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MOLECULAR EVIDENCE FOR POLYPLOID ORIGINS IN *SAXIFRAGA* (SAXIFRAGACEAE): THE NARROW ARCTIC ENDEMIC *S. SVALBARDENSIS* AND ITS WIDESPREAD ALLIES[1]

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The recently described polyploid *Saxifraga svalbardensis* is endemic to the arctic archipelago of Svalbard. We investigated relationships among four closely related species of *Saxifraga* in Svalbard and tested three previously proposed hypotheses for the origin of *S. svalbardensis*: (1) differentiation from the morphologically and chromosomally variable polyploid *S. cernua*; (2) hybridization between the diploid *S. hyperborea* and *S. cernua*; and (3) hybridization between the tetraploid *S. rivularis* and *S. cernua*. Fifteen populations were analyzed using random amplified polymorphic DNAs (RAPDs) and nucleotide sequences of the chloroplast gene *matK* and the internal transcribed spacers (ITS) of nuclear ribosomal DNA (rDNA). RAPD and *matK* data suggest that *S. svalbardensis* has originated from a hybrid with *S. rivularis* as the maternal parent and *S. cernua* as the paternal parent, possibly a single time, whereas ITS data could not be used to discriminate among the hypotheses. The data also suggest that the diploid *S. hyperborea* is a progenitor of the tetraploid *S. rivularis*. The four populations examined of *S. svalbardensis* were virtually identical for RAPD and ITS markers, whereas *S. cernua* showed high levels of variation, suggesting that the latter polyploid either has formed recurrently or has undergone considerable differentiation since its origin.

Key words: Arctic; ITS nucleotide sequences; *matK* nucleotide sequences; polyploid evolution; RAPDs; *Saxifraga cernua*; *Saxifraga svalbardensis*; Saxifragaceae.

Molecular approaches are well established as powerful tools for analysis of polyploids. In recent years, significant new insights into the dynamics of polyploid evolution in plants have been achieved using enzyme electrophoresis and restriction site analysis of chloroplast DNA (cpDNA) and nuclear ribosomal RNA genes (rDNA; e.g., Werth, Guttman, and Eshbaugh, 1985; Wyatt, Odrzykoski, and Stoneburner, 1988; Ranker et al., 1989; Soltis and Soltis, 1989; Doyle, Brown, and Grace, 1990; Ashton and Abbott, 1992; Brochmann, 1992; Brochmann and Elven, 1992; Brochmann, D. E. Soltis, and P. S. Soltis, 1992; Brochmann, P. S. Soltis, and D. E. Soltis, 1992a). In contrast, comparative gene sequencing (e.g., of the ITS [internal transcribed spacer] regions of nuclear rDNA) and RAPD analysis (random amplified polymorphic DNA) have rarely been applied to address questions of polyploid evolution (but see, e.g., Huff and Bara, 1993; van Houten, Scarlett, and Bachmann, 1993; Yu and Pauls, 1993; Kim and Jansen, 1994; Wendel, Schnabel, and Seelanan, 1995; Brochmann, Nilsson, and Gabrielsen, 1996).

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The use of ITS sequences for identification of the progenitors of polyploids is potentially problematic. Kim and Jansen (1994) examined ITS sequences of two tetraploids in *Krigia* but observed no additivity of putative parental genomes, and concluded that the polyploids were autoploids. Other workers have found additivity of parental genomes in the ITS sequences of polyploids (e.g., van Houten, Scarlett, and Bachmann, 1993; Baldwin et al., 1995). However, Wendel, Schnabel, and Seelanan (1995) found that ITS sequences in allotetraploid *Gossypium* have been homogenized via interlocus concerted evolution in the direction of one or the other progenitor species. Notably, an experimentally synthesized allotetraploid of *Gossypium* exhibited the ITS sequences of both of its parents (Wendel, Schnabel, and Seelanan, 1995). These problems are avoided, however, with the mainly biparentally inherited RAPD markers, which have been used, for example, in studies of the tetraploid *Medicago sativa* (Yu and Pauls, 1993) and the variable polyploid *Poa pratensis* (Huff and Bara, 1993). In a recent analysis of the Scandinavian endemic tetraploid *Saxifraga osloensis* and its diploid progenitors *S. adscendens* and *S. tridactylites*, we have shown that the ITS sequence of this tetraploid has been homogenized in the direction of *S. adscendens*, whereas RAPD markers revealed almost complete additivity in *S. osloensis* relative to its diploid progenitors (Brochmann, Nilsson, and Gabrielsen, 1996).

Herein we use a multifaceted molecular approach involving comparative sequencing of chloroplast (*matK*) and nuclear (ITS) DNA as well as analysis of RAPD markers to address the origin of the polyploid *Saxifraga*

svalbardensis D. O. Øvstedal. This recently described polyploid is one of the few species that are endemic to the arctic archipelago of Svalbard (Øvstedal, 1975). It is morphologically most similar to the circumpolar *S. cernua* L. Both of these species are common in Svalbard and largely asexual, reproducing by bulbils; *S. svalbardensis* also has subterranean runners. There has been controversy regarding the recognition of *S. svalbardensis* as a distinct species. It has been suggested that it only represents one of numerous races within the morphologically variable *S. cernua* (e.g., Rønning, 1979). Species rank for *S. svalbardensis* seems to be accepted by most recent authors (e.g., Elvebakk, 1979; Elven and Elvebakk, 1996), and it can easily be distinguished from Svalbard populations of *S. cernua* based on several morphological and ecological characteristics.

Saxifraga svalbardensis and *S. cernua* belong to *Mesogyne*, a section of eight species, which also is represented by two other species in Svalbard: the tetraploid *S. rivularis* L. and the diploid *S. hyperborea* R. Brown (Webb and Gornall, 1989; Elven and Elvebakk, 1996). *Saxifraga hyperborea* can be distinguished from *S. rivularis* by its lack of runners and its anthocyanic stems, leaves, and petals. *Saxifraga cernua* and *S. svalbardensis* are distinguished from the two morphologically similar species *S. hyperborea* and *S. rivularis* by their leafy, usually one-flowered stems with bulbils in the axils of the stem leaves. *Saxifraga svalbardensis* is confined to deep, wet moss vegetation and often occurs along small brooks. In contrast, *S. cernua* has a wide ecological amplitude and occurs in habitats such as unstable river banks, wet cliffs, moraines, snow beds, wet and dry moss vegetation, and *Dryas* heaths. *Saxifraga hyperborea* and *S. rivularis* have fairly narrow, overlapping ecological amplitudes and typically grow in river banks and late snowbeds, often intermingled at the same site.

Chromosome numbers have been determined for all of these species based on Nordic material. *Saxifraga hyperborea* is diploid with $2n = 26$ (seven counts from Svalbard: Flovik, 1940 (as *S. rivularis*, refers to *S. hyperborea* according to Engelskjøn, 1979); Engelskjøn, 1979; Borgen and Elven, 1983), whereas *S. rivularis* is tetraploid with $2n = 52$ (given as 52, 50–52, or 51–53; nine counts from mainland Norway and six counts from Svalbard: Engelskjøn and Knaben, 1971; Engelskjøn, 1979; Borgen and Elven, 1983). These chromosome numbers are consistent with those reported from other arctic areas for these species (Löve and Löve, 1975). The putative hybrid *S. hyperborea* × *rivularis* has also been reported from Svalbard ($2n = 3x = 39$; Borgen and Elven, 1983).

