A comparison of pollen-siring ability and life history between males and hermaphrodites of subdioecious *Silene acaulis*

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ABSTRACT

Background: *Silene acaulis* is an evergreen, very long-lived cushion plant. Populations of *S. acaulis* in Greenland are subdioecious, consisting of female, hermaphrodite, and male individuals. The sex expression of males and hermaphrodites can vary over years for the same individual, while females are always females. Previous studies have shown that outcrossed seeds from females become seedlings with higher survival and growth rates than those from outcrossed seeds from hermaphrodites.

Questions: (1) Do pollen grains from males exhibit some advantage over pollen from hermaphrodites? In particular, do they sire more seeds than hermaphrodites? (2) Is the reproductive system of *S. acaulis* stable or is it evolving towards one with fewer morphs (i.e. dioecy or gynodioecy)?

Hypothesis: Pollen from male plants is better at siring seeds on females than pollen from hermaphrodites.

Study system: A subdioecious population of *S. acaulis* in Greenland, containing male individuals that produce pollen and rarely or never set seed, hermaphrodites that produce both pollen and seeds, and females that produce only seeds.

Methods: A pollen-competition experiment was performed in which females were hand pollinated with a mixture of pollen from males and hermaphrodites, all with known isozyme alleles, which allowed determination of who sired each seed. We recorded plant size, flower morphology, fruit and seed set, as well as pollen per anther for the individuals used in the experiment, as well as for a large number of other individuals to allow us to make comparisons between the three types of individuals.

Conclusions: Well-developed pollen grains from males or hermaphrodites did not differ in their seed-siring capacity, although males had significantly more well-developed pollen grains per anther than hermaphrodites. Hence, on a per flower basis, males would be predicted to sire more seeds than hermaphrodites. However, given that males and hermaphrodites produce similar numbers of flowers per square centimetre of cushion and that male plants were significantly smaller than hermaphrodites, hermaphrodites have the potential to contribute relatively more to the seed pool than males. Calculations based on our quantitative

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results suggest that the dynamics of the population should develop slowly towards gynodioecy. However, this conclusion applies only as long as the population does not experience a significant change in the environmental conditions over time.

INTRODUCTION

An impressive array of different reproductive systems is present in flowering plants (Barrett, 2003). Hermaphroditism is most common, but evolution has created several other combinations of female and male function. Generally, hermaphroditism is the ancestral state, with gynodioecy and dioecy being derived (Charlesworth, 1999, 2006; Delph and Wolf, 2005). In some cases, detailed studies of gynodioecy and dioecy reveal an inconstancy in individuals with male function, with some individuals reproducing only via male function and others via both sex functions. Such systems are denoted as being subdioecious. They are often interpreted as stages in the evolutionary pathway from hermaphroditism to dioecy, but may be also stable (Desfeux *et al.*, 1996; Delph and Wolf, 2005).

The genus *Silene* is one of the genera in which several breeding systems are represented, ranging from gynodioecy to dioecy and hermaphroditism (Desfeux *et al.*, 1996; Brown and Kephart, 1999). Sex determination in the gynodioecious species *Silene vulgaris* is caused by the presence of cytoplasmic male-sterility factors and nuclear restorers of male sterility (McCauley and Olson, 2003), and this is likely to be the case for other gynodioecious species of *Silene*.

Within the genus, *Silene acaulis* possesses an unusual reproductive system. At some locations only females and hermaphrodites are found, whereas at others females, hermaphrodites, and males co-occur (Shykoff, 1992; Hermanutz and Innes, 1994; Alatalo and Totland, 1997; Philipp, 1997; Maurice *et al.*, 1998; Delph and Mutikainen, 2003). Sex expression has been found to vary from year to year for the same individual and also among different parts of the same individual (Alatalo and Molau, 1995; Delph and Mutikainen, 2003). Most often the females do not vary, whereas individuals with male function vary between male and hermaphrodite status. This capability to switch between different functional genders has the advantage of being a flexible reproductive system where the amount of resources can influence the functional gender of flowers or individuals. The selective advantage of plastic reproductive systems might prevent the evolution of dioecy, which often happens in gynodioecious species (Delph, 2003). On the other hand, such labile reproductive systems are prone to evolve towards a less flexible reproductive system if selection in a given direction prevails for a longer period.

