent. We also included *S. svalbardensis* in the present study because previous molecular analyses of Svalbard material have shown that this species had probably also originated as a hybrid between *S. cernua* and *S. rivularis*. The chloroplast genome of *S. svalbardensis* was identical to that of *S. opdalensis*, but the two species differed in many RAPD markers. Although these two endemics probably have been derived from the same hybrid combination, they are morphologically and genetically distinct and should be referred to separate species. Differences between such independently originated hybrid taxa may result from intraspecific variation in their parental taxa. *Saxifraga cernua* comprises, for example, several highly divergent evolutionary lineages.

© 2000 The Linnean Society of London

ADDITIONAL KEY WORDS:—cpDNA – PCR-RFLP – RAPD – Scandinavia – Svalbard – universal primers.

### CONTENTS

Introduction . . . . . 154

\* Corresponding author. E-mail: christian.brochmann@toyen.uio.no

Material and methods . . . . .	156
Plant material and DNA isolation . . . . .	156
DNA sequencing . . . . .	156
PCR-RFLP analysis . . . . .	157
RAPD analysis . . . . .	157
Results . . . . .	157
Sequences and PCR-RFLPs of cpDNA . . . . .	157
RAPD analysis . . . . .	158
Discussion . . . . .	160
Origin of <i>Saxifraga opdalensis</i> . . . . .	160
Same parental species, but different taxa: <i>Saxifraga opdalensis</i> and <i>S. svalbardensis</i> . . . . .	162
Acknowledgements . . . . .	163
References . . . . .	163

## INTRODUCTION

In 1888, some tiny, peculiar-looking plants of *Saxifraga* were discovered in open pioneer vegetation at high altitudes on the mountain Leirtjørnkollen in Oppdal, southern Norway (Blytt, 1892). The plants were characterized by white terminal flowers on stems carrying yellowish-green bulbils in the leaf axils. They were most similar to the locally abundant species *S. cernua* L., but this species is more robust and has dark red bulbils and longer petals. Other characters, such as the degree of fusion between the receptacle and the ovary, suggested a possible relationship to the small-grown and small-flowered *S. rivularis* L., which also occurred on the same mountain. The aberrant plants on Leirtjørnkollen were described as *S. opdalensis* A. Blytt, and were suggested to have originated as the hybrid *S. cernua* × *rivularis* (Blytt, 1892).

*Saxifraga opdalensis* was later searched for in vain and almost forgotten, until it was rediscovered on Leirtjørnkollen by Holaker, Nordhagen & Berg (1960). They concluded that it represents a distinct taxon that probably reproduces entirely asexually via bulbils, and that Blytt's (1892) hybrid hypothesis was probably well founded. Later, based on detailed morphometric and pollen stainability analyses of several populations of the species, Flugsrud (1985) concluded that the hybrid hypothesis was most likely. However, the possibility that *S. opdalensis* had originated directly from *S. cernua*-like progenitors without hybridization, could not be disregarded (Flugsrud, 1985; cf. also Øvstedal, 1998).

Seed set has never been observed in *S. opdalensis*, although it flowers luxuriantly in the field as well as in cultivation (Holaker *et al.*, 1960; Flugsrud, 1985). It is polyploid with the chromosome number  $2n = 48-50$  (Engelskjøn, 1979). Although *S. opdalensis* was placed between its proposed parental species in multivariate analyses of the morphometric data, it appeared very close to *S. cernua*. However, the low pollen stainability of *S. opdalensis* (0–3%) was taken as support for the hybrid hypothesis (Flugsrud, 1985). It is noteworthy that *S. cernua* itself shows considerable variation in pollen stainability; some clones of this species may be virtually sterile (Flugsrud, 1985; C. Brochmann, unpublished data). This variation in fertility is probably associated with the large variation in chromosome numbers in *S. cernua*, including many aneuploid ones ( $2n = 24-72$ ; Löve & Löve, 1975; Webb & Gornall, 1989). The chromosome number of this circumpolar species also varies within Norway ( $2n \approx 33, 44-46, 55-57$ ; Engelskjøn, 1979), and thus, neither chromosome

numbers nor pollen stainability data provide direct information which can be used to discriminate between the two hypotheses on the origin of *S. opdalensis*. In contrast to *S. cernua*, the circumpolar species *S. rivularis* is fully sexual and predominantly autogamous (Molau, 1993), with pollen stainabilities regularly above 90% (C. Brochmann, unpublished data) and a stable polyploid chromosome number ( $2n=52$ ; Löve & Löve, 1975; Engelskjøn, 1979; Borgen & Elven, 1983).