A range of chromosome numbers has been reported within *S. cernua* ($2n = 24$, ~33, 36, 44, 44–46, 48, 50, 52, ~54, 55–57, 56, 60, 62, 64, ~66, ~68, 70, 72; Löve and Löve, 1975; Webb and Gornall, 1989). These numbers probably represent diploid to hexaploid levels (including aneuploidy) based on $x = 12$, but it is also possible that some numbers represent a combination of $x = 12$ and $x = 13$. The chromosome number of *S. cernua* has not yet been determined in Svalbard, but $2n \approx 33$, 44–46, and 55–57 have been recorded from mainland Norway (Skovsted, 1934; Engelskjøn, 1979). *Saxifraga svalbardensis* is approximately pentaploid with $2n \approx 64$ (Borgen and Elven, 1983).

The four species show considerable variation in reproductive strategies (C. Brochmann, unpublished data). In Svalbard, both the diploid *S. hyperborea* and the tetraploid *S. rivularis* are fully sexual and mainly autogamous; the only means of asexual reproduction is by short runners in *S. rivularis*. In contrast, the chromosomally variable *S. cernua* varies from fully sexual (pollen stainabilities >90% and considerable seed set) to almost sterile; it largely reproduces by bulbils. *Saxifraga svalbardensis* was believed to reproduce entirely via bulbils and runners, but it has recently been shown that this species has partly stainable pollen and sets some viable seeds after hand-selfing (C. Brochmann, unpublished data).

Three hypotheses have been advanced to explain the origin of *S. svalbardensis* (Øvstedal, 1975; see also Borgen and Elven, 1983, and Elven and Elvebakk, 1996): (1) the *cernua* hypothesis—differentiation within *S. cernua*; (2) the *hyperborea* hybrid hypothesis—hybridization between *S. cernua* and *S. hyperborea*; and (3) the *rivularis* hybrid hypothesis—hybridization between *S. cernua* and *S. rivularis*. Because of the close morphological similarity between *S. svalbardensis* and *S. cernua*, the role of *S. cernua* in the origin of *S. svalbardensis* has never been questioned. It is, however, difficult to discriminate among the abovementioned hypotheses based solely on morphological (Øvstedal, 1975) and chromosomal data (Borgen and Elven, 1983), although it has been claimed that morphological data support the *hyperborea* hybrid hypothesis (Elvebakk, 1979; Elven and Elvebakk, 1996). Also, anthocyanin patterns were first reported to support the *hyperborea* hybrid hypothesis (Andersen and Øvstedal, 1983), but a subsequent, more detailed analysis of anthocyanins showed that these data could not be used to discriminate among the three hypotheses (Andersen and Øvstedal, 1988).

To address the origin of *S. svalbardensis* we sequenced the ITS regions of nuclear rDNA (cf. Baldwin, 1992, 1993) and the chloroplast gene *matK* (cf. Johnson and Soltis, 1994, 1995; Steele and Vilgalys, 1994). We also analyzed RAPD variation among populations of these species. Using these three independent molecular data sets, we: (1) test the three hypotheses for the origin of *S. svalbardensis*, (2) attempt to determine whether *S. svalbardensis* has originated one or several times, and (3) evaluate relationships among all four species (in particular, we hoped to determine whether the diploid *S. hyperborea* might be a progenitor of the morphologically and ecologically similar tetraploid *S. rivularis*).

MATERIALS AND METHODS

Plant materials and DNA isolation—Living plants from four populations of *S. svalbardensis*, seven populations of the morphologically variable *S. cernua* (including two populations of an unusual, small-flowered type), and several populations each of *S. hyperborea* and *S. rivularis* were collected in Nordenskiöld Land, Dickson Land, and Haakon VII Land in Spitsbergen, Svalbard and cultivated in a phytotron at the University of Oslo (Table 1, Fig. 1). Only two populations of each of the two latter species survived in cultivation. Chromosome counts were not carried out in this study because either no variation has been reported within species, or so much variation has been reported that another sample of chromosome numbers probably would be uninformative for our purpose (*S. cernua*; cf. above). Fresh leaves from cultivated plants were dried in silica gel for subsequent analysis at Washington

TABLE 1. Collection data for investigated populations of *Saxifraga*. Collectors are C. Brochmann and Arnodd Håpnes.

S. cernua pop. no. 12 (large-flowered type)—Svalbard, Spitsbergen, Haakon VII Land: Blomstrandhalvøya N of Kongsfjorden, between Hansneset and Sørvågen. VH 3671. Alt. 100 m. 21 July 1992.
S. cernua pop. no. 31 (large-flowered type)—Svalbard, Spitsbergen, Nordenskiöld Land: Adventdalen, Bolterdalen, W side. WG 2177. Alt. 100 m. 28 July 1992.
S. cernua pop. no. 45 (large-flowered type)—Svalbard, Spitsbergen, Nordenskiöld Land: Bjørndalen, E of the river. WG 0783. Alt. 30 m. 1 August 1992.
S. cernua pop. no. 66 (small-flowered type)—Svalbard, Spitsbergen, Dickson Land: W of Siklarhallet, N of Svenskehuset. WH 1512. Alt. 220 m. 5 August 1992.
S. cernua pop. no. 67 (large-flowered type)—data as for pop. no. 66.
S. cernua pop. no. 81 (large-flowered type)—Svalbard, Spitsbergen, Dickson Land: Siklarhallet between Kapp Thordsen and Svenskehuset, N of trig. point 113. WH 1312. Alt. 240 m. 7 August 1992.
S. cernua pop. no. 82 (small-flowered type)—data as for pop. no. 81.
S. hyperborea pop. no. 17—Svalbard, Spitsbergen, Nordenskiöld Land: Adventdalen, Bolterdalen, E side of Bolterelva. WG 2276. Alt. 90 m. 27 July 1992.
S. hyperborea pop. no. 24—Svalbard, Spitsbergen, Nordenskiöld Land: Adventdalen, Breinosa, NW of summit. WG 2476. Alt. 410 m. 29 July 1992.
S. rivularis pop. no. 26—Svalbard, Spitsbergen, Nordenskiöld Land: Adventdalen, Breinosa, NW of summit. WG 2375. Alt. 400 m. 29 July 1992.
S. rivularis pop. no. 47—Svalbard, Spitsbergen, Nordenskiöld Land: Bjørndalen, E of the river. WG 0783. Alt. 30 m. 1 August 1992.
S. svalbardensis pop. no. 20—Svalbard, Spitsbergen, Nordenskiöld Land: Adventdalen, Bolterdalen, E side of Bolterelva. WG 2276. Alt. 90 m. 27 July 1992.
S. svalbardensis pop. no. 28—Svalbard, Spitsbergen, Nordenskiöld Land: Adventdalen, Breinosa, NW of summit. WG 2375. Alt. 400 m. 29 July 1992.
S. svalbardensis pop. no. 40—Svalbard, Spitsbergen, Nordenskiöld Land: Bjørndalen, E of the river. WG 0783. Alt. 30 m. 1 August 1992.
S. svalbardensis pop. no. 59—Svalbard, Spitsbergen, Dickson Land: SE slope of Siklarhallet, 800 m SW of Svenskehuset. WH 1511. Alt. 140 m. 4 August 1992.

State University (sequencing) and University of Idaho (RAPDs). Vouchers of field-collected and cultivated plants are deposited at the Botanical Museum, University of Oslo (O).