Several aspects of the reproductive system in *S. acaulis* have been studied. Most studies have focused on differences between the morphs to elucidate how the system evolved and is maintained. The frequency of the different morphs has been studied at a number of localities showing around 30% females in gynodioecious populations in Scandinavia (Alatalo and Molau, 1995) and in the Rocky Mountains (Shykoff, 1988; Delph and Carroll, 2001), whereas on Baffin Island (Hermanutz and Innes, 1994) and Greenland (Philipp, 1997) females usually exceed 50%. Fruit set is probably the main difference between females and hermaphrodites, with females producing three to four times as many fruits per flower as hermaphrodites (Hermanutz and Innes, 1994; Alatalo and Molau, 1995; Morris and Doak, 1998; Delph and Carroll, 2001). Seed number, seed weight, and seed content have all been studied, but seed weight is the only factor that differs between females and hermaphrodites. Seeds from females are sometimes found to weigh less than

those from hermaphrodites (Shykoff, 1988; Delph *et al.*, 1999; Keller and Schwaegerle, 2006). In spite of this, seedlings from females show a higher survival and growth rate than seedlings from hermaphrodites (Shykoff, 1988; Delph and Mutikainen, 2003). Inbreeding depression has been shown to exist (Delph, 2004; Keller and Schwaegerle, 2006) and may explain the lower fitness when the seeds from hermaphrodites are the result of selfing. A difference in fitness is, however, also found between outcrossed seeds from hermaphrodites and females, with offspring from females performing better than outcrossed offspring from hermaphrodites. A cost of restoring male fertility is believed to be the most likely explanation for this (Delph *et al.*, 1999; Delph and Mutikainen, 2003; Keller and Schwaegerle, 2006).

This difference in performance of offspring from females and hermaphrodites was the background for the idea of comparing the performance of pollen from hermaphrodites and males on female plants. To improve our understanding of the maintenance and evolution of this reproductive system, we addressed the following question: Does pollen from males sire more seeds than pollen from hermaphrodites when competing in the styles of flowers on females? To have similar fitness between males and hermaphrodites, pollen from males would need to fertilize more ovules than pollen from hermaphrodites, because in addition to fertilizing ovules in females, hermaphrodites also produce seeds.

MATERIALS AND METHODS

The study species

Silene acaulis L. subsp. *acaulis* DC (hereafter *Silene acaulis*) has a circumpolar arctic and alpine distribution (Hultén, 1968). *Silene acaulis* is found throughout Greenland (Bay, 1992), particularly in fell-field and heath vegetation (Böcher *et al.*, 1978). Generally, established individuals live for 337 years on average, and life spans of more than 500 years have been estimated in a projection matrix study of a population in the Wrangell Mountains (Morris and Doak, 1998; Ehrlén and Lehtila, 2002). The individuals are evergreen and form dense cushions, often totally covered by short-stalked pink flowers. There is no vegetative propagation. Males, hermaphrodites, and females occur as described above.

Study site

The study site is located east of the nearby Arctic Station (69°15′N, 50°27′W), University of Copenhagen, in a plain and uniform area previously used as a football ground. Plants that have established after the area was abandoned are still spatially separated and mostly circular in circumference. The area is dominated by *S. acaulis*, but *Salix arctica*, *Armeria scabra*, and *Papaver radicatum* are also frequent. The study site was selected because a previous investigation had shown relatively high frequencies of males (31%) and hermaphrodites (12%), and at the same time the three alleles of the enzyme system GPI were all represented with suitable frequencies (Philipp, 1997). The study was mainly carried out in 2002, but supplemented with further observations in 2004.

Morphology

Based on the morphology of flowers at the onset of the study, each plant was categorized as being female, hermaphrodite or male. Females possess smaller flowers, longer stigmas, and

often retain capsules from the current year or previous years. Males do not have capsules, and flowers that have been open for several days possess stigmas that do not protrude from the corolla tube. Hermaphrodites possess capsules and/or their stigmas are longer than the corolla tube. To document the size of these traits, we used digital callipers to measure five flowers from each of the individuals used in the pollen-competition experiment (Table 1).

Pollen-competition experiment

Different allozymes of GPI were used to identify which pollen donors sired seeds harvested from specific females. A number of individuals were genotyped by electrophoresis (see below) and a number of pollen donors as well as pollen-receiving individuals were selected based on which alleles they were carrying. The selected individuals were bagged to prevent pollen removal from pollen donors and pollen deposition on flowers used as pollen recipients.