*Saxifraga opdalensis* appears to be morphologically uniform in the Leirtjørnkollen area, where it is well-established at several sites along a 15 km long mountain ridge (Leirtjørnkollen–Sissihø; Holaker *et al.*, 1960; Flugsrud, 1985). It is therefore possible that the species is composed of a single clone that has originated once locally after the last glaciation. Some morphologically similar populations have later been reported from northern Scandinavia, and it is possible that these populations had originated independently of the south Norwegian one (Holaker *et al.*, 1960; Rune, 1988; Øvstedal, 1998).

We have recently reported molecular analyses of a similar taxon, *S. svalbardensis* D. O. Øvstedal, which was described from the arctic archipelago of Svalbard and probably is endemic to that area (Brochmann *et al.*, 1998). *Saxifraga svalbardensis* differs from *S. opdalensis* in chromosome number ( $2n \approx 64$ ; Borgen & Elven, 1983) and in several morphological characters, e.g. presence of subterranean runners, reddish petals, flower shape, and red bulbils. In that study, evidence from nucleotide sequences of the chloroplast DNA (cpDNA) gene *matK* and random amplified polymorphic DNAs (RAPDs) suggested that *S. svalbardensis* has originated once from a hybrid with *S. cernua* as the paternal parent and *S. rivularis* as the maternal parent, whereas sequences of the internal transcribed spacers (ITS) of the nuclear ribosomal DNA (rDNA) could not be used to discriminate between various hypotheses on the origin of the species. The populations of *S. svalbardensis* analysed were virtually identical for all molecular markers, whereas *S. cernua* showed remarkably high levels of variation in Svalbard (see also Gabrielsen & Brochmann, 1998). The widespread and variable *S. cernua* is probably composed of several, divergent lineages of separate polyploid origins, and it is possible that participation of two such divergent lineages in independent hybridizations with *S. rivularis* may have produced *S. svalbardensis* and *S. opdalensis*, respectively (Brochmann *et al.*, 1998).

In the present study, we used various types of molecular data to test two previously proposed hypotheses on the origin of *S. opdalensis* at the type locality at Leirtjørnkollen (origin directly from *S. cernua*-like progenitors *vs* origin from *S. cernua* × *rivularis*; cf. Flugsrud, 1985). One reference sample of the virtually invariable *S. svalbardensis* was reanalysed here to allow for direct comparison with *S. opdalensis* and the local populations of its proposed parental species. Because only a few variable sites were observed among the sequences of the chloroplast gene *matK* in the *S. svalbardensis* study, we sequenced two potentially more variable cpDNA regions in the present study (the intron *trnL* and the spacer *trnL-trnF*; cf. Taberlet *et al.*, 1991). In addition, we wanted to examine the usefulness of PCR-RFLP analysis (restriction fragment length polymorphisms in PCR-amplified fragments) of cpDNA using 'universal' primers (Taberlet *et al.*, 1991; Demesure, Sodzi & Petit, 1995; Dumolin-Lapegue, Pemonge & Petit, 1997) to address questions at the population and species complex levels in arctic-alpine plants. In addition to these cpDNA analyses, which provide maternally inherited markers in the Saxifragaceae (Soltis, Soltis & Ness, 1990), we also used RAPD analysis to provide mainly biparentally inherited markers.

## MATERIAL AND METHODS

*Plant material and DNA isolation*

*Saxifraga opdalensis* was collected at the type locality (cf. Blytt, 1892 and Holaker *et al.*, 1960) at the mountain Leirtjørnkollen in Oppdal, Sør-Trøndelag, southern Norway (population CB 94-383; S slope, alt. 1520–1590 m, UTM NQ 385238, 27. vii. 1994). One population of each of the putative parental species was collected as close as possible to this site. *Saxifraga cernua* (pop. CB 94-381) occurred intermingled with *S. opdalensis*, and *S. rivularis* was found close by (pop. CB 94-382; S slope, alt. 1500 m, UTM NQ 387236). Only flowering plants were collected to ensure correct identification. Leaves from 5–8 plants from each population were dried in fine-grained silica gel, and some additional plants were taken as herbarium vouchers (deposited in O) or maintained in cultivation at the phytotron at the University of Oslo. For comparison, we also included one sample of previously analysed material of *S. svalbardensis* from Svalbard (pop. CB 92-20; Brochmann *et al.*, 1998), which was considered sufficient for this purpose, since the species was virtually invariable in all previous analyses (Brochmann *et al.*, 1998). Total DNA was isolated from 2–10 mg of dried leaf tissue from field-collected or cultivated plants using the Qiagen DNeasy Plant Mini Kit (Qiagen Inc.; following the recommendations of the producer).