Total DNAs were isolated from 0.02 to 0.06 g of dried leaf tissue following the CTAB method of Doyle and Doyle (1987) as modified by Soltis et al. (1991). DNA was isolated from one plant from most populations; leaves from several plants were pooled for a few populations (*S. cernua* pop. 67, *S. rivularis* pop. 26, and *S. hyperborea* pop. 24) to obtain sufficient amounts of DNA. DNAs were purified using S & S Elu-quik DNA purification kit (Schleicher & Schuell, Keene, New Hampshire).

RAPDs—DNA isolates were quantified using a Hoefer TKO-100 fluorometer and diluted to 10 ng/μL. Amplifications were performed in a MJ Research thermocycler using 10 ng of template DNA and following Williams et al. (1990). Four DNA samples (one per species) were initially run with 80 different 10-mer primers (kits A, B, C, and D; Operon Technologies, Alameda, California), and 24 of these primers were selected for analysis of all populations. Only robust, well-separated amplification products were scored, and each of them was tested several times for repeatability. For six markers (5, 7, 16, 25, 30, and 32; cf. Table 2), the homology of comigrating amplification products was confirmed by cutting the bands from low melting-point agarose gels, purifying using GELase (Epicentre Technologies, Madison, Wisconsin), and digesting with restriction endonucleases to reveal shared restriction sites. Polymorphic markers were scored as present or absent and analyzed using NTSYS-pc (Rohlf, 1990) as follows: (1) UPGMA analysis based on Dice's similarity between populations, given by $2a/(2a+b)$, where a is number of positive matches (i.e., shared bands) and b is number of mismatches; negative matches (i.e., absence of a band in both populations) were excluded because of the high possibility of nonhomologous absence of RAPD products in interspecific comparisons (see Skroch, Tivang, and Nienhus, 1992 for discussion); (2) correspondence analysis (CA), which is based on chi-square distances between populations and gives most weight to bands present in low frequencies; and (3) nonmetric multidimensional scaling (MDS) based on Dice's similarity. Minimum spanning trees (MSTs) were superimposed on the CA and MDS plots to reveal distortions (cf. Rohlf, 1990).

DNA sequencing and sequence analyses—For both *matK* and ITS, double-stranded DNA was amplified from total DNA via PCR using Replitherm DNA polymerase (Epicentre Technologies, Madison, Wisconsin), and single-stranded DNA was subsequently synthesized using the double-stranded PCR product as template and the two PCR primers individually in separate PCR reactions. Single-stranded DNA was precipitated with 20% PEG/2.5 mol/L NaCl, washed first with 70% and then with 95% ethanol, and resuspended in 1X TE buffer. Direct sequencing of these purified, single-stranded PCR products was performed using the dideoxy method with Sequenase version 2.0 (U.S. Biochemical Corp., Cleveland, Ohio) and ^{35}S dATP. Labeled fragments were separated in 6% acrylamide gels with 1X TBE buffer at 70 W (constant power).

The *matK* region, which is located within the intron of the gene *trnK* and evolves at least three times faster than *rbcL*, was amplified using primers *trnK*-3914F and *trnK*-2R (Johnson and Soltis, 1994, 1995). The single-stranded DNA amplified using *trnK*-3914F (the forward strand) was sequenced using *matK*-1168R and *matK*-1470R; the strand amplified using *trnK*-2R (the reverse strand) was sequenced using *matK*-1412F (see Johnson and Soltis, 1994 for details). This approach yielded 963 bp of the 1500-bp *matK* region. The entire ITS region including the 5.8S subunit was amplified using primers ITS-1 and ITS-4. The ITS 1 region was sequenced using primers ITS-1 and ITS-2; the ITS 2 region was sequenced using primer ITS-3 (Baldwin, 1992).

In the *matK* analysis, one population of *Saxifraga cernua* from Alaska (Soltis et al., 1993) was included in addition to the Svalbard populations. *Saxifraga oppositifolia* L. (section *Porphyrion*) was selected as an outgroup because broad analyses of *matK* sequences for Saxifragaceae indicate that sections *Porphyrion* and *Mesogyne* are closely allied (Soltis et al., 1996). The monophyly of the species group analyzed herein was also confirmed by these broad analyses of *matK* sequences (Soltis et al., 1996). Sequences were aligned manually. The nuclear ITS sequences were not subjected to cladistic analysis because inclusion of taxa of hybrid origin may distort such analyses. The relationships among species were therefore evaluated based on ITS sequence divergence values. The *matK* sequences, which were derived from haploid, and thus nonhybrid, chloroplast genomes, were analyzed cladistically using PAUP 3.1.1 (Swofford, 1993) with characters specified as unordered and equally weighted. Parsimony analysis was conducted using the exhaustive branch-and-bound search strategy. Bootstrap analysis (Felsenstein, 1985) using 100 replicates (branch-and-bound search) was performed to obtain estimates of reliability for monophyletic groups.

RESULTS

RAPD analysis—A total of 42 reliable amplification products was scored for the 24 primers that were run for all populations. Thirty-eight of these products were polymorphic (Table 2). The four populations analyzed of *S. svalbardensis* showed identical RAPD patterns. The two populations analyzed of *S. hyperborea* differed by two markers, and the two populations analyzed of *S. rivularis*

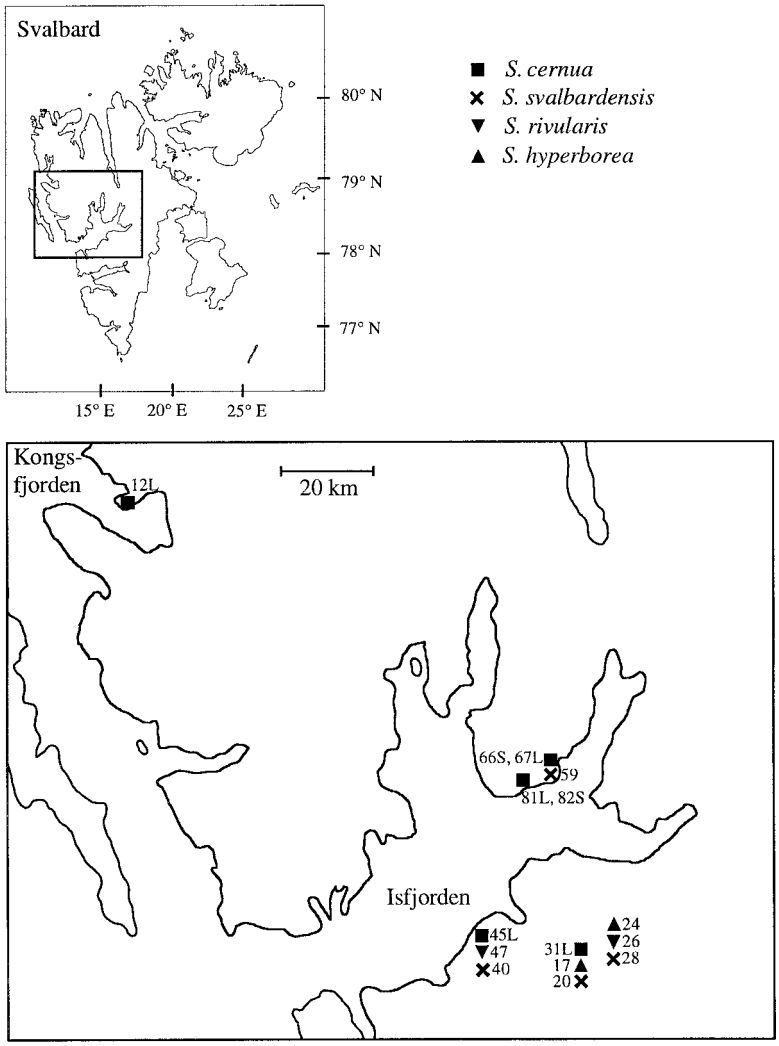

Fig. 1. Map of the arctic archipelago of Svalbard showing the sampling sites for the 15 populations analyzed of *Saxifraga*. Population numbers and flower size in *S. cernua* (L—large-flowered, S—small-flowered) are indicated (cf. Table 1).

differed by three markers. In contrast, there was large RAPD variation among the seven populations analyzed of *S. cernua*. In this species, 19 of the 42 markers varied among populations. Thus, the level of intraspecific RAPD polymorphism ranged from 0 to 45% among the four species analyzed (Table 2).

