



# Reproductive strategies in some arctic *Saxifraga* (Saxifragaceae), with emphasis on the narrow endemic *S. svalbardensis* and its parental species

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Arctic saxifrages show conspicuous reproductive and chromosomal variation. We examined sexual and asexual traits in 43 phytotron-cultivated Svalbard populations of nine species, including the endemic, supposedly entirely asexual and aneuployploid *S. svalbardensis* and its parental species, *S. cernua* and *S. rivularis*. All species were self-compatible hermaphrodites with low pollen/ovule ratios, including the strongly protandrous *S. cernua*, which previously has been reported as self-incompatible with an androdioecious mating system. Spontaneous selfing resulted in considerable seed set in several species and a few seeds in *S. svalbardensis* and *S. cernua*; hand-selfing and cross-pollination often increased seed set in the two latter species. Self-fertilized seeds of *S. svalbardensis* and *S. cernua* were viable and developed into normal, vigorous plants. *Saxifraga rivularis* and its close relative *S. hyperborea* were strongly autogamous. The bulbil-reproducing *S. svalbardensis* and *S. cernua* showed extreme variation in fertility, probably because of frequent aneuploidy. Many plants of *S. cernua* were fully fertile, suggesting that although natural seed set rarely has been observed, sexual reproduction is frequent enough to maintain its previously reported high levels of clonal diversity. Some plants of *S. svalbardensis* were also fairly fertile. This species may have considerable evolutionary potential; sexual events can lead to increasingly fertile genets with euploid chromosome numbers.

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ADDITIONAL KEY WORDS: clonal plants – fertility – polyploidy – P/O ratios – seed set – self-pollination.

## INTRODUCTION

The genus *Saxifraga* is characterized by complex chromosomal evolution and conspicuous reproductive variation. In their comprehensive account of the genus, Webb & Gornall (1989) emphasized that more experimental work on breeding systems is badly needed and might yield interesting results. A number of studies have later addressed reproductive characteristics and variation in *Saxifraga* (Olesen & Warncke, 1990; Molau, 1992; Molau & Prentice, 1992; Stenström & Molau, 1992; Warncke *et al.*, 1993; Berggren & Haugset, 1994; Lindgaard-Hansen & Molau, 1994; Dahlgaard & Warncke, 1995; Vargas & Feliner, 1996; Holderegger, 1996, 1998; Gugerli, 1997, 1998; Gabrielsen & Brochmann, 1998; Hollingsworth *et al.*, 1998; Meier & Holderegger, 1998; Gugerli, Eichenberger & Schneller, 1999).

Species of *Saxifraga* are important components of arctic floras. Arctic saxifrages are phylogenetically diverse and represent a number of different infrageneric taxa, which suggests that the Arctic has been colonized independently by several divergent evolutionary lineages within the genus (Savile, 1975; Rebristaya & Yurtsev, 1984; Zhmylev, 1997a, b; Zhmylev *et al.*, 1999). The arctic taxa are also diverse in reproductive strategies and chromosome numbers. They span ranges of variation from various sexual to asexual strategies, from autogamy to allogamy, and from diploid to high polyploid levels (Rebristaya & Yurtsev, 1984; Webb & Gornall, 1989).

Thirteen species of *Saxifraga* occur in the Norwegian arctic archipelago of Svalbard (Elven & Elvebakk, 1996), representing six of the 15 sections enumerated by Webb & Gornall (1989). Chromosome numbers have been determined in Svalbard material of all species except *S. aizoides* and *S. cernua*, for which counts are available from Norwegian mainland populations (summarized in Brochmann & Steen, 1999). The Svalbard taxa, their chromosome numbers and inferred

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ploidal levels are listed in Table 1. The Svalbard species vary in chromosome number from  $2n=20$  (*S. tenuis*) to  $2n=112$  (*S. hieracifolia*). The ancestral base number in *Saxifraga* is unknown; the lowest gametophytic chromosome number reported in each section varies from  $n=5$  to  $n=18$  (Webb & Gornall, 1989). Some of the ploidal levels listed in Table 1 are inferred based on data from enzyme electrophoresis (Brochmann & Steen, 1999). *Saxifraga tenuis* and *S. hyperborea* are, for example, probably tetraploids rather than diploids as traditionally assumed (by e.g. Rebristaya & Yurtsev, 1984) because they exhibit duplications at enzyme electrophoretic loci and are fully fertile, suggesting that they are eupolyploid with original base numbers of  $x=5$  and  $x=6$ , respectively. Thus, all Svalbard species of *Saxifraga* are probably polyploid, varying from tetraploid to approximately 22-ploid.

Five of the Svalbard species regularly reproduce clonally via runners and/or bulbils (*S. cernua*, *S. flagellaris*, *S. foliolosa*, *S. rivularis*, and *S. svalbardensis*). The unspecialized, usually hermaphroditic flowers of *Saxifraga* are insect-pollinated. Most species are self-compatible (Webb & Gornall, 1989), but self-incompatibility has been reported for some species, including *S. cernua* and *S. foliolosa* (Molau, 1992; Molau & Prentice, 1992). There are no precise reproductive data available for Svalbard populations of *Saxifraga*, but some of the species occurring in Svalbard have been studied in other arctic or arctic-alpine areas, where they vary from strongly autogamous with low pollen/ovule ratios (e.g. *S. rivularis*; P/O ratio 30–160) to predominantly allogamous with high pollen/ovule ratios (e.g. *S. oppositifolia*; P/O ratio 1470; Tikhmenev, 1984; Molau & Prentice, 1992; Stenström & Molau, 1992; Molau, 1993; Berggren & Haugset, 1994). There are also reports of gynodioecy (e.g. *S. cespitosa*; Warming, 1909; Molau & Prentice, 1992) and even of the extremely rare androdioecious mating system (*S. cernua* and *S. foliolosa*; Molau, 1992; Molau & Prentice, 1992).

Two of the species occurring in Svalbard, the local polyploid endemic *S. svalbardensis* and one of its parental species, the widespread *S. cernua*, have been the focus of research in recent years. Both species reproduce regularly via bulbils produced in the axils of the cauline leaves, and although they usually flower luxuriantly, seed set has only very rarely been observed in *S. cernua* (reports summarized by Molau, 1992) and never in *S. svalbardensis* (cf. Øvstedal, 1975; Elven & Elvebakk, 1996).

*Saxifraga svalbardensis* is one of only three species that possibly are endemic to this arctic archipelago (Elven & Elvebakk, 1996; Zhmylev *et al.*, 1999), which was fully glaciated at the Weichselian maximum (Landvik *et al.*, 1998). It occurs on several of the islands, and it is common in some areas (Elven &

Elvebakk, 1996). The species is virtually invariable for all molecular markers examined (internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA and random amplified polymorphic DNA (RAPD) markers), and it has probably originated postglacially in Svalbard as a hybrid between local populations of the circumpolar species *S. cernua* and *S. rivularis* (Brochmann *et al.*, 1998; Steen *et al.*, 2000). The four populations investigated of *S. svalbardensis* were identical for all RAPD markers examined, and only a single nucleotide polymorphism was observed in their ITS sequences. Assuming it is possible that this polymorphism has arisen as a somatic mutation, the apparently entirely sterile *S. svalbardensis* may consist of only two clones derived from a single hybridization event, unable to reproduce sexually because of its aneuploid chromosome number.

The mating system of the variable and unstable polyploid *S. cernua* appears to be complex and is poorly understood (Wehrmeister & Bonde, 1977; Godfree, 1979; Webb & Gornall, 1989; Molau, 1992). A wide range of chromosome numbers has been reported in this species, including  $2n=24, 26, c. 33, 36, 44, 44-46, 48, 50, 52, c. 54, 55-57, 56, 60, 62, 64, c. 66, c. 68, 70, 72$  (Löve & Löve, 1975; Zhukova, 1980; Zhukova & Petrovsky, 1987; Webb & Gornall, 1989). If the base number is  $x=6$ , as inferred for its close relatives *S. hyperborea* and *S. rivularis* because of their duplicated loci (Brochmann & Steen, 1999), these numbers include euploid levels from tetra- to 12-ploid. Regardless of its correct base number, the series of chromosome numbers also includes many aneuploid ones, and it is obvious that *S. cernua* has an extremely dynamic and complex history of chromosomal evolution.