*DNA sequencing*

Double stranded DNA amplification was performed in a volume of 25 µL containing 2.5 mM MgCl<sub>2</sub>, 200 µM of each dNTP, 1 µM of each primer and 1 U AmpliTaq Gold polymerase (Perkin Elmer), 1 × buffer, and 3 µL DNA template. The *tmL* intron was amplified with primers 'c' (5'-CGA AAT CGG TAG ACG CTA CG-3') and 'd' (5'-GGG GAT AGA GGG ACT TGA AC-3'); and the *tmL-tmF* intergenic spacer was amplified with primers 'e' (5'-GGT TCA AGT CCC TCT ATC CC-3') and 'f' (5'-ATT TGA ACT GGT GAC ACG AG-3') (Taberlet *et al.*, 1991). PCR reactions were performed in a PE 2400 thermocycler (Perkin Elmer) programmed for an activation step for the enzyme of 10 min at 95°C (Perkin Elmer specification), followed by 35 cycles, each consisting of 30 s at 95°C, 30 s at 50°C, and 1 min at 72°C. To remove excess primers and dNTPs after amplification, PCR products were purified on QiaQuick PCR columns (Qiagen). Sequencing of both strands was performed using the ABI PRISM Dye terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer) in a volume of 20 µL containing 20 ng of purified DNA and 3.2 pmol of primer. Sequencing reactions underwent 25 cycles of 30 s at 96°C, 30 s at 50°C, and 4 min at 60°C on a PE 2400 thermocycler (Perkin Elmer). Excess dye terminators were removed by a spin-column purification, and sequencing reactions were electrophoresed for 6 h on a ABI PRISM 377 DNA sequencer (Perkin Elmer) in a 5% Long Ranger Gel (FMC). Multiple alignments of the sequences were obtained using the Clustal program (Higgins, Fuchs & Blesby, 1992) implemented in the Sequence Navigator 1.0.1 software (Perkin Elmer).

As a first test, the intron *tmL* and the spacer *tmL-tmF* of the cpDNA were sequenced for one plant of *S. opdalensis* and one of each of its putative parental species. The spacer *tmL-tmF* was most variable, and this region was therefore sequenced for seven additional plants, including *S. svalbardensis* (Table 1).

*PCR-RFLP analysis*

The 19 universal cpDNA primer-pairs of Taberlet *et al.* (1991), Demesure *et al.* (1995), and Dumolin-Lapegue *et al.* (1997) were tested for one plant of each of the four species. The primers for the regions *tmH-trnK*<sup>1</sup> (Demesure *et al.*, 1995), *tmD-trnT* (Demesure *et al.*, 1995), *tmK*<sup>2</sup>-*trnQ* (Dumolin-Lapegue *et al.*, 1997), and *tmT-psbC* (Dumolin-Lapegue *et al.*, 1997) gave the best PCR products for all species and were chosen for further analyses. PCR reactions were performed in volumes of 25 µL containing 2.5 mM MgCl<sub>2</sub>, 200 µM of each dNTP, 1 U AB-Taq polymerase (Advanced Biotechnologies), 1 × buffer, 1 µM of each primer, and 5 µL DNA template. PCR reactions were performed in a Techne Genius thermocycler programmed for 10 min initial denaturation at 95°C, followed by 35 cycles, each consisting of 30 s at 95°C, 30 s at 50°C, and 5 min at 72°C.

Digestions were performed in volumes of 25 µL containing 10 µL PCR product, 5 U of each of two restriction enzymes (Biolabs Inc.), and 1 × buffer. Each PCR product was cut by two sets of restriction enzymes (*Hae*III/*Hinf*I and *Dde*I/*Mbo*I). After mixing at 0°C, samples were incubated for at least 2 h at 37°C. Restriction digests were first run on 1.4% agarose gels to check for digestion quality and later on 3% agarose gels for increased resolution. Agarose gels were stained with ethidium bromide and visualized in UV-light.