No RAPD markers were observed exclusively within *S. svalbardensis* or within *S. rivularis*. Four markers were observed only within *S. hyperborea*, and these markers were observed in both populations of this species. Eight markers were observed only within *S. cernua*, and six of these markers were observed in all populations of this species. Six of the RAPD markers that were shared between *S. svalbardensis* and *S. cernua* were not detected in any other species. Two of the markers that were shared between *S. svalbardensis* and *S. rivularis* were not detected in any other species. In contrast, no markers were exclusively shared between *S. svalbardensis* and *S. hyperborea* (Table 2).

The result of the MDS analysis of the RAPD data (not shown; stress 0.024) was very similar to that of the CA analysis (Fig. 2), which extracted most of the variance in the data matrix (89.0% by the first three axes). In these analyses, *S. svalbardensis* appeared intermediate between *S. cernua* and *S. rivularis* but closest to the latter species, whereas *S. hyperborea* was distinctly different. Notably, the minimum spanning tree connected the four populations of *S. svalbardensis* to one population of *S. rivularis* (no. 26) as well as to one population of *S. cernua* (no. 31).

The UPGMA analysis produced results similar to those of the CA and MDS analyses (Fig. 3; cophenetic correlation coefficient 0.87). In the UPGMA analysis, the populations of *S. svalbardensis* clustered with those of *S. rivularis* at the 0.83 level and with those of *S. cernua* at the 0.54 level, whereas the populations of *S. hyperborea* clustered with the remaining taxa at the 0.32 level. Notably, the UPGMA analysis indicated that the RAPD differentiation within *S. cernua* was larger than the differentiation between *S. svalbardensis* and *S. rivularis*: population no. 12 of *S. cernua* was, for example, very distinct and clustered with the other populations of *S. cernua* at the 0.70 level. The two deviating, small-flowered pop-

TABLE 2. Polymorphic RAPD markers scored for 24 different primers in 15 populations of *Saxifraga.*

Species	Pop. no.	A2 1	A2 2	A2 3	A2 4	A4 5	A9 6	A10 7	A11 8	A11 9	A13 10	A15 11	A15 12	B7 13	B7 14	B10 15	B13 16	B16 17	B16 18	C1 19	C5 20	C5 21	C5 22	C8 23	C8 24	C18 25	C19 26	C20 27	C20 28	D2 29	D3 30	D7 31	D7 32	D8 33	D11 34	D11 35	D13 36	D13 37	D16 38
S. cernua	12	1	0	0	1	0	0	0	0	0	1	0	0	0	1	1	0	1	1	1	0	1	0	0	1	0	0	0	1	1	1	0	0	1	0	0	0	0	0
S. cernua	31	1	0	1	1	0	1	0	0	0	1	0	1	0	1	1	0	1	1	1	1	1	1	0	1	0	0	1	1	1	1	1	0	1	1	0	0	1	0
S. cernua	45	1	0	0	1	0	1	0	0	0	1	0	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	0	1	1	1	1	0	1	1	0	1	1	0
S. cernua	66	1	0	1	1	0	1	0	0	0	1	0	1	0	1	1	0	0	1	1	1	1	1	0	1	0	1	0	1	1	1	1	0	1	1	0	1	1	0
S. cernua	67	1	0	0	1	1	1	1	0	0	1	0	0	0	1	1	0	1	1	1	0	0	0	0	1	1	1	0	1	1	1	1	0	1	0	1	0	1	0
S. cernua	81	1	0	0	1	0	0	1	0	0	1	0	1	0	1	1	0	1	0	1	1	1	0	0	1	1	1	0	1	1	1	1	0	1	0	1	0	1	0
S. cernua	82	1	0	1	1	0	1	0	0	0	1	0	1	0	1	1	0	0	1	1	1	1	1	0	1	1	1	0	1	1	1	1	0	1	1	0	0	1	0
S. svalbardensis	20	1	1	1	0	1	1	1	0	0	1	0	1	0	1	1	0	1	1	1	0	1	1	0	1	1	1	0	1	1	1	1	0	1	1	0	1	1	0
S. svalbardensis	28	1	1	1	0	1	1	1	0	1	0	1	1	0	1	1	0	1	1	1	0	1	1	0	1	1	1	0	1	1	1	1	0	1	1	0	1	1	0
S. svalbardensis	40	1	1	1	0	1	1	1	0	1	0	1	1	0	1	1	0	1	1	1	0	1	1	0	1	1	1	0	1	1	1	1	0	1	1	0	1	1	0
S. svalbardensis	59	1	1	1	0	1	1	1	0	1	0	1	1	0	1	1	0	1	1	1	0	1	1	0	1	1	1	0	1	1	1	1	0	1	1	0	1	1	0
S. rivularis	26	1	1	1	0	0	1	1	0	1	0	1	1	1	0	0	1	0	0	0	1	0	1	1	0	0	1	0	0	1	0	0	0	1	1	0	1	1	0
S. rivularis	47	1	1	1	0	0	1	0	0	1	0	1	1	1	0	0	1	0	0	0	1	0	1	1	0	0	1	0	0	1	0	0	0	1	1	0	1	1	0
S. hyperborea	17	0	1	1	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	1	1	1
S. hyperborea	24	0	1	0	0	0	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	1	1	1

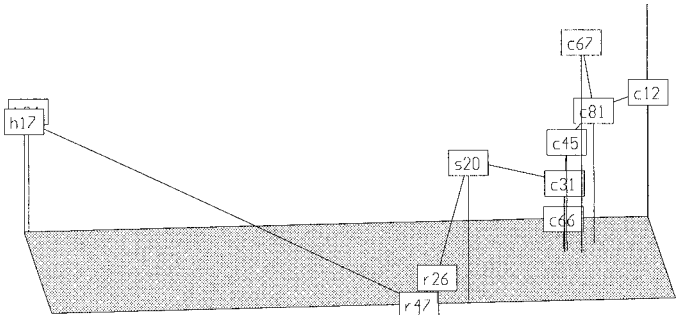

Fig. 2. Correspondence analysis (axes 1–3; minimum spanning tree superimposed) of RAPD data for 15 populations of *Saxifraga.* s—*S. svalbardensis* (four identical populations); c—*S. cernua* (seven populations, partly overlapping in the plot); r—*S. rivularis* (two populations); h—*S. hyperborea* (two populations, partly overlapping in the plot). Axis 1—50.8%, axis 2—30.0%, axis 3—8.2%.

ulations of *S. cernua* were not differentiated from the large-flowered ones in RAPD patterns (Fig. 3).

matK sequences—Forty-seven (4.9%) of the 963 *matK* base positions in the six populations sequenced (five taxa, including the outgroup *S. oppositifolia*) were variable. When the outgroup was excluded, only nine (0.9%) base positions were variable. Four of these nine characters were autapomorphic, and the remaining five (0.5%) characters (ten character states) were potentially informative. The exhaustive search option in PAUP provided a single most parsimonious tree of 47 steps without homoplasy (Fig. 4).