Although *S. cernua* is described as reproducing exclusively via bulbils in most contemporary floras, Webb & Gornall (1989) and Molau (1992) drew attention to a few records of successful sexual reproduction, also in Svalbard (Euroala, 1972). Seed set was also occasionally observed in recent field studies in northern Sweden and Greenland and in a survey of herbarium material by Molau (1992) and Molau & Prentice (1992). Following Godfree (1979), Webb & Gornall (1989) suggested that the species is self-incompatible, and that large populations consist of a single clone. They suggested that the rarity of seed set was caused by a combination of self-incompatibility and monoclonality of the populations; only in extremely rare cases, a pollinating insect may fly far enough to cross the boundary between one clone and another. On the basis of field experiments, Molau (1992) and Molau & Prentice (1992) reported that the species was self-incompatible in northern Sweden and that it had an androdioecious mating system, with rare functional hermaphrodites and with most populations consisting

**Table 1.** Survey of sections and subsections (Webb & Gornall, 1989) and species (Elven & Elvebakk, 1996) of *Saxifraga* occurring in the arctic Norwegian archipelago of Svalbard, and their chromosome numbers and inferred ploidal levels (Brochmann & Steen, 1999). Chromosome numbers outside parentheses are based on populations from Svalbard except for those of *S. aizoides* and *S. cernua*, which are based on Norwegian mainland populations. If different, data based on non-Norwegian populations are given in parentheses

Section	Subsection	Species/subspecies	2n	Ploidal level
<i>Ciliatae</i> Haw.	<i>Hirculoidea</i> Engl. & Irmsch.	<i>S. hirculus</i> L.	32 (16)	4x (2x)
<i>Ciliatae</i> Haw.	<i>Flagellares</i> (C. B. Clarke) Engl. & Irmsch.	<i>S. flagellaris</i> Sternb. & Willd. ssp. <i>platysepala</i> (Trautv.) A. E. Forsild	32	4x
<i>Micranthes</i> (Haw.) D. Don	<i>Stellares</i> (Engl. & Irmsch.) Gornall	<i>S. foliolosa</i> R. Br.	56	8x?
<i>Micranthes</i> (Haw.) D. Don	<i>Micranthes</i> (Haw.) Gornall	<i>S. tenuis</i> (Wahlenb.) H. Smith ex Lindm.	20	4x
<i>Micranthes</i> (Haw.) D. Don	<i>Micranthes</i> (Haw.) Gornall	<i>S. nivalis</i> L.	60	12x
<i>Micranthes</i> (Haw.) D. Don	<i>Micranthes</i> (Haw.) Gornall	<i>S. hieracifolia</i> Waldst. & Kit. ex Willd.	112 (100–120)	c. 22x (20x–24x)
<i>Porphyryon</i> Tausch	<i>Oppositifoliae</i> Hayek	<i>S. oppositifolia</i> L.	52 (26)	8x? (4x?)
<i>Xanthizoon</i> Griseb.		<i>S. aizoides</i> L.	26	4x?
<i>Mesogyne</i> Sternb.		<i>S. hyperborea</i> R. Br.	26	4x?
<i>Mesogyne</i> Sternb.		<i>S. rivularis</i> L.	52	8x?
<i>Mesogyne</i> Sternb.		<i>S. svalbardensis</i> Øvstedal	c. 64	c. 11x?
<i>Mesogyne</i> Sternb.		<i>S. cernua</i> L.	44–66 (24–72)	c. 7x?–11x? (4x?–12x?)
<i>Saxifraga</i>	<i>Triplinervium</i> (Caudin) Gornall	<i>S. cespitosa</i> L.	80	10x

exclusively of males with rudimentary styles and stigmatic surfaces without papillae.

We have recently examined molecular genetic variation within and among Svalbard populations of *S. cernua*. Some populations were extremely divergent in RAPD markers and ITS sequences; 21 divergent sites (4.9%) were observed in ITS between the two most divergent populations (Brochmann *et al.*, 1998). RAPD analysis at different spatial scales within two populations of *S. cernua* identified 13 putative clones among 93 plants, and nine of these were detected among 39 plants within a single 3 × 3 m sample plot (Gabrielsen & Brochmann, 1998). The plants analysed represented only 3.7% of the 1058 plants observed in this sample plot. Thus, even very local patches of *S. cernua* can be highly multiclonal, suggesting that sexual reproduction is more frequent than indicated by the rarity of seed set observations.

In the present study, we investigated reproductive characteristics of the Svalbard species of *Saxifraga* based on field-collected plants cultivated under uniform 'arctic conditions' in a phytotron. All of the 13 species occurring in Svalbard were sampled, and nine species were successfully maintained in cultivation where they flowered luxuriantly. Four species were excluded from the experiments (*S. foliolosa* and *S. tenuis*, because most plants of these species died early in cultivation; *S. oppositifolia*, because it flowered poorly under our cultivation conditions; and *S. flagellaris*, because it was not flowering when most of the experiments were carried out). Our intention was to provide basic information on breeding systems in *Saxifraga* in Svalbard, and to compare the results with data available from other geographic areas. Of particular interest were the potential and modes of sexual reproduction in the endemic *Saxifraga svalbardensis* and its parental species, *S. cernua* and *S. rivularis*.

## MATERIAL AND METHODS

The final set of material used included 43 populations of nine species, of which *S. cernua* and *S. cespitosa* each comprised two morphologically divergent forms (Table 2): *S. aizoides* (three populations), *S. cernua*, large-flowered morph (11 populations), *S. cernua*, small-flowered morph (two populations), *S. cespitosa*, white-flowered morph (six populations), *S. cespitosa*, yellow-flowered morph (two populations), *S. hieracifolia* (two populations), *S. hirculus* (three populations), *S. hyperborea* (three populations), *S. nivalis* (one population), *S. rivularis* (five populations), and *S. svalbardensis* (five populations). Vouchers of cultivated and field-collected plants are deposited in O.

The differences observed in the field between the morphs of *S. cernua* and *S. cespitosa* were retained in cultivation. The small-flowered morph of *S. cernua* was observed only in Dickson Land and had several-flowered inflorescences with few, light red bulbils, urn-shaped flowers, and small petals. The large-flowered morph of this species was widespread and had one-flowered inflorescences with many, dark red bulbils and more spreading, larger petals. The yellow-flowered morph of *S. cespitosa* was also limited to Dickson Land, exclusively occurring in damp sites with dense moss vegetation, and it had urnshaped flowers with deep yellow, small petals (referred to as *S. aurea* (Hadac) Rønning by Rønning, 1996). The white-flowered morph of *S. cespitosa*, which was widespread and mainly occurred in gravelly pioneer sites, was characterized by larger, more spreading petals that usually were purely white, but some plants of this morph had yellowish white or pale yellow petals.

Samples of 20–30 plants were taken from some populations to assess intrapopulation variation; 5–10 plants were sampled from the other populations. The plants were cultivated in an insect-free phytotron with two flowering seasons per year. To induce flowering, the plants were vernalized at 6(–9)°C with 10 h daylength for 12 weeks. In the flowering season, the plants were cultivated at 12–14°C with 24 h daylength for 12 weeks (see Brochmann, Soltis & Soltis, 1992 for further details). A first screening of pollen stainability and pilot tests for the other experiments were carried out during the first flowering season; complete experiments were carried out during the second flowering season to avoid effects of potential differences between seasons. It was not possible to obtain identical sample sizes in all experiments because some plants died and because all plants did not flower at the same time. One flower was used from each plant in each experiment if not otherwise stated.

Ability to set asexual seeds was tested by emasculating buds of all species before the anthers reached maturity. Capacity for vegetative reproduction was examined in *S. cernua* and *S. svalbardensis*. Presence of subterranean runners was scored twice for each plant; in the field and after some months in cultivation. Number of bulbils was counted in two leaf axils on the middle part of one stem and on one entire stem in each cultivated plant.