*RAPD analysis*

Template DNA concentrations were estimated by visual inspection of staining intensities relative to a molecular weight marker and found to be approximately 0.1 ng/µL. PCR reactions were performed in volumes of 25 µL containing 1 × PCR buffer (HT Biotechnology), 100 µM of each dNTP, 0.24 µM of a decamer primer (Operon Technologies), 0.75 U Super Taq polymerase (HT Biotechnology), and 0.5 ng genomic DNA (cf. Gabrielsen *et al.*, 1997). Amplifications were performed in a MJ Research PTC 100 thermocycler programmed for 3 min initial denaturation at 94°C followed by 35 cycles, each consisting of 15 s at 94°C, 30 s at 40°C, and 60 s at 72°C, ending with a final step of 5 min at 72°C. Three plants from each population of the three species from *locus classicus* of *S. opdalensis*, and one plant of *S. svalbardensis* were run for 15 primers (Table 2), that previously had shown to be useful in RAPD analyses of *S. svalbardensis* (Brochmann *et al.*, 1998) and *S. osloensis* (Brochmann, Nilsson & Gabrielsen, 1996). PCR products were separated on 1.4% agarose gels stained with ethidium bromide and visualized in UV-light. The gels were conservatively interpreted (i.e. only very distinct markers were scored) and samples were isolated and run twice to check for reproducibility. Polymorphic markers were scored as present (1) or absent (0). Based on Dice similarity, the RAPD data were subjected to principal coordinate (PCO) analysis, minimum spanning tree (MST) analysis, and UPGMA analysing using NTSYS-pc (Rohlf, 1990).

## RESULTS

*Sequences and PCR-RFLPs of cpDNA*

In the spacer *tmL-trnF*, *Saxifraga opdalensis* was identical to *S. rivularis* as well as to *S. svalbardensis* at all of the 414 base positions (bp) sequenced (Table 1). *Saxifraga*

*cernua* differed from the other species at 8 bp in the spacer (1.9%), including two single-base substitutions, and one single-base insertion and one five-base deletion relative to the sequences of the other species. *Saxifraga opdalensis* and *S. rivularis* were also identical at all of the 566 bp sequenced of the intron *tmL*, whereas the intron sequence of *S. cernua* differed from those of the other species by two single-base substitutions (0.4%). No cpDNA sequence variation was observed within species. The complete sequences are deposited in the EMBL database (see Table 1 for accession numbers).

*Saxifraga opdalensis*, *S. rivularis*, and *S. svalbardensis* were also identical for the PCR-RFLP banding patterns obtained for the four selected cpDNA regions. *Saxifraga cernua* differed from the other species in the PCR-RFLP patterns obtained for two of these regions, including both of the double digestions of *tmH-tmK*<sup>1</sup> and *tmD-tmT*, whereas the double digestions of *tmT-psbC* and *tmK*<sup>2</sup>-*tmQ* showed no variation among the four species. The double digestion of *tmH-tmK*<sup>1</sup> with the restriction enzymes *Hae*III/*Hinf*I yielded four distinct bands in all species, but one band was slightly longer (~420 bp) in *S. cernua* compared to the other species (~410 bp). This indel was also detected with the double digestion with the restriction enzymes *Dde*I/*Mbo*I of the same region, which gave a similar profile with four bands, and where one band differed between *S. cernua* (~500 bp) and the other species (~490 bp). The double digestion of *tmD-tmT* with *Hae*III/*Hinf*I resulted in a partly blurred profile, but one distinct band differed between *S. cernua* (~390 bp) and the other species (~380 bp). This indel was also detected in the double digestion of *tmD-tmT* with *Dde*I/*Mbo*I, which produced five bands, one of which was longer in *S. cernua* (~300 bp) than in the other species (~290 bp).

Thus, the sequence analysis and the PCR-RFLP analysis revealed two rather divergent cpDNA haplotypes in the material, one in *S. cernua*, and another shared by *S. opdalensis*, *S. rivularis*, and *S. svalbardensis*. The differences observed between these two haplotypes included single-base substitutions and insertions/deletions at a total of 10 bp in the sequences of the spacer *tmL-tmF* and the intron *tmL*, including one five-base deletion in *S. cernua* relative to the other species, and two restriction fragment length differences in the regions *tmH-tmK*<sup>1</sup> and *tmD-tmT*.