In the *matK* analysis, *S. hyperborea* and *S. rivularis* were sister species differing by a single base substitution. The *hyperborea–rivularis* clade was supported by one synapomorphic mutation and had a bootstrap value of 76%. These two species in turn formed a well-supported monophyletic group with *S. svalbardensis* (bootstrap value 95%; supported by three synapomorphic mutations; Fig. 4). *Saxifraga svalbardensis* had no autapomorphic mutations. The two populations sequenced of *S. cernua* were sisters differing by three autapomorphic mutations, and this clade was supported by one synapomorphic mutation and had a bootstrap value of 61%. The *cernua*

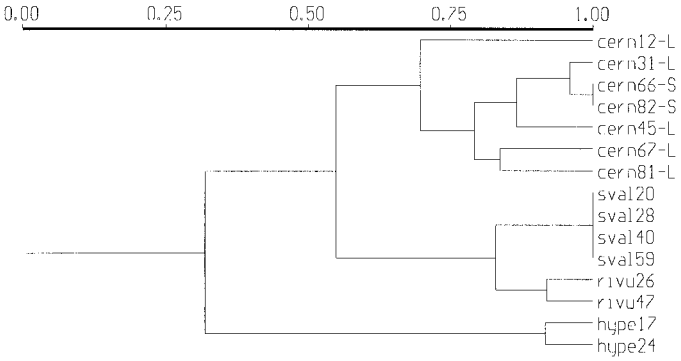

Fig. 3. Cluster analysis (UPGMA based on Dice's similarity index) of RAPD data for 15 populations of *Saxifraga.* sval—*S. svalbardensis*; cern—*S. cernua*; rivu—*S. rivularis*; hype—*S. hyperborea.* Population numbers and flower size in *S. cernua* (L—large-flowered, S—small-flowered) are indicated (cf. Table 1).

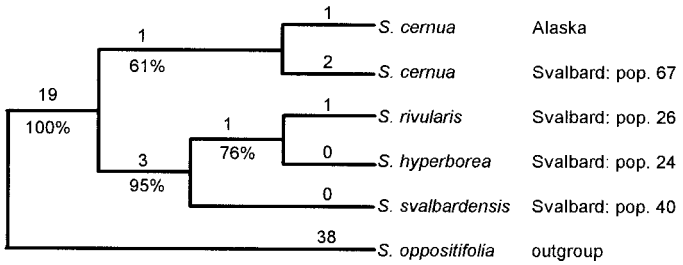

Fig. 4. The single most parsimonious tree derived from analysis of 963 bp *matK* sequences of five populations (four species) of *Saxifraga* and one outgroup (*S. oppositifolia*). The number above each branch is the number of base substitutions. Below branches, bootstrap support is given as percentages based on 100 bootstrap replications. Tree length = 47, consistency index = 1.00, retention index = 1.00.

clade was sister to the *hyperborea–rivularis–svalbardensis* clade (Fig. 4).

ITS sequences—ITS sequences were obtained for three populations of *S. svalbardensis,* four populations of *S. cernua,* and one population each of *S. rivularis* and *S. hyperborea.* A total of 538 base positions was obtained, of which 34 (6.3%) were variable. The ITS data did not resolve the relationship between *S. svalbardensis* and the other species. The ITS sequence divergence values, which were evaluated directly rather than carrying out a cladistic analysis involving these polyploids, are shown in Table 3.

Two of the populations of *S. svalbardensis* had identical ITS sequences, and the third population of this species differed from the other two only by a single base substitution. In contrast to *S. svalbardensis,* the four populations sequenced of *S. cernua* showed high levels of ITS divergence, with the two most divergent populations differing by 21 base substitutions (Table 3). This is greater than the amount of ITS divergence between any other populations in the ingroup, for which a maximum of 20 divergent sites was observed (Table 3). *Saxifraga rivularis* and *S. hyperborea* differed from each other by five substitutions, and these two species differed from the populations of *S. cernua* by 6–20 substitutions.

The ITS divergence between *S. svalbardensis* and *S. cernua* ranged from five to 20 substitutions. The divergence between *S. svalbardensis* and *S. hyperborea* ranged from six to seven substitutions, and the divergence between *S. svalbardensis* and *S. rivularis* ranged from nine to ten substitutions (Table 3).

DISCUSSION

Polyploid origins and relationships among species: molecular evidence—Origin of *S. svalbardensis* from *S. cernua* alone, without any contribution from another species (the *cernua* hypothesis), can be disregarded with reasonable certainty based on the *matK* data as well as the RAPD data. In the analysis of the *matK* sequences, *S. svalbardensis* was included in a well-supported clade with *S. rivularis* and *S. hyperborea,* suggesting that the chloroplast of *S. svalbardensis* was donated by one of these two species rather than *S. cernua* (Fig. 4). The *cernua* hypothesis is also very unlikely based on the RAPD data. In the RAPD analyses, *S. svalbardensis* was closer to *S. rivularis* than to *S. cernua* (Figs. 2, 3).

The *hyperborea* hybrid hypothesis, involving hybridization between *S. cernua* and *S. hyperborea,* is also unlikely based on the RAPD data. Although *S. rivularis* and *S. hyperborea* are almost equally probable as one of the progenitors (the maternal parent) of *S. svalbardensis* based on *matK* sequences (Fig. 4), *S. hyperborea* is more divergent from *S. svalbardensis* than is *S. rivularis* in overall RAPD pattern (Figs. 2, 3). Furthermore, four of the RAPD markers were only observed in *S. hyperborea,* and no markers were exclusively shared between *S. hyperborea* and *S. svalbardensis.*

In contrast, the RAPD data support the *rivularis* hybrid hypothesis, origin of *S. svalbardensis* by hybridization between *S. cernua* and *S. rivularis.* This hypothesis is supported by (1) the presence of two RAPD markers exclusively shared between *S. rivularis* and *S. svalbardensis,* (2) that all RAPD markers observed in *S. rivularis* also were observed in *S. svalbardensis,* (3) the presence of five RAPD markers exclusively shared between *S. svalbardensis* and *S. cernua,* and (4) the intermediate position of *S. svalbardensis* between *S. cernua* and *S. rivularis* in the analyses of overall RAPD pattern (Figs. 2, 3).

That *S. svalbardensis* appeared closer to *S. rivularis* than to *S. cernua* in the analyses of the RAPD data (Figs. 2, 3) is mainly caused by the presence of six apparently species-specific and constant (i.e., occurring in all populations analyzed) RAPD markers in *S. cernua* (Table 2). The absence of these RAPD markers, which usually are dominantly inherited (cf. Williams et al., 1990), in *S. svalbardensis* can most easily be explained by the large variation in other RAPD markers observed among different populations of *S. cernua.* It is possible that the

TABLE 3. Pairwise divergence between ITS sequences of populations of *Saxifraga cernua, S. svalbardensis, S. rivularis,* and *S. hyperborea.* Divergence values in the upper right half of the matrix are the proportion of divergent sites in each comparison (adjusted for missing data). Actual numbers of divergent sites are given in the lower left half of the matrix.