Earlier observations on pollen number per anther had shown that male plants have more well-developed pollen grains per anther than hermaphrodites (M. Philipp, personal observation; see also below). We therefore used two different mixtures of pollen from hermaphrodites and males. Immediately before pollinations, an equal number of open anthers with plenty of pollen grains was harvested from a hermaphrodite and a male individual and then mixed in an Eppendorf tube. Alternatively, the mixture consisted of twice as many anthers from hermaphrodite as from male individuals.

The bagged flowers on females were pollinated once with the pollen mixture when they had receptive stigmas. After pollination, the flowers were marked (with sewing thread and labels), re-bagged, and left for maturation. When the capsules were well developed but not yet ripe, we placed a drop of glue on top of them to prevent opening of the capsules and seed loss.

Ripe seeds were harvested and brought to the Botanical Gardens in Copenhagen to be germinated in Petri dishes. Seedlings were transferred to plant pots and harvested when they had grown some additional leaves. By means of electrophoresis the genotype of each seedling was determined and the pollen donor identified.

At the Arctic Station, genotyping of individuals was undertaken by mini-electrophoresis with equipment from Helena Laboratories (Beaumont, TX) using cellulose-acetate plates soaked in Tris-glycine pH 8.5 at least 20 min before the run. Leaves were homogenized in three drops of Tris-HCl grinding buffer-PVP solution (Soltis *et al.*, 1983). The extract was transferred to an Eppendorf tube and spun down. Then, 13.5 μl of the extract was placed in each of 12 wells and subsequently transferred by an applicator to the humid but not wet cellulose plate. The running buffer was Tris-glycine pH 8 and the run time was 20 min at 225 V. The bands appeared following staining for GPI (phosphoglucoisomerase E.C.5.3.1.9) in an agar solution according to a recipe from Weeden and Wendel (1989).

In Copenhagen, starch-gel electrophoresis was performed by homogenization as mentioned above, and the extract was absorbed onto filter paper wicks. Electrophoresis was carried out on 12% gels using sodium-borate, pH 8.5/Tris-citrate, pH 7.8 (Torres and Berg, 1978) run for 4 h at 75–35 mA. GPI was stained as mentioned above, but without agar.

A single undehisced anther from three different flowers was removed from each of the individuals used as pollen donors and its content stained in carmine acetic acid. The numbers of stained and non-stained pollen grains were counted under a microscope.

Table 1. Values for morphological traits measured on five flowers from each of 9 female, 12 hermaphrodite, and 8 male plants (mean ± *s*)

Morph	Calyx (mm)	Corolla (mm)	Ovule (mm)	Style (mm)	Filament (mm)	Pollen per anther	Well-developed pollen
Female	5.37 ± 0.38 (a)	3.07 ± 0.30 (a)	2.48 ± 0.29 (a)	4.81 ± 0.35 (a)			
Herm.	6.19 ± 0.34 (b)	3.87 ± 0.34 (b)	1.82 ± 0.18 (b)	1.65 ± 0.29 (b)	6.75 ± 0.49 (a)	1349.9 ± 264.1 (a)	823.7 ± 421.1 (a)
Male	6.22 ± 0.63 (b)	3.99 ± 0.28 (b)	1.80 ± 0.18 (b)	1.48 ± 0.38 (b)	6.88 ± 0.85 (a)	1633.9 ± 255.3 (b)	1341.9 ± 192.5 (b)

Note: Letters in brackets indicate statistically different (ANOVA or *t*-test, $P < 0.05$) and non-different values across the sexual morphs.

Flower and fruit production

In 2004, flower and seed production were recorded for 345 individuals at the same locality as studied in 2002. Data for size of individuals, number of flowers, capsules, and seeds per capsule were obtained.

Data analysis

The proportion of offspring from males (p) among all offspring when flowers were sired by a mixture of male pollen (m) and hermaphroditic pollen (h) was analysed by logistic regression (Hosmer and Lemeshow, 1989) using the model

$$p = \frac{M}{M+H} = \frac{e^{\beta_0 + \beta_1 x}}{1 + e^{\beta_0 + \beta_1 x}},$$

where M and H denote the number of offspring sired by males and hermaphrodites respectively. β_0 and β_1 are constants and $x = \ln(m/h)$ is seen to be 0 when $m = h$ and to vary between $-\infty$ and $+\infty$. If the two types of pollen are similar with respect to siring β_1 should be 1, whereas values larger than unity indicate that pollen from males is superior to pollen from hermaphrodites and vice versa when $\beta_1 < 1$. The empirical value of β_1 is compared with the expected value of 1 by means of a t-test, i.e.

$$t_{n-2} = \frac{\hat{\beta}_1 - 1}{SE(\hat{\beta}_1)},$$

where n is the number of pollination experiments (37). Logistic-regression analysis was carried out by PROC GENMOD in SAS/Enterprise version 4.1 (SAS Institute, Inc., 2006).