Floral gender and development were observed regularly in the entire flowering period, including timing of maturation of anthers and stigmas, movement of stamens, and autodeposition of pollen on the stigma. Pollen/ovule-ratios (P/O-ratios; Cruden, 1977) were calculated separately for individual flowers. Pollen counts included all grains, mis-shapen as well as well-developed ones. The total number of pollen grains per flower was estimated by crushing one anther in 0.4 ml

**Table 2.** Collection data for the populations analysed of *Saxifraga*. All populations were collected on the island of Spitsbergen in the arctic Norwegian archipelago of Svalbard

Species	Pop. no.	Main geographic area	Collection data
<i>S. aizoides</i>	78	Dickson Land	Norskedalen E, SW slope of Rotundafjellet. Alt. 60 m. 6.vii.1992.
	163	Olav V Land	Billefjorden, Rudmosepynten. WH 3537. Alt. 20–30 m. 9.vii.1993.
	250	Sabine Land	Sassendalen NW, 1 km SE of Nøisdalen. WG 4397. Alt. 25–50 m. 19.vii.1993.
<i>S. cernua</i> (large-flowered)	12	Haakon VII Land	Blomstrandhalvøya, Hansneset—Sørvågen. VH 3671. Alt. 100 m. 21.vii.1992.
	31	Nordenskiöld Land	Adventdalen, Bolterdalen, W side. WG 2177. Alt. 100 m. 28.vii.1992.
	45	Nordenskiöld Land	Bjørndalen, E of the river. WG 0783. Alt. 30 m. 1.viii.1992.
	58	Nordenskiöld Land	Adventdalen, Longyearbyen—Isdammen. Alt. 1 m. 2.viii.1992.
	60	Dickson Land	Siklarhallet, SE slope. WH 1511. Alt. 140 m. 4.viii.1992.
	67	Dickson Land	W of Siklarhallet, N of Svenskehuset. WH 1512. Alt. 220 m. 5.viii.1992.
	76	Dickson Land	Studentdalen, SE of Rotundafjellet. Alt. 30 m. 6.viii.1992.
	81	Dickson Land	Siklarhallet, N of trig. point 113. WH 1312. Alt. 240 m. 7.viii.1992.
	134	Prins Karls Forland	Fuglehuken, below Fuglehukfjellet. VH 0363. Alt. 100 m. 7.vii.1993.
	207	Nordenskiöld Land	Adventdalen, NE slope of Bayfjellnosa. WG 1979. Alt. 50–150 m. 17.vii.1993.
	248	Sabine Land	Sassendalen NW, 1 km SE of Nøisdalen. WG 4397. Alt. 25–50 m. 19.vii.1993.
<i>S. cernua</i> (small-flowered)	66	Dickson Land	W of Siklarhallet, N of Svenskehuset. WH 1512. Alt. 220 m. 5.viii.1992.
	82	Dickson Land	Siklarhallet, N of trig. point 113. WH 1312. Alt. 240 m. 7.viii.1992.
<i>S. cespitosa</i> (white-flowered)	32	Nordenskiöld Land	Adventdalen, Bolterdalen, W side. WG 2177. Alt. 100 m. 29.vii.1992.
	50	Nordenskiöld Land	Bjørndalen, E of the river. WG 0783. Alt. 30 m. 1.viii.1992.
	65	Dickson Land	Svenskehuset, SE slope of Siklarhallet. WH 1511. Alt. 200 m. 5.viii.1992.
	77	Dickson Land	Studentdalen, SE of Rotundafjellet. Alt. 30 m. 6.viii.1992.
	83	Dickson Land	Siklarhallet, N of trig. point 113. WH 1312. Alt. 240 m. 7.viii.1992.
<i>S. cespitosa</i> (yellow-flowered)	212	Nordenskiöld Land	Adventdalen, NE slope of Bayfjellnosa. WG 1979. Alt. 50–150 m. 17.vii.1993.
	63	Dickson Land	Svenskehuset, SE slope of Siklarhallet. WH 1511. Alt. 200 m. 5.viii.1992.
	64	Dickson Land	Svenskehuset, SE slope of Siklarhallet. WH 1511. Alt. 200 m. 5.viii.1992.
<i>S. hieracifolia</i>	4	Haakon VII Land	Osian Sars mountain. Alt. 50 m. 19.vii.1992.
	21	Nordenskiöld Land	Adventdalen, Bolterdalen, W side. WG 2177. Alt. 100 m. 28.vii.1992.
<i>S. hirculus</i>	30	Nordenskiöld Land	Adventdalen, Bolterdalen, W side. WG 2177. Alt. 100 m. 31.vii.1992.
	48	Nordenskiöld Land	Bjørndalen, E of the river. WG 0783. Alt. 30 m. 1.viii.1992.
	219	Nordenskiöld Land	Adventdalen, NE slope of Bayfjellnosa. WG 1979. Alt. 50–150 m. 17.vii.1993.

continued

Table 2 – continued

Species	Pop. no.	Main geographic area	Collection data
<i>S. hyperborea</i>	17	Nordenskiöld Land	Adventdalen, Bolterdalen, E of Bolterelva. WG 2276. Alt. 90 m. 27.vii.1992.
	24	Nordenskiöld Land	Adventdalen, Breinosa, NW of summit. WG 2476. Alt. 410 m. 29.vii.1992.
	119	Nordenskiöld Land	Kapp Starostin NE of Isfjord Radio. VG 7369. Alt. 50–100 m. 8.vii.1993.
<i>S. nivalis</i>	5	Haakon VII Land	Ossian Sars mountain. Alt. 50 m. 19.vii.1992.
<i>S. rivularis</i>	26	Nordenskiöld Land	Adventdalen, Breinosa, NW of summit. WG 2375. Alt. 400 m. 29.vii.1992.
	47	Nordenskiöld Land	Bjørndalen, E of the river. WG 0783. Alt. 30 m. 1.viii.1992.
	133	Prins Karls Forland	Fuglehukén, below Fuglehukfjellet. VH 0363. Alt. 100 m. 7.vii.1993.
	204	Albert I Land	Magdalenafjorden, Gravneset. VJ 2036. Alt. 5–50 m. 14.vii.1993.
	229	Nordenskiöld Land	Bjørndalen. WG 0785. Alt. 20–100 m. 18.vii.1993.
<i>S. svalbardensis</i>	20	Nordenskiöld Land	Adventdalen, Bolterdalen, E of Bolterelva. WG 2276. Alt. 90 m. 27.vii.1992.
	28	Nordenskiöld Land	Adventdalen, Breinosa, NW of summit. WG 2375. Alt. 400 m. 29.vii.1992.
	40	Nordenskiöld Land	Bjørndalen, E of the river. WG 0783. Alt. 30 m. 1.viii.1992.
	59	Dickson Land	Siklarhallet, SW of Svenskehuset. WH 1511. Alt. 140 m. 4.viii.1992.
	233	Nordenskiöld Land	Bjørndalen. WG 0785. Alt. 20–100 m. 18.vii.1993.

70% ethanol with one droplet cotton blue in lactophenol, shaking, and counting the number of grains in five separate 10 µl subsamples under a light microscope (100×; cf. Dafni, 1992). The mean number of grains obtained for the subsamples was multiplied by the dilution factor (40) and number of stamens. Ovule numbers were determined in unfertilized ovaries.

Male fertility was estimated by pollen stainability in cotton blue in lactophenol (Radford *et al.*, 1974), determined in a sample of 200 grains from each plant. A second pollen sample was taken from another flower of the same plant after three or more days if the first sample contained less than 90% stained pollen, and the higher percentage was used.

Plants with the highest pollen stainability were selected as pollen donors in pollination experiments. Flowers were (1) left undisturbed to test for ability to set seed after spontaneous self-pollination ('auto-deposition' of pollen; cf. Molau, 1993), (2) pollinated by hand with excess self-pollen to test for self-compatibility and to test whether autogamous seed set was pollen limited, or (3) emasculated at an early stage and later cross-pollinated by hand with excess pollen (section *Mesogyne* only). Plants from different populations were used in the cross-pollinations to reduce the possibility for intraclonal crosses (i.e. selfing) in *S. cernua* and *S. svalbardensis*. One-tailed *t*-tests (SPSS

for Windows; Norusis, 1993) were used to test for differences in seed set obtained after different treatments. We used one-tailed tests because seed set is not expected to decrease when excess pollen is applied.

The seeds obtained after selfing in *Saxifraga svalbardensis* and *S. cernua* were tested for viability in a germination experiment. Ten seedlings (if available) were selected from each maternal population, raised to maturity, and examined for vigour and morphology.