#### RAPD analysis

The result of the RAPD analysis was immediately obvious from visual inspection of the gels, as well as from visual inspection of the matrix containing the 37 polymorphic markers that were scored (Table 2). No RAPD variation was observed within the species. Additivity in *S. opdalensis* relative to its postulated parental species was observed in 27 (73%) of the RAPD markers. Eleven markers (30%) were present in both *S. opdalensis* and *S. cernua*, but absent in *S. rivularis*, whereas 16 markers (43%) were present in both *S. opdalensis* and *S. rivularis*, but absent in *S. cernua*. One marker was only observed in *S. opdalensis*, and eight markers were only observed in *S. cernua*. *Saxifraga svalbardensis* showed almost no additivity relative to these south-Norwegian populations of *S. rivularis* and *S. cernua* (but see Brochmann *et al.*, 1998 for comparison with Svalbard populations). In the present analysis, *S. svalbardensis* shared 15 RAPD markers with *S. rivularis*, only a single marker with *S. cernua*, and only a single marker with *S. opdalensis*.

TABLE 1. Fragments of nucleotide sequences of the intron *tml*L and the spacer *tml*L-*tm*F of cpDNA showing sites that varied among the species of *Saxifraga*. The complete sequences are available from the EMBL database (cf. accession numbers below). Some invariable sites on both sides of the variable ones are shown to visualize the alignment of the sequences. The range in base positions indicates the placement of each fragment within the entire sequence. Intron and spacer sequences were first obtained for three plants; more plants were subsequently sequenced for the spacer because it revealed more variation

Species	Population	Plant no.	Intron <i>tml</i> L			Spacer <i>tml</i> L- <i>tm</i> F				
			EMBL no.	117-125	315-323	EMBL no.	92-100	223-232	260-268	270-279
<i>S. cernua</i>	CB 94-381	2				AJ238768	GTCGTTATG	TCCAG-----	CTTTACATA	AAAAAATGCA
<i>S. cernua</i>	CB 94-381	4					.....	.....	.....	.....
<i>S. cernua</i>	CB 94-381	6	AJ238765	AACATACAA	AAATCAAAT		.....	.....	.....	.....
<i>S. cernua</i>	CB 94-381	7					.....	.....	.....	.....
<i>S. opdalensis</i>	CB 94-383	2				AJ238769	.....G.....	.....TCCAG	.....C.....	.....-
<i>S. opdalensis</i>	CB 94-383	5	AJ238766	.....C.....	.....A.....		.....G.....	.....TCCAG	.....C.....	.....-
<i>S. rivularis</i>	CB 94-382	1	AJ238767	.....C.....	.....A.....	AJ238770	.....G.....	.....TCCAG	.....C.....	.....-
<i>S. rivularis</i>	CB 94-382	3					.....G.....	.....TCCAG	.....C.....	.....-
<i>S. rivularis</i>	CB 94-382	5					.....G.....	.....TCCAG	.....C.....	.....-
<i>S. svalbardensis</i>	CB 92-20	7				AJ238771	.....G.....	.....TCCAG	.....C.....	.....-

In the PCO analysis of the RAPD data (Fig. 1), the first axis extracted 75.4% of the variation and separated *S. cernua* from *S. rivularis*, whereas *S. opdalensis* was placed in an intermediate position. The second PCO axis (22.4%) separated *S. opdalensis* from its putative parental species. *Saxifraga svalbardensis* was placed closest to *S. rivularis* along both axes. *Saxifraga opdalensis* was connected to *S. cernua* as well as to *S. rivularis* (via *S. svalbardensis*) by the minimum spanning tree (Fig. 1). In the UPGMA analysis of the RAPD data (not shown), *S. opdalensis* clustered with *S. rivularis* and *S. svalbardensis* at the 0.7 level, and this cluster was connected to *S. cernua* at the 0.2 level.

## DISCUSSION

### *Origin of Saxifraga opdalensis*

The molecular data provide unambiguous support for the hypothesis that the population of *S. opdalensis* at its type locality has originated as a hybrid between *S. cernua* and *S. rivularis*. The alternative hypothesis that *S. opdalensis* has originated directly from *S. cernua*-like ancestors, which was forwarded because *S. opdalensis* is morphologically most similar to *S. cernua* (Flugsrud, 1985), can be disregarded with reasonable certainty because of the taxonomic distribution of the two well-differentiated chloroplast genomes observed in the material. Based on a considerable number of markers from various parts of the chloroplast genome, obtained by sequencing of two regions and PCR-RFLP analysis of two regions, it appears that *S. opdalensis* has inherited its chloroplast directly from *S. rivularis*. *Saxifraga cernua* possesses the other chloroplast type (Table 1). Chloroplasts are usually inherited from the maternal parent in angiosperms, including the Saxifragaceae (Soltis *et al.*, 1990), and *S. rivularis* is therefore probably the maternal parent of *S. opdalensis*. The contribution from *S. cernua* to *S. opdalensis*, as well as from *S. rivularis* to *S. opdalensis*, is evident from the distinct additivity observed for the majority of the RAPD markers (Table 2, Fig. 1). RAPD markers are mainly dominantly inherited, and many of them usually represent loci from the biparentally inherited, nuclear genome (Williams *et al.*, 1993). In summary, the RAPD markers obtained clarify that both *S. cernua* and *S. rivularis* have contributed to the genome of *S. opdalensis*, and the uniparentally inherited chloroplast markers identify *S. rivularis* as the chloroplast donor, thus probably the maternal parent, of *S. opdalensis*. *Saxifraga cernua* has therefore most probably acted as the paternal parent of the original hybrid *S. cernua* × *S. rivularis*, which gave rise to *S. opdalensis*.