RESULTS

Morphology

We found that females were significantly different from hermaphrodites and males in all flower characters measured (Table 1). Females had shorter petals, longer styles, and initially larger ovaries than hermaphrodites and males. Comparison of hermaphrodites and males showed that they were not significantly different in flower traits apart from the total number of pollen grains and the number of well-developed pollen grains in anthers (t-test, $P = 0.0282$ and $P = 0.0045$, respectively), with males having more of each. However, since seven morphological characters were analysed by independent t-tests, only the latter P-value can be considered significant after a sequential Bonferroni test [i.e. the Dunn-Sidak method according to Sokal and Rohlf (1995)].

Gender plasticity in the individuals with male function was exposed by observing 20 pollen-producing individuals included in the experiment in 2002 again in 2004. Five of the plants had disappeared in 2004. Seven individuals changed from male to hermaphrodite or vice versa ($4M \rightarrow H$, $3H \rightarrow M$) and eight ($2M$, $6H$) remained the same. The female individuals remained female – they were never seen to produce pollen. The conclusion that a change from male to hermaphrodite had occurred was based on the presence of a few capsules found on plants that previously had relatively short styles and no capsules present.

Pollination-competition experiment

In the pollen-competition experiment, the total number of pollinated female flowers was 254, distributed over 37 combinations of 9 females with 12 hermaphrodite and 8 male pollen donors (Table 2). The parameters of the logistic model and their standard errors (in parentheses) were estimated as $\hat{\beta}_0 = -0.1107$ (0.1529) and $\hat{\beta}_1 = 0.9404$ (0.2840). Figure 1 indicates that pollen from hermaphrodites was slightly more efficient than pollen from males, but the value of $\hat{\beta}_1$ is not significantly different from 1 ($t_{35} = 0.210$, $P = 0.834$). The model predicts that if $m = h$, the proportion of offspring sired by males will be 0.472 (95% CL: 0.399–0.547).

Flower and seed production

The population-based recording of numbers of flowers, capsules, and seeds per plant obtained in 2004 showed that the median sizes of the three morphs were significantly different [females ($n = 149$): 95.0 cm^2; hermaphrodites ($n = 32$): 153.9 cm^2; males ($n = 54$): 56.7 cm^2] (Kruskal-Wallis one-way analysis of variance on ranks, $H = 10.8$. $P = 0.0046$). The median numbers of *flowers* per cm^2 cushion for females ($n = 149$), hermaphrodites ($n = 32$), and males ($n = 54$) were 0.70, 0.58, and 0.63 respectively. These values are not significantly different (Kruskal-Wallis one-way analysis of variance on ranks, $H = 0.532$, $P = 0.77$). The number of *capsules per flower* was significantly different between females and hermaphrodites [median values (25 and 75 percentiles)]: 0.48 (0.25–0.70) and 0.02 (0.0–0.09) respectively (Mann-Whitney, $U = 1036.5$, $n_1 = 32$, $n_2 = 149$, $P < 0.001$). On the other hand, the number of *seeds per capsule* was not different between females and hermaphrodites [median values (25 and 75 percentiles)]: 5.2 (3.3–7.2) and 4.6 (2.1–7.7) respectively (Mann-Whitney, $U = 1754.5$, $n_1 = 23$, $n_2 = 141$, $P = 0.50$).
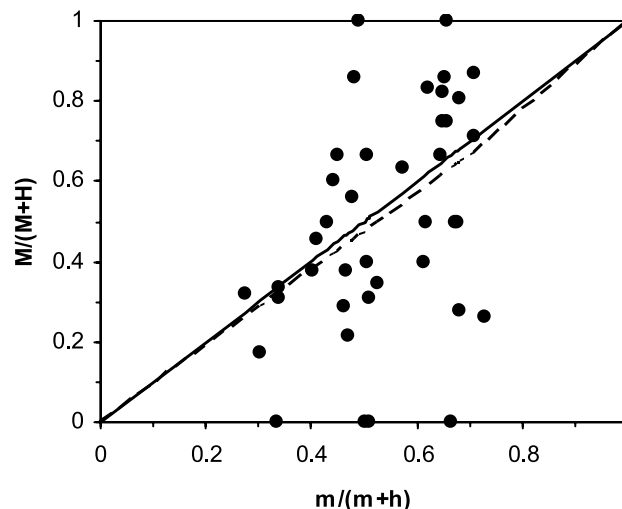
Fig. 1. The proportion of seeds sired by males relative to the total amount of seeds produced with a mixture of pollen from males and hermaphrodites. *m* and *h* are the amount of pollen grains from males and hermaphrodites in the mixture; *M* and *H* are the amount of seeds sired by males and hermaphrodites. The broken line represents the case where pollen from males and hermaphrodites has the same capacity of siring seeds. The solid line shows the result of the modelling.