## RESULTS

### CLONAL REPRODUCTION

No seeds were obtained in the tests for agamospermous seed production (Tables 3 and 4). All plants of *S. svalbardensis* (Table 5) and *S. rivularis* possessed subterranean runners in the field as well as in cultivation. Runners were absent in all field-collected plants examined of *S. cernua* and *S. hyperborea*, and runners were not produced by these species in cultivation. The large-flowered morph of *S. cernua* had, on the average, 14.46 bulbils per leaf axil, whereas the small-flowered morph of *S. cernua* and *S. svalbardensis* had considerably smaller numbers (means 4.35 and 2.53, respectively; Table 3). The total number of bulbils produced by each plant varied from 409–1325 in large-flowered *S. cernua* via 57–121 in small-flowered *S.*

*cernua* to 24–97 in *S. svalbardensis* (population means; Table 5).

#### FLORAL GENDER AND DEVELOPMENT

All plants were phenotypically hermaphroditic. Based on timing of dehiscence of anthers relative to emergence of fully developed papillae at the stigmatic surface, the flowers were slightly to strongly protandrous in five species (*S. cernua*, *S. cespitosa*, *S. hieracifolia*, *S. hirculus*, and *S. nivalis*), slightly protogynous in *S. hyperborea* and *S. rivularis*, and homogamous in *S. svalbardensis* (Table 3). Spontaneous bending of the stamens inwards in the flower was regularly observed in plants of all species examined (no observations were made in *S. aizoides* and *S. hirculus*). Autodeposition of pollen on the stigma occurred frequently in all populations except in the large-flowered morph of *S. cernua*; in this morph, the styles were often too short and the stigmas not fully developed when the stamens bent inwards.

The degree of protandry varied considerably in *S. cernua*. Most plants of the large-flowered morph were strongly protandrous, whereas the small-flowered morph was slightly protandrous. The initial male phase of individual flowers of the large-flowered morph usually lasted for 6–10 days. In this period, the gynoecium was conspicuously under-developed with short styles without stigmas, but longer styles with papillose stigmas were later developed in all plants that were observed throughout the entire flowering period (Table 5). Most flowers had a phenotypically hermaphroditic phase; the gynoecia reached full maturity before all anthers had dehisced. Autodeposition of pollen was occasionally observed in the large-flowered morph and regularly in the small-flowered morph.

In *S. svalbardensis*, the flowers were often irregular with 6–11 stamens and 1–3 stigmas. The flowers were homogamous with rapid maturation of stigmas and anthers, and autodeposition was regularly observed. In *S. rivularis* and *S. hyperborea*, all plants had rapidly selfing flowers, which sometimes were irregular with three stigmas and 8–12 stamens. These species were slightly protogynous; the stigmas appeared receptive before flower opening, but some anthers had already dehisced when the flower was half open.

In *S. cespitosa*, autodeposition first occurred some hours or even days after full flower opening ('delayed selfing'). Floral irregularities (1–3 stigmas and 6–11 stamens) were frequent, and variation was sometimes observed between flowers on the same plant. Some stamens were short with empty pollen sacs and some stigmas were reduced, but at least some morphologically normal flowers were observed in most plants. In *S. hieracifolia*, the anthers dehisced very early and autodeposition was observed, but the stamens were sometimes short and did not come into

contact with the stigma. In *S. nivalis*, the flowers were protandrous with anthers occasionally dehiscing at the bud stage, before the stigmas were receptive, but autodeposition was observed also in this species.

#### POLLEN/OVULE RATIOS

The mean number of ovules per flower varied from 105 in *S. hieracifolia* to 500 in *S. hirculus*, but there was large variation within several species (Tables 3 and 6). The pollen/ovule ratios were low; species means ranged from 61 in *S. hyperborea* and 93 in *S. svalbardensis* to 292 in *S. aizoides* and 715 in *S. hieracifolia*.

#### POLLEN STAINABILITY

Six species had fully stainable pollen (means >88%; Tables 3 and 6). Lower and variable stainabilities were observed in three species, *S. svalbardensis* (46.2%), *S. cernua* (64.2–74.1%), and *S. cespitosa* (65.5–70.7%). In section *Mesogyne*, most plants of *S. rivularis* and *S. hyperborea* had fully stainable pollen, whereas large variation was observed among individual plants of *S. cernua* and *S. svalbardensis* (Fig. 1). The two latter species contained entirely or almost male sterile plants as well as some fully or almost fully male fertile plants (up to 99.5% in *S. cernua* and 85.0% in *S. svalbardensis*). Within a single population of *S. cernua*, the pollen stainabilities ranged from 0% to 99.5% (mean 61.1%), and within one population of *S. svalbardensis*, the stainabilities varied from 21.5% to 85.0% (mean 46.1%; Fig. 2).

#### SELF-COMPATIBILITY AND SEED SET

All of the eight species tested were self-compatible, setting at least some seeds when pollinated by self-pollen (Tables 3 and 4, Fig. 3). Spontaneous selfing resulted in considerable, but variable, seed set in five species (means 33.1–78.7%), and in none or very little in *S. cernua* (morph means 0.1% and 5.5%) and *S. svalbardensis* (mean 0.7%). In large-flowered *S. cernua*, seeds were only obtained after spontaneous selfing in one population, whereas both populations of the small-flowered morph set some seeds (2.5–8.5%).

Hand-selfing with excess pollen resulted in seed set in all species, with means varying from 5.5% in *S. svalbardensis* and 10.3% in large-flowered *S. cernua* to 100% in *S. nivalis*. Seed-set after hand-selfing increased relative to that after spontaneous selfing in most populations (Table 4), but there was large variation and the sample sizes were low. The increases were significant (one-tailed *t*-tests,  $P < 0.05$ ) in two populations (numbers 31 and 76) of *S. cernua*, *S. nivalis*, and one population (number 20) of *S. svalbardensis*.

**Table 3.** Species-level synopsis of reproductive strategies observed in arctic *Saxifraga*, based on the population data given in Tables 4–6. All plants observed were hermaphrodites. Mode of clonal reproduction is indicated as B, bulbils produced in stem leaf axils, or R, subterranean runners.  $N_1$ , number of populations examined;  $N_2$ , number of plants examined

Species/morph	Sex maturation (sl—slightly, st—strongly)	Pollen stainability (%)				Seed set per flower after various treatments														
		Spont. selfing		Hand-selfing		Spont. selfing		Hand-selfing												
		$N_1$	$N_2$	Mean	Mean	$N_1$	$N_2$	Mean	Mean											
<i>S. aizoides</i>		3	8	88.6																
<i>S. cernua</i> (large-flw.)	(sl-)st protandrous	11	80	74.1	8	39	0.3	0.1	7	35	22.9	41.8	19.7							
<i>S. cernua</i> (small-flw.)	sl protandrous	2	12	64.2	2	9	12.1	5.5	2	14	38.7	22.9	10.3							
<i>S. cespitosa</i> (white-flw.)	protandrous	6	23	65.5	3	12	80.9	33.2	3	12	78.6	38.7	17.4							
<i>S. cespitosa</i> (yellow-flw.)	protandrous	2	10	70.7	2	17	31.2	14.6	2	5	80.9	78.6	31.6							
<i>S. hieracifolia</i>	protandrous	2	9	97.4	2	24	45.6	44.7	1	5	48.0	80.9	38.0							
<i>S. hirculus</i>	protandrous	3	7	88.2								48.0	52.2							
<i>S. hyperborea</i>	sl protogynous	3	22	95.0	2	22	88.2	32.8	2	13	87.8	48.0	52.2							
<i>S. nivalis</i>	protandrous	1	6	90.6	1	15	57.3	33.1	1	4	185.0	87.8	32.6							
<i>S. rivularis</i>	sl protogynous	4	24	97.1	4	23	169.9	78.7	4	16	152.7	185.0	100.0							
<i>S. svalbardensis</i>	homogamous	5	22	46.2	4	12	1.8	0.7	4	13	14.0	152.7	78.0							
Total sample size		42	223		28	173			27	122										