The present analyses did not give a definite answer to the question of whether *S. opdalensis* has originated locally from still existing populations of both of its parental species at Leirtjønkollen. The RAPD data are consistent with direct contribution to *S. opdalensis* from the present, local population of *S. rivularis*, since all of the markers observed in *S. rivularis* were also present in *S. opdalensis*. However, although the population analysed of *S. cernua* also had a considerable number (11) of RAPD markers that were uniquely shared with *S. opdalensis*, this population of *S. cernua* also contained eight RAPD markers absent in *S. opdalensis* (Table 2). *Saxifraga cernua* has, however, been shown to comprise several highly divergent clones at small spatial scales in Svalbard (e.g. within 3 × 3 m; Gabrielsen & Brochmann, 1998), and it is possible that *S. cernua* also varies considerably within the Leirtjønkollen area. The



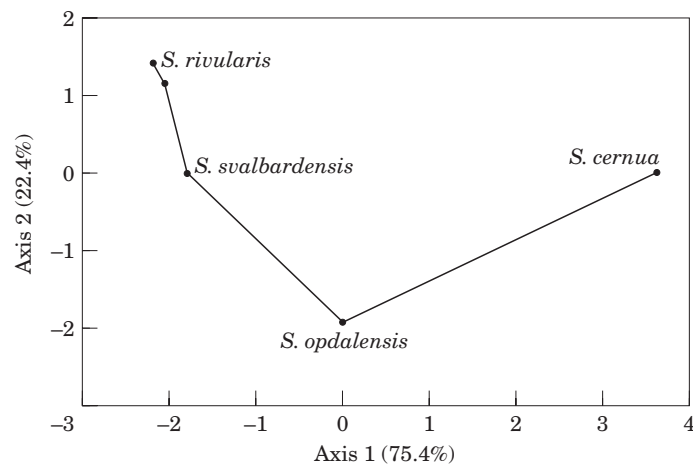


Figure 1. Principal coordinate (PCO) analysis with minimum spanning tree (MST) superimposed of RAPD phenotypes observed in *Saxifraga opdalensis* and its putative parental species from *locus classicus* (Leirtjønkollen in Oppdal, southern Norway; cf. Table 2). *Saxifraga svalbardensis* from Svalbard, which probably has originated independently from other populations of the same parental species, was included for comparison in the analysis (for full treatment of that species, see Brochmann *et al.*, 1998).

limited material analysed in the present study may thus have been insufficient to identify the particular genotype of *S. cernua*, which contributed to the formation of *S. opdalensis*. The high level of distinct additivity observed in the material analysed nevertheless suggests that *S. opdalensis* has a recent, probably postglacial origin, in southern Norway.

*Same parental species, but different taxa: Saxifraga opdalensis and S. svalbardensis*

It is intriguing that two different Nordic endemics in *Saxifraga* probably have been independently derived from hybridization between the same two parental taxa, *S. cernua* and *S. rivularis* (this study and Brochmann *et al.*, 1998). *Saxifraga opdalensis* and *S. svalbardensis* are so conspicuously different in morphology that it is unreasonable to refer them to the same taxonomic species (compare the description in Øvstedal, 1975, 1998; Flugsrud, 1985; Elven, 1994), and they also differ in chromosome number (Engelskjøn, 1979; Borgen & Elven, 1983) and in several RAPD markers (this study).