		1	2	3	4	5	6	7	8	9
1 *S. cernua*	pop. 12	—	0.015	0.049	0.021	0.041	0.042	0.042	0.036	0.040
2 *S. cernua*	pop. 31	7	—	0.033	0.004	0.018	0.017	0.017	0.017	0.027
3 *S. cernua*	pop. 67	21	14	—	0.023	0.025	0.035	0.035	0.040	0.047
4 *S. cernua*	pop. 81	10	2	10	—	0.011	0.010	0.010	0.013	0.023
5 *S. svalbardensis*	pop. 40	18	8	10	5	—	0.002	0.002	0.016	0.021
6 *S. svalbardensis*	pop. 20	20	8	15	5	1	—	0.000	0.013	0.019
7 *S. svalbardensis*	pop. 28	20	8	15	5	1	0	—	0.013	0.021
8 *S. hyperborea*	pop. 24	17	8	17	6	7	6	6	—	0.011
9 *S. rivularis*	pop. 26	19	13	20	11	9	9	10	5	—

particular population of *S. cernua* that contributed genomes to *S. svalbardensis* lacked these six RAPD markers, and that the sample size used in this study was insufficient to detect this particular variation within *S. cernua.*

The ITS data do not provide strong independent evidence for or against any of the hypotheses on the origin of *S. svalbardensis.* It is noteworthy that we neither found distinct additivity of two parental ITS types in *S. svalbardensis,* nor that the ITS of *S. svalbardensis* had been homogenized via concerted evolution in the direction of either *S. cernua* or *S. rivularis,* the most probable progenitors of *S. svalbardensis* as inferred from the RAPD and *matK* data. The absence of clear ITS additivity in the material analyzed may be caused by undetected variation in *S. cernua,* which showed large variation in ITS (cf. Table 3). In addition, it appears that homogenizing of ITS sequences via concerted evolution requires many sexual generations for completion (cf. Wendel, Schnabel, and Seelanan, 1995; Brochmann, Nilsson, and Gabrielsen, 1996), and it is possible that *S. svalbardensis* may have existed as a single or a few clones since its origin.

In conclusion, when analyzed in concert, the data suggest that the endemic polyploid *Saxifraga svalbardensis* originated as a hybrid between *S. cernua* and *S. rivularis.* Based on the *matK* sequences, *S. rivularis* was the likely chloroplast donor (i.e., maternal parent); chloroplasts have been shown to be maternally inherited in two other genera of the Saxifragaceae (Soltis, Soltis, and Ness, 1990). Because the RAPD data indicate that *S. svalbardensis* has been derived from *S. rivularis* and *S. cernua,* it is likely that *S. cernua* is the paternal parent of *S. svalbardensis.* This hypothesis is also supported by the observation that many plants of *S. cernua* are female-sterile with rudimentary styles (Molau, 1992). Thus, *S. cernua* would be the likely pollen donor.

The virtual absence of molecular genetic variation in *S. svalbardensis* in spite of the considerable variation in one of its progenitors (*S. cernua*) suggests that *S. svalbardensis* may have originated only once. Hence, *S. svalbardensis* may be one of the few examples of a polyploid examined with molecular markers that appears to have originated a single time (cf. review in Soltis and Soltis, 1993). Its low level of variation also suggests that *S. svalbardensis* is a neopolyploid of very recent, most likely postglacial, origin.

The high levels of ITS and RAPD divergence among the seven populations analyzed of *S. cernua* suggest that this polyploid, in contrast to *S. svalbardensis,* may have formed recurrently. This conclusion is not unexpected because *S. cernua* also shows very high levels of chromosomal and morphological variation. It cannot be entirely excluded, however, that the variation within *S. cernua* has been caused by differentiation since its origin rather than multiple origins. *Saxifraga cernua* may thus add to the already numerous examples of recurrently originated, polyploid plant species (reviewed in Soltis and Soltis, 1993). Multiple origins of arctic polyploids have previously been demonstrated for several species of *Draba,* and this feature of polyploid evolution is probably of particular importance in arctic-alpine floras because of their high percentage of polyploidy (Brochmann, P. S. Soltis, and D. E. Soltis, 1992a, b; Brochmann, D. E. Soltis, and P. S. Soltis, 1992).

The molecular data also provide strong evidence for the hypothesis that *S. hyperborea* is one of the diploid progenitors of the tetraploid *S. rivularis.* These two species are very similar in both *matK* and ITS sequences, in agreement with the high degree of morphological and ecological similarity between them. Thus, the virtually identical *matK* sequences of *S. hyperborea, S. rivularis,* and *S. svalbardensis* (cf. Fig. 4) suggest that the diploid *S. hyperborea* was the maternal parent of the tetraploid *S. rivularis,* which in turn was the maternal parent of the pentaploid *S. svalbardensis.*

The origin of S. svalbardensis: other lines of evidence—Under the *rivularis* hybrid hypothesis, the chromosome number reported in *S. svalbardensis* ($2n \approx 64$; Borgen and Elven, 1983) may be explained by fertilization of a normal, reduced egg in the tetraploid *S. rivularis* ($n = 2x = 26$; $x = 13$) by a normal, reduced pollen grain from hexaploid *S. cernua* or an unreduced pollen grain from triploid *S. cernua* ($3x = 36$; $x = 12$), i.e., the exact chromosome number of *S. svalbardensis* may be $2n = 5x = 62$ based on a combination of $x = 12$ and $x = 13$. Alternatively, an unbalanced pollen grain from an aneuploid race of *S. cernua* may have been involved. Although chromosome counts have not been obtained for *S. cernua* in Svalbard, this species probably shows a wide range of chromosomal variation also in this area because the pollen stainability shows large variation both among and within geographically delimited ''populations,'' each of which probably has a multiclonal structure (C. Brochmann, unpublished data). In contrast, *S. svalbardensis* always has lower levels of pollen stainability, shows very low levels of molecular genetic variation, and is morphologically uniform throughout its distribution area in Svalbard (C. Brochmann, personal observation). These characteristics suggest that the single chromosome count of *S. svalbardensis* (Borgen and Elven, 1983) is representative and that the species invariably is approximately pentaploid.

It appears that the only morphological character used to support the *hyperborea* hybrid hypothesis (e.g., by Elvebakk, 1979; Elven and Elvebakk, 1996) is the anthocyanic-colored petals shared by *S. svalbardensis* and *S. hyperborea*; *S. cernua* and most populations of *S. rivularis* have white petals (cf. Øvstedal, 1975; Andersen and Øvstedal, 1983). The bulbils of *S. cernua* are, however, also anthocyanic-colored, and detailed analysis has revealed that all of the four species of section *Mesogyne* in Svalbard contain the same five anthocyanin compounds although in different quantities and proportions (Andersen and Øvstedal, 1988). Thus, petal color probably cannot be used to discriminate between the hypotheses for the origin of *S. svalbardensis.*

The *rivularis* hybrid hypothesis, on the other hand, is supported by two obviously independent morphological characters: the presence of subterranean runners and semi-inferior ovary in *S. svalbardensis.* The presence of runners in *S. svalbardensis* can be explained as a contribution from *S. rivularis,* which also has runners, whereas runners are absent from both *S. hyperborea* and *S. cernua.* The ovary is immersed to about one-third in *S. sval-*

bardensis, less immersed in *S. hyperborea,* more immersed in *S. rivularis,* and only slightly immersed in *S. cernua* (Øvstedal 1975). Thus, the degree of ovary immersion in *S. svalbardensis* is intermediate between that of *S. cernua* and *S. rivularis.* It should be noted, however, that both runners and ovary inferiority may have a simple genetic basis, and these characters may vary within species if sufficient material is examined. Small (1–2 mm) runners have, for example, been observed in plants that otherwise resemble *S. hyperborea* very closely (C. Brochmann, unpublished data).