continued



Table 3 — continued

Species/morph	Seed set per flower after various treatments						Pollen/ovule ratio						Clonal reproduction						
	Cross-pollination			Emasculation			No. of ovules			P/O-ratio			Mode	Bulbils/leaf axil					
	$N_1$	$N_2$	Mean	Seed set (%)	Mean	Mean	$N_1$	$N_2$	Mean	$N_1$	$N_2$	Mean	$N_1$	$N_2$	Mean				
<i>S. aizoides</i>																			
<i>S. cernua</i> (large-flw.)	7	23	15.4	5.2	8	43	0.0	1	8	0.0	1	3	212	1	3	292	—		
<i>S. cernua</i> (small-flw.)	2	6	75.8	33.5	2	10	0.0	2	8	0.0	2	8	292	8	21	180	B	5	46
<i>S. cespitosa</i> (white-flw.)					4	16	0.0	2	4	0.0	2	4	242	2	6	116	B	2	12
<i>S. cespitosa</i> (yellow-flw.)					2	10	0.0	1	2	0.0	1	2	239	2	3	195	—		
<i>S. hieracifolia</i>					1	4	0.0	2	6	0.0	2	6	213	1	2	233	—		
<i>S. hirculus</i>					2	6	0.0	1	1	0.0	1	1	105	2	5	715	—		
<i>S. hyperborea</i>	1	2	63.5	21.2	3	12	0.0	2	5	0.0	2	5	500	1	1	214	—		
<i>S. nivalis</i>					1	4	0.0	1	3	0.0	1	3	278	2	4	61	—		
<i>S. rivularis</i>	2	4	163.9	81.2	3	9	0.0	3	7	0.0	3	7	173	1	3	280	—		
<i>S. svalbardensis</i>	2	5	21.8	11.5	3	13	0.0	2	3	0.0	2	3	196	3	5	124	R	3	13
Total sample size	14	40			30	135		25	70		25	70	240	2	3	93	B, R	10	71

**Table 4.** Seed set per flower after various treatments in populations of arctic *Saxifraga* cultivated under uniform conditions. One flower was used for each plant in each experiment. Per cent seed set was calculated for each population based on mean number of seeds obtained and mean ovule number per flower (cf. Table 6). *N* = number of flowers examined

Species/morph	Pop. no.	Spontaneous selfing			Hand-selfing			Cross-pollination			Emasculation				
		No. of seeds		Seed set (%)	No. of seeds		Seed set (%)	No. of seeds		Seed set (%)	No. of seeds		Seed set (%)		
		<i>N</i>	Mean	SD	<i>N</i>	Mean	SD	<i>N</i>	Mean	SD	<i>N</i>	Mean	SD		
<i>S. aizoides</i>	78	1	0.0	—	5	41.8	30.61	19.7	—	—	—	—	8	0.0	0.00
<i>S. cernua</i> (large-flowered)	12	11	0.0	0.0	11	9.0	16.07	2.4	7	6.7	9.59	1.8	1	0.0	—
	45	4	0.0	0.0	4	54.8	63.68	34.2	3	0.0	0.00	0.0	2	0.0	0.00
	58	4	2.7	4.62	6	44.0	60.51	18.0	3	6.0	10.39	2.5	10	0.0	0.00
	60	5	0.0	0.0	4	0.0	0.00	0.0	2	0.0	0.00	0.0	7	0.0	0.00
	67	2	0.0	0.0	2	0.0	0.00	0.0	4	18.8	37.50	7.8	4	0.0	0.00
	76	5	0.0	0.0	5	52.6	31.43	17.1	3	40.3	12.06	13.1	5	0.0	0.00
	248	7	0.0	0.0	3	0.0	0.00	0.0	1	36.0	—	11.3	4	0.0	0.00
<i>S. cernua</i> (small-flowered)	66	6	17.2	25.21	7	53.3	71.76	26.2	3	95.0	77.00	46.8	5	0.0	0.00
	82	3	7.0	11.27	7	24.1	47.08	8.6	3	56.7	98.15	20.2	5	0.0	0.00
<i>S. cespitosa</i>	32	3	5.3	6.66	1	62.0	—	31.0	—	—	—	—	4	0.0	0.00
(white-flowered)	50	7	84.4	127.32	6	157.8	80.90	57.0	6	157.8	80.90	57.0	9	0.0	0.00
	65	5	16.0	22.63	5	16.0	22.63	7.0	—	—	—	—	2	0.0	0.00
	77	2	153.0	35.36	—	—	—	—	—	—	—	—	1	0.0	—
<i>S. cespitosa</i>	63	14	54.4	49.80	4	109.8	92.02	51.5	4	109.8	92.02	51.5	5	0.0	0.00
(yellow-flowered)	64	3	8.0	13.86	1	52.0	—	24.4	—	—	—	—	5	0.0	0.00
<i>S. hieracifolia</i>	4	8	41.9	3.44	—	—	—	—	—	—	—	—	4	0.0	0.00
	21	16	49.4	10.98	5	48.0	9.90	52.2	—	—	—	—	4	0.0	0.00
<i>S. hirculus</i>	30	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	48	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>S. hyperborea</i>	17	16	60.3	43.66	9	63.7	48.87	21.2	2	63.5	16.26	21.2	10	0.0	0.00
	24	6	116.2	55.18	4	112.0	11.40	43.9	—	—	—	—	1	0.0	—
	119	5	57.3	23.28	4	185.0	64.94	100.0	—	—	—	—	1	0.0	—
<i>S. nivalis</i>	5	15	101.7	79.79	6	86.7	52.41	41.7	—	—	—	—	4	0.0	0.00
<i>S. rivularis</i>	26	6	101.7	79.79	6	86.7	52.41	41.7	3	161.7	83.12	77.7	6	0.0	0.00
	133	4	224.8	29.17	3	190.3	64.49	100.0	—	—	—	—	1	0.0	—
	204	4	224.0	34.96	3	190.7	24.68	97.3	—	—	—	—	—	—	—
	229	9	129.2	52.38	4	143.3	20.27	73.1	1	166.0	—	84.7	2	0.0	0.00
<i>S. svalbardensis</i>	20	6	0.0	0.00	6	26.2	24.13	8.6	4	7.5	15.00	2.5	9	0.0	0.00
	40	1	4.0	—	1	20.0	—	8.3	—	—	—	—	—	—	—
	59	1	0.0	—	1	6.0	—	3.4	1	36.0	—	20.6	3	0.0	0.00
	233	4	1.3	2.50	5	4.0	3.08	1.7	—	—	—	—	1	0.0	—
Total sample size	173	—	—	—	122	—	—	—	40	—	—	—	135	—	—

**Table 5.** Gender observations and capacity for clonal reproduction in populations of *Saxifraga cernua* and *S. svalbardensis* cultivated under uniform conditions. Observations of gender were made several times throughout the flowering period. N = number of plants examined

Species/morph	Pop. no.	Gender observations		Presence of runners		No. of bulbils produced in axils of stem leaves								
		N	Proportion of hermaphrodites (%)	N	Proportion with runners (%)	Per leaf axil			Per stem			Per plant		
						Mean	SD	N	Mean	SD	N	Mean	SD	Mean
<i>S. cernua</i> (large-flowered)	31	22	100	30	0	18	15.9	4.98	94.4	30.14	13.7	5.51	1325	695
	45	4	100	5	0	7	15.9	5.93	84.3	26.21	4.9	1.57	423	209
	58	4	100	30	0	6	12.4	6.39	89.3	46.76	6.3	5.96	778	995
	60	3	100	5	0									
	67	5	100	5	0	10	13.6	4.70	88.5	29.35	5.7	5.52	409	356
<i>S. cernua</i> (small-flowered)	76	4	100	5	0	5	14.5	4.34	89.0	27.93	10.0	7.48	1041	913
	66	4	100	10	0	7	2.9	2.01	9.9	7.71	5.3	5.35	57	67
	82	4	100	10	0	5	5.8	2.28	20.6	10.60	4.8	4.76	121	154
<i>S. svalbardensis</i>	20	14	100	30	100	9	3.3	0.83	16.3	7.30	5.1	4.76	97	98
	28	1	100	5	100									
	40	3	100	5	100	3	2.8	0.58	15.3	0.58	1.7	0.58	26	9
	59	1	100	5	100	1	1.5	—	8.0	—	3.0	—	24	—