Repeated hybridization between two species and subsequent stabilization of the hybrids (in this case by asexual reproduction) may result in populations that may or may not be referable to a single taxonomic species. Taxonomically significant morphological differences between independently originated hybrid products of the same parentage may, for example, be caused by morphological differences between the particular parental populations involved in each hybridization event. This is probably the situation in the *Saxifraga* case reported here. The presence of runners in *S. svalbardensis* and the absence of runners in *S. opdalensis* may, for example, reflect intraspecific variation for this character in their maternal parent, *S. rivularis*. South Norwegian populations of this species often lack runners, whereas Svalbard populations usually have runners (pers. observ.; see also Elven, 1994). Most important is

probably the remarkable degree of intraspecific variation previously demonstrated within *S. cernua*. This species is variable in morphology, chromosome number, and molecular markers (RAPDs and ITS sequences; cf. Brochmann *et al.*, 1998, and Gabrielsen & Brochmann, 1998), and the most plausible explanation for the differences between *S. opdalensis* and *S. svalbardensis* is that two highly divergent lineages of *S. cernua* were involved in each hybridization event. This explanation is supported by the observation of a higher number of shared RAPD markers between *S. svalbardensis* and Svalbard populations of *S. cernua* (Brochmann *et al.*, 1998) than between *S. svalbardensis* and the south Norwegian population of *S. cernua* (this study). However, no direct comparison can be made between the data obtained in these two studies because RAPD data may have too low a degree of repeatability across studies. Further studies of the intraspecific variation in *S. cernua* on a broader geographic scale, combined with comparative studies of *S. svalbardensis*, south Norwegian *S. opdalensis*, and the putative north Scandinavian populations of the latter species are necessary to resolve the number of independent hybrid origins and variation among the hybrid products in this complex.

Multiple origins of hybridogenous taxa have most often been postulated in cases where the independently originated populations are referred to a single taxonomic species, usually a polyploid one (e.g. Werth, Guttman & Eshbaugh, 1985; Soltis & Soltis, 1989; Brochmann, Soltis & Soltis, 1992a, b; reviewed by Soltis & Soltis, 1993), but in one case also a diploid species has been reported to have originated recurrently by hybrid speciation (Brochmann, Borgen & Stabbetorp, 2000). In the presently reported case in *Saxifraga*, however, multiple hybridizations between the same parental species have produced two distinct species. A similar situation has been reported in the genus *Helianthus*, in which three different species (*H. paradoxus* Heiser, *H. anomalus* Blake, and *H. deserticola* Heiser) probably all have originated by hybridization between the same two parental taxa (*H. annuus* L. and *H. petiolaris* ssp. *fallax* Heiser; Rieseberg, 1991).

#### ACKNOWLEDGEMENTS

We thank Tove M. Gabrielsen and Mari Mette Tollefsrud for assistance, but most of all for a lot of fun on the field trip, Aslaug Hagen for isolating some of the DNAs, and Hanne Hegre Grundt, Richard Gornall, and an anonymous reviewer for commenting on the manuscript. We also thank Rolf Y. Berg, Ketil Flugsrud, and Dag Olav Øvstedal for previous discussions on this subject. DNA sequencing and initial PCR-RFLP analysis were carried out at the Laboratoire de Biologie des Populations d'Altitude at the Université Joseph Fourier, Grenoble, and final PCR-RFLP analysis and RAPD analysis were carried out at the DNA lab for Ecology and Biosystematics at the Department of Biology, University of Oslo. The study was partly supported by grant 101183/720 to C. B. from the Norwegian Research Council under the TERRØK program.

#### REFERENCES

- Blytt A. 1892. Nye bidrag til kundskaben om karplanternes udbredelse i Norge. *Forhandlinger i Videnskabs-selskabet i Christiania*, B: 1–73. (In Norwegian.)