The relationship between S. svalbardensis and S. opdalensis and their taxonomic status—It is intriguing that the results of this study suggest that *S. svalbardensis* has originated from the same progenitors as previously hypothesized (Flugsrud, 1985) for the southern Norwegian, narrow endemic *S. opdalensis,* which is morphologically (e.g., Elven, 1994) and cytologically ($2n$ = 48–50; Engelskjøn, 1979) quite different from *S. svalbardensis.* Notably, the diploid *S. hyperborea* does not occur in the Scandinavian mainland. Morphometric variation and pollen stainability in a number of populations of *S. opdalensis, S. cernua,* and *S. rivularis* from southern Norway suggested that *S. opdalensis* originated from *S. cernua* × *rivularis,* although the possibility that *S. opdalensis* differentiated directly from *S. cernua* could not definitely be disregarded (Flugsrud, 1985; see also Holaker, Nordhagen, and Berg, 1960). All of the populations that clearly could be referred to *S. opdalensis* were morphologically uniform and similar to each other, suggesting a single origin of these populations, whereas one deviating population resembled *S. cernua* more closely and may have another origin (Flugsrud, 1985). It is also possible that some northern Scandinavian populations that morphologically resemble *S. opdalensis* and/or *S. svalbardensis* (see Holaker, Nordhagen, and Berg, 1960; Nilsson, 1986; Rune, 1988; Elven, 1994) have similar, but independent origins, but these populations have not yet been investigated in detail.

That the same parental species probably have given rise to two morphologically and chromosomally distinguishable taxa by hybridization in different geographic areas may seem surprising but is nevertheless easily explained. Both *S. cernua* and *S. rivularis* are widespread, polyploid species, and there are, as noted, considerable morphological, chromosomal, and molecular genetic variations within *S. cernua.* It is likely that two very divergent races of *S. cernua* have been involved in the formation of *S. svalbardensis* and *S. opdalensis,* respectively. Further supporting this possibility is the fact that hybridization between polyploids sometimes produces unpredictable and varying hybrid morphologies because of complicated phenotypic effects of their duplicated genes, as demonstrated, for example, in artificial F_1 and F_2 hybrids between polyploids in *Draba* (Brochmann, Borgen, and Stedje, 1993). An interesting case parallel to that of *S. svalbardensis* and *S. opdalensis* has been suggested in *Helianthus* (Rieseberg, 1991), in which three species (*H. paradoxus, H. anomalus,* and *H. deserticola*) probably have originated by hybridization between the same two parental taxa (*H. annuus* and *H. petiolaris* ssp. *fallax*).

Implications for interpreting biodiversity in the Arctic—The genetic data reported herein for arctic *Saxifraga* species as well as those previously reported for arctic species of *Draba* (Brochmann and Elven, 1992; Brochmann, P. S. Soltis, and D. E. Soltis, 1992a, b; Brochmann, D. E. Soltis, and P. S. Soltis, 1992) have important consequences for interpreting biodiversity in the Arctic. Traditional conservation biology pays much attention to narrow endemic species such as the polyploids *Saxifraga svalbardensis* and *Draba cacuminum.* However, these endemics only contain a fraction of the genetic and ecological variation represented by their recurrently formed, polyploid progenitor species *S. cernua* and *D. norvegica,* respectively, which are both widespread in the Arctic (this study and Brochmann and Elven, 1992; Brochmann, P. S. Soltis, and D. E. Soltis, 1992a). A few individuals of *S. svalbardensis,* for example, sufficiently represent the genetic and ecological variation present in this species, whereas numerous populations of *S. cernua* are needed to represent the variation in this polymorphic species.

The traditional focus on endemism in conservation biology is particularly understandable in the case of arctic floras; these floras are probably relatively young and thus exhibit low levels of endemism. This focus is paradoxical, however, because arctic floras are characterized by highly reticulate neopolyploid evolution resulting in widely circumscribed, variable taxonomic species. The considerable width of the species concept necessarily used in the Arctic therefore leads to considerable underestimates of arctic biodiversity when endemism and mere species numbers are used as criteria.

LITERATURE CITED

ANDERSEN ("ANDERSØN"), Ø. M., AND D. O. ØVSTEDAL. 1983. Anthocyanin content in *Saxifraga svalbardensis* and some allied species. *Biochemical Systematics and Ecology* 11: 239–241.

———, AND ———. 1988. Anthocyanin patterns of European *Saxifraga* species. *Biochemical Systematics and Ecology* 16: 545–550.

ASHTON, P. A., AND R. J. ABBOTT. 1992. Multiple origins and genetic diversity in the newly arisen allopolyploid species, *Senecio cambrensis* Rosser (Compositae). *Heredity* 68: 25–32.

BALDWIN, B. G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* 1: 3–16.

———. 1993. Molecular phylogenetics of *Calycadenia* (Compositae) based on ITS sequences of nuclear ribosomal DNA: chromosomal and morphological evolution reexamined. *American Journal of Botany* 80: 222–238.

———, M. J. SANDERSON, J. M. PORTER, M. F. WOJCIECHOWSKI, C. S. CAMPBELL, AND M. J. DONOGHUE. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.

BORGEN, L., AND R. ELVEN. 1983. Chromosome numbers of flowering plants from northern Norway and Svalbard. *Nordic Journal of Botany* 3: 301–306.

BROCHMANN, C. 1992. Polyploid evolution in arctic-alpine *Draba* (Brassicaceae). *Sommerfeltia Supplement* 4: 1–37.

———, L. BORGEN, AND B. STEDJE. 1993. Crossing relationships and chromosome numbers of Nordic populations of *Draba* (Brassicaceae), with emphasis on the *D. alpina* complex. *Nordic Journal of Botany* 13: 121–147.

———, AND R. ELVEN. 1992. Ecological and genetic consequences of polyploidy in arctic *Draba* (Brassicaceae). *Evolutionary Trends in Plants* 6: 111–124.

———, T. NILSSON, AND T. M. GABRIELSEN. 1996. A classic example of postglacial allopolyploid speciation re-examined using RAPD

markers and nucleotide sequences: *Saxifraga osloensis* (Saxifragaceae). *Symbolae Botanicae Upsaliensis* 31(3): 75–89.

———, D. E. SOLTIS, AND P. S. SOLTIS. 1992. Electrophoretic relationships and phylogeny of Nordic polyploids in *Draba* (Brassicaceae). *Plant Systematics and Evolution* 182: 35–70.

———, P. S. SOLTIS, AND D. E. SOLTIS. 1992a. Multiple origins of the octoploid Scandinavian endemic *Draba cacuminum*: electrophoretic and morphological evidence. *Nordic Journal of Botany* 12: 257–272.