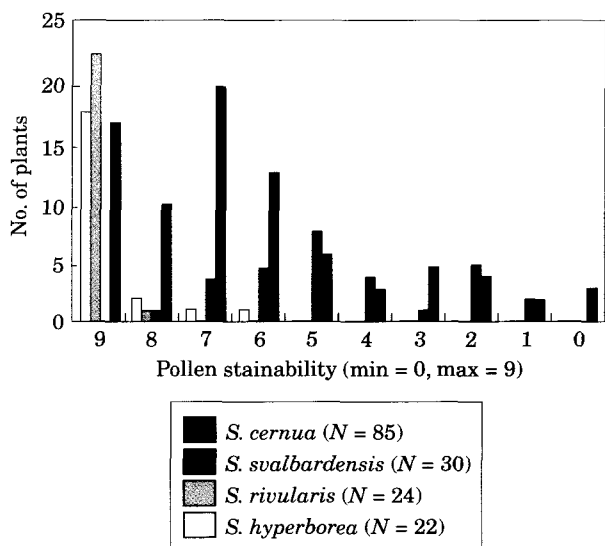
**Table 6.** Pollen stainabilities and pollen/ovule-ratios in populations of arctic *Saxifraga* cultivated under uniform conditions. One flower from each plant was used in each experiment, except that an additional pollen sample was taken after minimum 3 days from a new flower if the stainability of the first sample was below 90%, and the highest value was used

Species	Pop. no.	Pollen stainability (%)				Pollen and ovule number (N = no. of flowers examined)										
		$N_1 = \text{no. of plants examined}$ $N_2 = \text{no. of flowers examined}$				No. of pollen grains per flower				No. of ovules per flower				Pollen/ovule-ratio		
		$N_1$	$N_2$	Mean	SD	Min.	Max.	N	Mean	SD	N	Mean	SD	N	Mean	SD
<i>S. aizoides</i>	78	3	4	89.2	17.03	69.5	99.0	3	61 867	22 244	3	212	8	3	292	106
	163	3	3	94.5	5.68	88.0	98.5									
	250	2	2	82.0	20.51	67.5	96.5									
	12	2	2	97.5	0.71	97.0	98.0	1	72 133	—	1	380	—	1	190	—
	31	29	43	61.1	26.40	0.0	92.0	3	26 500	6 486	9	377	38	3	74	15
<i>S. cernua</i> (large-flw)	45	4	8	78.3	9.37	71.0	92.0	1	37 067	—	1	160	—	1	232	—
	58	11	17	56.3	31.51	0.0	96.5	5	61 693	21 952	6	244	83	5	294	175
	60	4	7	69.8	9.97	55.0	76.5	4	48 658	13 307	4	306	83	4	176	85
	67	5	6	76.3	29.96	24.5	97.0	2	32 533	5 091	2	240	85	2	141	28
	76	4	5	61.4	4.75	54.5	65.0	3	42 533	14 932	3	307	71	3	153	91
<i>S. cernua</i> (small-flw)	81	3	4	74.5	13.43	59.0	82.5									
	134	5	6	93.5	1.46	92.0	95.5									
	207	5	5	81.1	24.28	38.0	95.0									
	248	8	13	65.2	8.77	53.5	75.5	2	54 000	5 657	2	320	85	2	177	65
	66	6	8	70.3	4.69	66.5	78.5	3	23 611	5 450	5	203	33	3	117	23
<i>S. cespitosa</i> (white-flw)	82	6	8	58.0	22.20	19.5	78.5	4	33 117	5 400	3	280	66	3	114	35
	32	3	5	56.8	37.03	33.0	99.5	1	37 700	—	1	200	—	1	189	—
	50	5	8	57.1	25.50	22.0	93.0	2	58 267	14 519	3	277	50	2	200	77
	65	4	6	48.9	29.37	22.0	90.5									
	77	5	7	64.5	43.12	12.0	98.0									
<i>S. cespitosa</i> (yellow-flw)	83	2	2	94.8	3.89	92.0	97.5									
	212	4	5	71.0	15.35	58.5	93.0									
	63	6	7	80.5	11.83	69.5	97.0	2	49 133	5 563	2	213	18	2	233	46
	64	4	7	60.9	25.86	29.5	92.5									
	4	2	2	98.3	1.06	97.5	99.0	2	90 150	5 869	3	117	35	2	852	316
<i>S. hieracifolia</i>	21	7	8	96.5	3.87	89.5	100.0	3	52 411	6 256	3	92	8	3	578	113
	30	3	5	91.8	8.55	82.0	97.5									
	48	1	2	78.0	—	—	—	1	106 800	—	1	500	—	1	214	—
	219	3	3	94.8	1.26	93.5	96.0									
	17	17	20	92.1	9.56	66.0	99.5	3	16 878	10 027	3	300	20	3	58	38
<i>S. hyperborea</i>	24	2	2	95.0	4.24	92.0	98.0									
	119	3	3	97.8	1.26	96.5	99.0	1	15 400	—	2	255	21	1	64	—

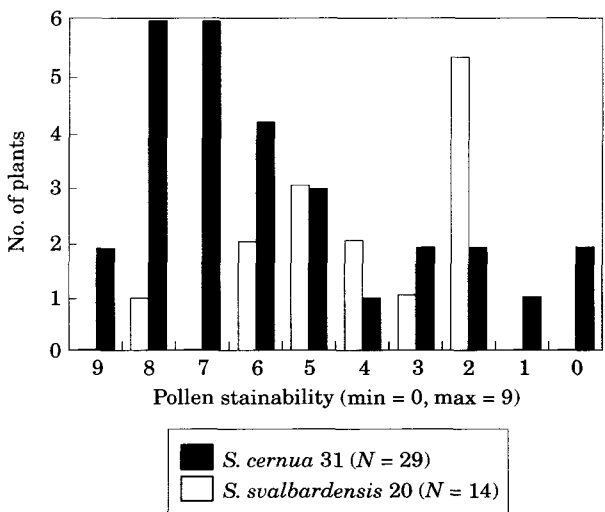
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Table 6 -- continued

Species	Pop. no.	Pollen stainability (%)						Pollen and ovule number (N = no. of flowers examined)								
		$N_1 = \text{no. of plants examined}$			$N_2 = \text{no. of flowers examined}$			No. of pollen grains per flower			No. of ovules per flower			Pollen/ovule-ratio		
		$N_1$	$N_2$	Mean	SD	Min.	Max.	N	Mean	SD	N	Mean	SD	N	Mean	SD
<i>S. nivalis</i>	5	6	6	90.6	13.95	62.5	99.0	3	47 644	3983	3	173	25	3	280	60
<i>S. rivularis</i>	26	9	9	95.9	3.94	88.0	99.0	4	20 725	7 322	4	208	19	3	114	33
	47							1	22 400	—	2	190	14	1	124	—
	133	7	8	94.9	2.34	92.5	99.0	1	25 300	—	1	190	—	1	133	—
	204	3	3	98.7	1.04	97.5	99.5									
	229	5	6	98.9	0.89	97.5	99.5									
<i>S. svalbardensis</i>	20	14	21	46.1	18.81	21.5	85.0	2	11 640	622	2	305	21	2	38	1
	28	1	1	55.0	—	—	—									
	40	3	5	42.3	25.58	18.0	69.0	1	25 800	—	1	175	—	1	147	—
	59	1	1	44.5	—	—	—									
	233	3	4	43.0	21.23	18.5	56.0	58			70					
Total sample size		223	297													

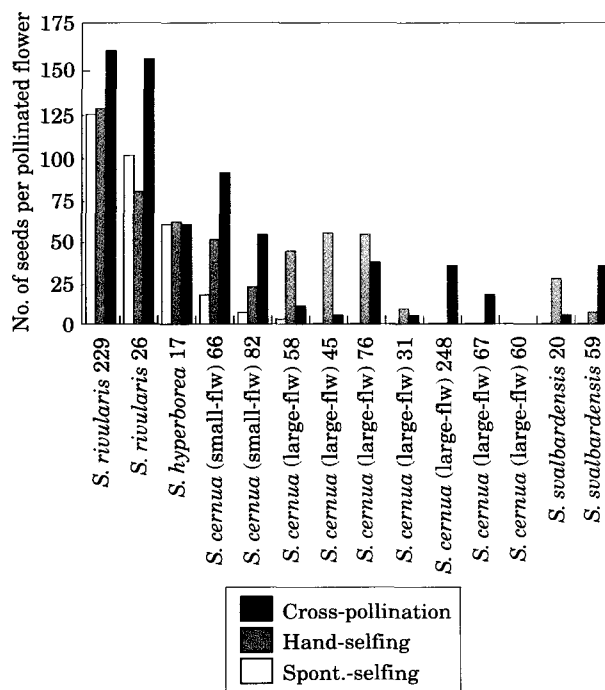


**Figure 1.** Pollen stainability (PS) in cultivated plants of *Saxifraga svalbardensis* and its close allies (*S. cernua*, *S. rivularis*, and *S. hyperborea*) from Svalbard. Individual plants are pooled for all populations examined. *N* = number of plants examined. (0) PS < 10.0%; (1) PS = 10.0–19.9%; (2) PS = 20.0–29.9%; ... (9) PS = 90.0–100.0%.