- Borgen L, Elven R. 1983.** Chromosome numbers of flowering plants from northern Norway and Svalbard. *Nordic Journal of Botany* **3**: 301–306.
- Brochmann C, Soltis PS, Soltis DE. 1992a.** Multiple origins of the octoploid Scandinavian endemic *Draba cacuminum*: electrophoretic and morphological evidence. *Nordic Journal of Botany* **12**: 257–272.
- Brochmann C, Soltis PS, Soltis DE. 1992b.** Recurrent formation and polyphyly of Nordic polyploids in *Draba* (Brassicaceae). *American Journal of Botany* **79**: 673–688.
- Brochmann C, Nilsson T, Gabrielsen TM. 1996.** A classic example of postglacial allopolyploid speciation re-examined using RAPD markers and nucleotide sequences: *Saxifraga osloensis* (Saxifragaceae). *Symbolae Botanicae Upsalensis* **31**: 75–89.
- Brochmann C, Xiang Q-Y, Brunnsfeldt SJ, Soltis DE, Soltis PS. 1998.** Molecular evidence for polyploid origins in *Saxifraga* (Saxifragaceae): the narrow arctic endemic *S. svalbardensis* and its widespread allies. *American Journal of Botany* **85**: 135–143.
- Brochmann C, Borgen L, Stabbetorp OE. 2000.** Multiple diploid hybrid speciation of the Canary Island endemic *Argyranthemum sundingii* (Asteraceae). *Plant Systematics and Evolution* **220**: 77–92.
- Demasure B, Sodзи N, Petit RJ. 1995.** A set of universal primers for amplifications of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology* **4**: 129–131.
- Dumolin-Lapegue S, Pemonge M-H, Petit RJ. 1997.** An enlarged set of consensus primers for the study of organelle DNA in plants. *Molecular Ecology* **6**: 393–397.
- Elven R. 1994.** *Norsk Flora*. 6th ed. of Lid J & Lid DT. Oslo: Det Norske Samlaget.
- Engelskjøn T. 1979.** Chromosome numbers in vascular plants from Norway, including Svalbard. *Opera Botanica* **52**: 1–38.
- Flugsrud K. 1985.** En morfologisk, økologisk og taksonomisk analyse av *Saxifraga opdalensis*. Unpublished thesis, University of Oslo. (In Norwegian.)
- Gabrielsen TM, Brochmann C. 1998.** Sex after all: high levels of diversity detected in the arctic clonal plant *Saxifraga cernua* using RAPD markers. *Molecular Ecology* **7**: 1701–1708.
- Gabrielsen TM, Bachmann K, Jakobsen KS, Brochmann C. 1997.** Glacial survival does not matter: RAPD phylogeography of Nordic *Saxifraga oppositifolia*. *Molecular Ecology* **6**: 831–842.
- Higgins DG, Fuchs R, Blesby A. 1992.** CLUSTAL: a new multiple sequence alignment program. *Computer Applied Bioscience* **8**: 189–191.
- Holaker P, Nordhagen R, Berg RY. 1960.** *Saxifraga opdalensis* A. B1. gjenfunnet. Foreløpig meddelelse. *Blyttia* **18**: 108–112.
- Löve Å, Löve D. 1975.** *Cytotaxonomical atlas of the arctic flora*. Vaduz: J. Cramer.
- Molau U. 1993.** Relationships between flowering phenology and life history strategies in tundra plants. *Arctic and Alpine Research* **25**: 391–402.
- Øvstedal DO. 1975.** A new *Saxifraga* from Svalbard. *Astarte* **8**: 23–27.
- Øvstedal DO. 1998.** Variation within some Nordic *Saxifraga* species (Saxifragaceae). *Nordic Journal of Botany* **18**: 171–181.
- Rieseberg LH. 1991.** Homoploid reticulate evolution in *Helianthus* (Asteraceae): evidence from ribosomal genes. *American Journal of Botany* **78**: 1218–1237.
- Rohlf FJ. 1990.** *NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 1.60*. Setauket, NY: Exeter Software.
- Rune O. 1988.** Serpentine flora of Scandinavia. *Blyttia* **46**: 43–51. (In Norwegian with English summary.)
- Soltis DE, Soltis PS. 1989.** Allopolyploid speciation in *Tragopogon*: insights from chloroplast DNA. *American Journal of Botany* **76**: 1119–1124.
- Soltis DE, Soltis PS. 1993.** Molecular data and the dynamic nature of polyploidy. *Critical Reviews in Plant Sciences* **12**: 243–273.
- Soltis DE, Soltis PS, Ness BD. 1990.** Maternal inheritance of the chloroplast genome in *Heuchera* and *Tolmiea* (Saxifragaceae). *Journal of Heredity* **81**: 168–170.
- Taberlet P, Gielly L, Pautou G, Bouvet J. 1991.** Universal primers for the amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**: 1105–1109.
- Webb DA, Gornall RJ. 1989.** *A manual of saxifrages and their cultivation*. Portland, OR: Timber Press.
- Werth CR, Guttman SI, Eshbaugh WH. 1985.** Recurring origins of allopolyploid species in *Asplenium*. *Science* **228**: 731–733.
- Williams JGK, Hanafey MK, Rafalski JA, Tingey SV. 1993.** Genetic analysis using random amplified polymorphic DNA markers. *Methods in Enzymology* **218**: 704–740.