———, ———, AND ———. 1992b. Recurrent formation and polyphyly of Nordic polyploids in *Draba* (Brassicaceae). *American Journal of Botany* 79: 673–688.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

———, ———, A. H. D. BROWN, AND J. P. GRACE. 1990. Multiple origins of polyploids in the *Glycine tabacina* complex inferred from chloroplast DNA polymorphism. *Proceedings of the National Academy of Sciences, USA* 87: 714–717.

ELVEBAKK, A. 1979. Plantesosiologi og-fenologi i eit arktisk område: Stuphallet, Brøggerhalvøya, Svalbard. Cand. real. thesis, University of Trondheim, Trondheim.

ELVEN, R. 1994. Norsk flora, 6d ed. of Lid, J., and D. T. Lid. Det Norske Samlaget, Oslo.

———, AND A. ELVEBAKK. 1996. Part 1. Vascular plants. *In* A. Elvebakk and P. Prestrud [eds.], A catalogue of Svalbard plants, fungi, algae, and cyanobacteria, 9–55. Norsk Polarinstitutt Skrifter 198, Oslo.

ENGELSKJØN, T. 1979. Chromosome numbers in vascular plants from Norway, including Svalbard. *Opera Botanica* 52: 1–38.

———, AND G. KNABEN. 1971. Chromosome numbers of Scandinavian arctic-alpine plant species. III. *Acta Borealia, A. Scientia,* 28: 1–30.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

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

FLUGSRUD, K. 1985. En morfologisk, økologisk og taksonomisk analyse av *Saxifraga opdalensis.* Cand. scient. thesis, University of Oslo, Oslo.

HOLAKER, P., R. NORDHAGEN, AND R. Y. BERG. 1960. *Saxifraga opdalensis* A. Bl. gjenfunnet. Foreløpig meddelelse. *Blyttia* 18: 108–112. (In Norwegian.)

HUFF, D. R., AND J. M. BARA. 1993. Determining genetic origins of aberrant progeny from facultative apomictic Kentucky bluegrass using a combination of flow cytometry and silver-stained RAPD markers. *Theoretical and Applied Genetics* 87: 201–208.

JOHNSON, L. A., AND D. E. SOLTIS. 1994. *matK* DNA sequences and phylogenetic reconstruction in Saxifragaceae s. str. *Systematic Botany* 19: 143–156.

———, AND ———. 1995. Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using *matK* sequences. *Annals of the Missouri Botanical Garden* 82: 149–175.

KIM, K.-J., AND R. K. JANSEN. 1994. Comparisons of phylogenetic hypotheses among different data sets in dwarf dandelions (*Krigia, Asteraceae*): additional information from internal transcribed spacer sequences of nuclear ribosomal DNA. *Plant Systematics and Evolution* 190: 157–185.

LÖVE, A., AND D. LÖVE. 1975. Cytotaxonomical atlas of the arctic flora. J. Cramer, Vaduz.

MOLAU, U. 1992. On the occurrence of sexual reproduction in *Saxifraga cernua* and *S. foliolosa* (Saxifragaceae). *Nordic Journal of Botany* 12: 197–203.

NILSSON, Ö. 1986. Nordisk fjällflora. Bonniers, Stockholm. (In Swedish.)

ØVSTEDAL, D. O. 1975. A new *Saxifraga* from Svalbard. *Astarte* 8: 23–27.

RANKER, T. A., C. H. HAUFLER, P. S. SOLTIS, AND D. E. SOLTIS. 1989. Genetic evidence for allopolyploidy in the neotropical fern *Hemionitis pinnatifida* (Adiantaceae) and the reconstruction of an ancestral genome. *Systematic Botany* 14: 439–447.

RIESEBERG, L. H. 1991. Homoploid reticulate evolution in *Helianthus* (Asteraceae): evidence from ribosomal genes. *American Journal of Botany* 78: 1218–1237.

ROHLF, F. J. 1990. NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 1.60. Exeter Software, Setauket, NY.

RØNNING, O. I. 1979. Svalbards flora, 2d ed. Norsk Polarinstitutt, Oslo.

RUNE, O. 1988. Serpentine flora of Scandinavia. *Blyttia* 46: 43–51. (In Norwegian with English summary.)

SKOVSTED, A. 1934. Cytological studies in the tribe *Saxifrageae. Dansk Botanisk Arkiv* 8(5): 1–52.

SKROCH, P., J. TIVANG, AND J. NIENHUIS. 1992. Analysis of genetic relationships using RAPD marker data. *In* Applications of RAPD Technology to Plant Breeding, Joint Plant Breeding Symposia Series, November 1, 1992, 26–30. Minneapolis, MN.

SOLTIS, D. E., R. K. KUZOFF, E. CONTI, R. GORNALL, AND K. FERGUSON. 1996. *matK* and *rbcL* gene sequence data indicate that *Saxifraga* (Saxifragaceae) is polyphyletic. *American Journal of Botany* 83: 371–382.

———, D. R. MORGAN, A. GRABLE, P. S. SOLTIS, AND R. KUZOFF. 1993. Molecular systematics of Saxifragaceae sensu stricto. *American Journal of Botany* 80: 1056–1081.

———, AND P. S. SOLTIS. 1989. Allopolyploid speciation in *Tragopogon*: insights from chloroplast DNA. *American Journal of Botany* 76: 1119–1124.

———, AND ———. 1993. Molecular data and the dynamic nature of polyploidy. *Critical Reviews in Plant Sciences* 12: 243–273.

———, ———, T. G. COLLIER, AND M. L. EDGERTON. 1991. Chloroplast DNA variation within and among genera of the *Heuchera* group (Saxifragaceae): evidence for chloroplast transfer and paraphyly. *American Journal of Botany* 78: 1091–1112.

———, ———, AND B. D. NESS. 1990. Maternal inheritance of the chloroplast genome in *Heuchera* and *Tolmiea* (Saxifragaceae). *Journal of Heredity* 81: 168–170.

STEELE, K. P., AND R. VILGALYS. 1994. Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene *matK*. *Systematic Botany* 19: 126–142.

SWOFFORD, D. L. 1993. PAUP: Phylogenetic analysis using parsimony, version 3.1.1. Illinois Natural History Survey, Champaign, IL.

VAN HOUTEN, W. H. J., N. SCARLETT, AND K. BACHMANN. 1993. Nuclear DNA markers of the Australian tetraploid *Microseris scapigera* and its North American diploid relatives. *Theoretical and Applied Genetics* 87: 498–505.

WEBB, D. A., AND R. J. GORNALL. 1989. A manual of saxifrages and their cultivation. Timber Press, Portland, OR.

WENDEL, J. F., A. SCHNABEL, AND T. SEELANAN. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). *Proceedings of the National Academy of Sciences, USA* 92: 280–284.

WERTH, C. R., S. I. GUTTMAN, AND W. H. ESHBAUGH. 1985. Recurring origins of allopolyploid species in *Asplenium. Science* 228: 731–733.

WILLIAMS, J. G. K., A. R. KUBELIK, K. J. LIVAK, J. A. RAFALSKI, AND S. V. TINGEY. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18: 6531–6535.

WYATT, R., I. J. ODRZYKOSKI, AND A. STONEBURNER. 1988. Allopolyploidy in bryophytes: recurring origins of *Plagiomnium medium. Proceedings of the National Academy of Sciences, USA* 85: 5601–5604.

YU, K. F., AND K. P. PAULS. 1993. Segregation of random amplified polymorphic DNA markers and strategies for molecular mapping in tetraploid alfalfa. *Genome* 36: 844–851.