**Figure 2.** Intrapopulation variation in pollen stainability (PS) in two populations of *Saxifraga svalbardensis* and *S. cernua* cultivated under uniform conditions. *N* = number of plants examined. (0) PS < 10.0%; (1) PS = 10.0–19.9%; (2) PS = 20.0–29.9%; ... (9) PS = 90.0–100.0%.

Most cross-pollinations (section *Mesogyne* only) resulted in similar or somewhat increased seed set relative to that after hand-selfing, but no differences were significant (Table 4, Fig. 3). Non-significant differences



**Figure 3.** Mean seed obtained after various treatments in cultivated populations of *Saxifraga svalbardensis* and its close allies (*S. cernua*, *S. rivularis*, and *S. hyperborea*) from Svalbard. Populations not included in all treatments are excluded (some seeds were, for example, obtained after spontaneous selfing in two other populations of *S. svalbardensis*; see Table 4).

were expected in *S. rivularis* and *S. hyperborea*, because these species fertilized most of their ovules with self-pollen. The results for *S. cernua* and *S. svalbardensis* reflected their large variation in fertility. The data nevertheless demonstrated a considerable potential for sexual reproduction (the highest populations means were 36 seeds in *S. svalbardensis*, 54.8 seeds in large-flowered *S. cernua*, 95.0 seeds in small-flowered *S. cernua*).

#### SEED GERMINATION AND OFFSPRING VIABILITY IN *SAXIFRAGA CERNUA* AND *S. SVALBARDENSIS*

Some seeds germinated one week after sowing and most of them during the third week. Mean germination was 23.0–51.2% in large-flowered *S. cernua*, 16.7–73.1% in small-flowered *S. cernua*, and 0.0–10.0% in *S. svalbardensis*, of which very few seeds were available (Table 7). In *S. cernua*, full samples of progeny (10 plants per population) were raised into adult, flowering plants. In *S. svalbardensis*, a total of seven seeds germinated, and four seedlings survived to the

**Table 7.** Germination of seeds obtained after selfing cultivated plants of *Saxifraga cernua* and *S. svalbardensis*. Ten offspring (if obtained) from each maternal population were cultivated until flowering

Species/morph	Pop. no.	No. of maternal plants	No. of seeds sown	No. of seedlings obtained	Germination (%)		No. of adult offspring raised
					Calculated per maternal plant Mean	Range	
<i>S. cernua</i> (large-flowered)	31	3	96	30	29.2	0–65	10
	45	1	196	46	23.0	—	10
	58	2	209	77	37.3	22–53	10
	66	2	318	164	51.2	45–57	10
	76	3	258	71	25.4	18–35	10
<i>S. cernua</i> (small-flowered)	66	1	26	19	73.1	—	10
	82	1	18	3	16.7	—	3
	82	1	116	25	21.6	—	10
<i>S. svalbardensis</i>	20	4	143	6	6.9	0–12	4
	40	2	20	0	0.0	—	—
	59	1	4	0	0.0	—	—
	233	2	11	1	10.0	0–20	0
Total sample size		23	1415	442			77

flowering stage. All offspring appeared to be morphologically normal and fully vigorous.

## DISCUSSION

### SAXIFRAGA SVALBARDENSIS: EVOLUTIONARY POTENTIAL AFTER ALL?

Although seeds never have been observed in its natural populations, we have shown that the bulbiferous Svalbard endemic *S. svalbardensis* is not entirely sterile. Some plants were semifertile, and we obtained vigorous, normal offspring from self-fertilized seeds. Autodeposition of pollen occasionally resulted in a few seeds, but more seeds were set after hand-selfing, demonstrating that the plants are self-compatible but that seed-set is pollen-limited. However, the seed-set was low even after hand-selfing and cross-pollination, suggesting that the main limiting factor is the poor quality of the pollen and, presumably, of the ovules. The variable fertility and frequent floral irregularities in *S. svalbardensis* are probably associated with aneuployploidy, resulting in high frequencies of unbalanced gametes.

Sexual reproduction is commonly believed to be a prerequisite for long-term survival in a changing environment. Thus, *S. svalbardensis* does not necessarily represent an evolutionary dead-end; it is certainly able to reproduce sexually in good seasons with high insect activity. A clone of *S. svalbardensis* can survive for thousands of years via runner and bulbil propagation, producing numerous partly fertile flowers during its lifespan. The gametes probably vary in chromosome

number, and it is thus possible that fertilization occasionally results in euploid and more fertile genets (cf. Ramsey & Schemske, 1998). The number of chromosomes has only been determined in a single plant of *S. svalbardensis* (Borgen & Elven, 1983). This plant was aneuploid ( $2n=c. 64$ ), but euploid lineages may also exist in Svalbard. The large variation we observed in fertility suggests that the species consists of several clones with different chromosome numbers. Although the species is virtually invariable for molecular markers investigated so far (Brochmann *et al.*, 1998), it may consist of several clones that are very closely related, and the limited genetic variation can be explained by a single, recent origin via hybridization between two genotypes, one of *S. cernua* and one of *S. rivularis*.

### SAXIFRAGA CERNUA: A SELF-COMPATIBLE HERMAPHRODITE IN SVALBARD

In contrast to reports from other geographic areas (Molau, 1992; Molau & Prentice, 1992), *Saxifraga cernua* is self-compatible, invariably hermaphroditic, and usually strongly protandrous in Svalbard. In our cultivated plants, mature anthers and apparently reduced gynoecia were observed for several days at the early floral stage, but later the flowers invariably developed normal styles and papillose stigmas. Similar field observations have been reported from the Dovre mountains in southern Norway (described and illustrated by Lindman, 1887).

The previous reports of high frequencies of male plants of *S. cernua* were partly based on herbarium

specimens or conserved flowers; this material may have been preserved at the long-lasting protandrous stage in hermaphrodites. However, many plants in northern Sweden and Greenland that were observed in entire field seasons did not develop mature gynoecea (U. Molau, pers. comm.). In late-melting habitats, the flowering season may be too short in most years to allow for development of gynoecea in strongly protandrous species such as *S. cernua*. Thus, the plants may function as males for many years but as hermaphrodites in good years with extended flowering seasons, although the season nevertheless may be too short for completion of seed maturation. In *Dryas octopetala* L., the frequency of phenotypically and functionally male flowers was highest in late-melting habitats, but it is not known whether this was genetically determined (Wada, Kudo & Kojima, 1999).

The Svalbard populations of *S. cernua* occasionally can set small amounts of seeds after spontaneous selfing, but hand-selfing and cross-pollination often increased the seed set (depending on the fertility of the parental plant). The most widespread, large-flowered morph of *S. cernua* is largely dependent on insects for pollen transfer, whereas the rare, small-flowered morph is less protandrous and can set more seeds autogamously.

We have demonstrated that *S. cernua* has a considerable capacity for sexual reproduction, but the fertility varies conspicuously within and among populations in Svalbard. The variation in fertility suggests that this multiclonal species (cf. Gabrielsen & Brochmann, 1998) consists of clones that have different chromosome numbers. Fully fertile plants are probably euploid. The previous reports of self-incompatibility in *S. cernua* (Godfree, 1979; Webb & Gornall, 1989; Molau, 1992; Molau & Prentice, 1992) may have been based on experiments with more or less sterile, aneuploid genotypes. Large variation in pollen stainability has also been reported in *S. cernua* from other geographic areas (northern Sweden, Molau & Prentice, 1992; southern Norway, Oppdal, Flugrud, 1985; southern Norway, Finse, Berggren & Haugset, 1994). The species is widespread and abundant in the Arctic, but several small, isolated populations are known outside its main distribution area (Hultén & Fries, 1986). In contrast to the local multiclinality observed in Svalbard, Bauert *et al.* (1998) found that plants from entire geographic regions of the Alps had identical RAPD phenotypes and may belong to a single clone. A small Scottish population consisted of entirely male-sterile plants (Godfree, 1979). Wehrmeister & Bonde (1977) obtained viable seeds after hand-pollination between different plants from Alaska, but plants from Colorado were entirely pollen-sterile. It is possible that many of the small, isolated populations of *S. cernua* are monoclonal today because of bottlenecks during

the Quaternary climatic changes, and the surviving clone may be aneuploid and largely sterile.

#### SAXIFRAGA RIVULARIS AND *S. HYPERBOREA*: THE SELFING SPECIALISTS

These species are genetically, morphologically and ecologically very similar (Brochmann *et al.*, 1998; Øvstedal, 1998) and also have similar breeding systems. *Saxifraga rivularis* is mat-forming, propagating via short subterranean runners in addition to seeds, whereas *S. hyperborea* reproduces exclusively sexually, forming small tufts without runners. Both species are fully fertile with small, slightly protogynous flowers which autodeposit pollen at an early stage, consistent with their very low pollen/ovule ratios. They set abundant seed (up to 100%) after spontaneous selfing, and the pollination experiments suggest that sufficient amounts of pollen are deposited autogamously. Predominant autogamy has previously been reported in Scandinavian *S. rivularis* (selfing efficiency 0.86 and P/O ratio 30, Berggren & Haugset, 1994; selfing efficiency 1.00 and P/O ratio 160, Molau, 1993).

#### OTHER SPECIES STUDIED

*Saxifraga aizoides*, *S. cespitosa*, *S. hieracifolia*, *S. hirculus*, and *S. nivalis* are protandrous, self-compatible, and fully fertile in Svalbard (except that some plants with low pollen stainability occur in most populations of *S. cespitosa*). *Saxifraga cespitosa*, *S. hieracifolia*, and *S. nivalis* are moderately autogamous; they have relatively low pollen/ovule ratios and set abundant seed after spontaneous selfing. The low pollen/ovule ratios of *Saxifraga aizoides* and *S. hirculus* suggest that also these species are autogamous in Svalbard. *Saxifraga hieracifolia* and *S. hirculus* have previously been reported as autogamous in northeastern Russia (90–100% seed set after spontaneous selfing; Tikhmenev, 1984), and *S. nivalis* has a low pollen/ovule ratio and is probably autogamous in southern Norway (P/O = 100; Berggren & Haugset, 1994).

It is possible that the breeding system of *S. aizoides* varies among geographic areas. This species has been reported as mainly insect-pollinated in the Arctic, Scandinavia, and the Alps by Warming (1909), and as self-compatible but with no seed set after spontaneous self-pollination in the Alps (Meier & Holderegger, 1998). Molau (1993) reported, however, that *S. aizoides* set abundant seed autogamously in northern Sweden and Greenland (selfing efficiency = 0.60). Pollen/ovule ratios of 700 (Molau, 1993) and 160 (Berggren & Haugset, 1994) have previously been reported for this species.

The widespread and polymorphic *S. cespitosa* has been reported as more or less autogamous in several geographic areas, and it has frequently been observed



with floral irregularities and variable pollen stainability. In Svalbard, the flowers of *S. cespitosa* showed frequent but distinctly delayed selfing (cf. Brochmann, 1993), thus increasing the possibility for cross-pollination. Previously reported pollen/ovule ratios are fairly similar to our values (220 in north Sweden, Molau & Prentice, 1992; 100 in south Norway, Berggren & Haugset, 1994), and high autogamous seed set was also reported from these areas (selfing efficiencies 0.74 and 0.78, respectively). Variable pollen stainability has been reported from southern Norway (mean 75%, Berggren & Haugset, 1994), and Molau & Prentice (1992) reported that plants from northern Sweden were either perfectly male fertile (mean stainability 91.8%) or entirely male sterile. The floral irregularities and variable male fertility in *S. cespitosa* may be associated with some variation in chromosome number; in addition to  $2n=10x=80$ , there is a report of  $2n=78$  (Webb & Gornall, 1989). Another possibility is that *S. cespitosa* has an unstable gynodioecious mating system (cf. Molau & Prentice, 1992). All plants we observed were phenotypically and at least somewhat functionally hermaphroditic (i.e. with some stainable pollen). Chromosome number and female fertility must be examined in the more or less male-sterile plants to determine whether they are functional females or sterile because of aneuploidy.

#### SYNOPSIS AND CONCLUSIONS: THE STRATEGY OF MIXED STRATEGIES

We have shown that interpretation of experimental reproductive data in arctic *Saxifraga* can be complicated by large variation in protogyny/protandry and in fertility, which probably is associated with unstable chromosome numbers.

The Svalbard endemic *S. svalbardensis* is not entirely sterile, although no seeds have been observed in natural populations. The species varies in fertility. Viable seeds were obtained after selfing and cross-pollinating some plants, suggesting that although it mainly reproduces via bulbils and runners, it can occasionally reproduce sexually. Sexual reproduction in aneuployploids such as *S. svalbardensis* often involves gametes with different chromosome numbers, resulting in genets with new chromosome numbers. Sexual reproduction in *S. svalbardensis* can therefore lead to increasingly more fertile genets.

In contrast to reports from other geographic areas, the chromosomally unstable polyploid *S. cernua* is self-compatible and hermaphroditic in Svalbard, but the fertility varies conspicuously within and among populations. The high levels of local clonal diversity previously observed in this species suggest that sexual reproduction is more frequent than indicated by the rarity of seed set observations. Some clones are fully

fertile and probably euploid, and crosses among clones with different chromosome numbers may repeatably form new clones with variable fertility. The Svalbard populations set some seeds after spontaneous selfing; hand-selfing and cross-pollination can increase seed set, but the result is probably highly dependent on the meiotic stability of the parental genotype. Sexual reproduction in the common large-flowered morph is largely insect-dependent; the rare small-flowered morph also reproduces autogamously.

The other results of this study are largely in agreement with reports from other geographic areas. The Svalbard saxifrages do not set seeds asexually; agamospermy is unknown in the entire genus (Webb & Gornall, 1989; Meier & Holderegger, 1998). All species examined herein are self-compatible, as all other species investigated in Svalbard (Brochmann & Steen, 1999). The species have low pollen/ovule ratios, and spontaneous selfing resulted in considerable seed set in *S. cespitosa*, *S. hieracifolia*, *S. hyperborea*, *S. nivalis*, and *S. rivularis*.

The species examined reproduce mainly clonally (*S. svalbardensis* and *S. cernua*), by a combination of clonality (runners) and predominant autogamy (*S. rivularis*), or more or less autogamously (the remaining species), but they can obviously also set seed after cross-pollination and thereby maintain intrapopulation genetic variation. There is definite evidence for outcrossing even in the most strongly autogamous species, *S. rivularis*; it acted as the maternal parent in the cross that gave rise to *S. svalbardensis*. In Svalbard, we have observed high levels of intrapopulation molecular variation in the moderately autogamous *S. cespitosa* (41% of the total variation in AMOVA analysis of RAPD data; Tollefsrud *et al.*, 1998). The high levels of genotypic diversity observed at small spatial scales in *S. cernua* (Gabrielsen & Brochmann, 1998) and in one strongly autogamous Svalbard species, *Papaver dahlianum* Nordh. (Steen, Spjelkavik & Brochmann, unpublished data), suggest that input of allogamously produced seeds can be sufficient to maintain genetic variation even in highly clonal or autogamous species. The reproductive strategy of arctic saxifrages, as well as of many other arctic plants, is, therefore, a strategy of mixed strategies. The recent data accumulated for arctic plants have thus verified the suggestions of Murray (1987), who emphasized that sexual/asexual strategies and self-compatibility/cross-pollination are not mutually exclusive, and that mixed mating is common and highly advantageous in the diverse arctic environment.

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