Table 2. Results of the pollination experiments on *Silene acaulis* to study the ability of male and hermaphrodite individuals to sire ovules when competing in styles of female flowers

Female ID	Male ID	Hermaphrodite ID	Total offspring (n)	Offspring from male donors (n)	Offspring from hermaphrodite donors (n)	Proportion in pollen mixture (M:H)	Pollen per anther in male donors (n)	Pollen per anther in hermaphrodite donors (n)	Expected offspring from male donors (n)	Expected offspring from hermaphrodite donors (n)
F8	M20	H6	2	1	1	1:1	1303.3	1719.7	0.9	1.1
F8	M20	H6	25	8	17	1:2	1303.3	1719.7	6.9	18.1
F8	M7	H10	15	6	9	1:1	1533.0	963.7	9.2	5.8
F8	M7	H10	23	8	15	1:2	1533.0	963.7	12.1	10.9
F8	M7	H2	14	3	11	1:2	1533.0	861.0	6.6	7.4
F10	M20	H10	16	6	10	1:2	1303.3	963.7	6.5	9.5
F10	M20	H25	29	11	18	1:2	1303.3	742.3	13.6	15.4
F10	M8	H20	15	13	2	1:1	1569.7	648.7	10.6	4.4
F13	M20	H22	51	41	10	1:2	1303.3	306.3	34.7	16.3
F13	M20	H6	14	12	2	1:1	1303.3	693.7	9.1	4.9
F13	M20	H9	12	8	4	1:1	1303.3	714.3	7.8	4.2
F13	M20	H9	25	14	11	1:2	1303.3	714.3	11.9	13.1
F13	M7	H10	10	6	4	1:2	1533.0	963.7	4.4	5.6
F14	M6	H3	4	3	1	1:1	1338.0	693.7	2.6	1.4
F14	M6	H3	14	14	0	1:2	1338.0	693.7	6.9	7.1

F14	M8	H20	7	5	2	1:1	1569.7	648.7	5.0	2.0
F15	M13	H20	17	14	3	1:1	1206.5	648.7	11.1	5.9
F15	M22	H15	22	10	12	1:2	1173.5	840.5	9.0	13.0
F15	M33	H32	55	22	33	1:1	1551.0	1506.0	27.9	27.1
F15	M33	H32	29	9	20	1:2	1551.0	1506.0	9.9	19.1
F15	M8	H21	23	6	17	1:1	1569.7	584.3	16.8	6.2
F15	M8	H21	52	33	19	1:2	1569.7	584.3	29.8	22.2
F19	M20	H18	29	8	21	1:2	1303.3	304.0	19.8	9.2
F19	M20	H32	21	6	15	1:1	1303.3	1506.0	9.7	11.3
F19	M20	H32	41	7	34	1:2	1303.3	1506.0	12.4	28.6
F25	M13	H21	4	2	2	1:1	1206.5	584.3	2.7	1.3
F25	M13	H21	6	4	2	1:2	1206.5	584.3	3.0	3.0
F25	M21	H20	12	10	2	1:1	1060.5	648.7	7.4	4.6
F25	M21	H20	6	4	2	1:2	1060.5	648.7	2.7	3.3
F25	M7	H25	13	4	9	1:2	1533.0	742.3	6.6	6.4
F41	M13	H20	4	3	1	1:1	1206.5	648.7	2.6	1.4
F41	M13	H20	7	6	1	1:2	1206.5	648.7	3.4	3.6
F41	M33	H32	3	1	2	1:2	1551.0	1506.0	1.0	2.0
F41	M33	H25	2	1	1	1:1	1551.0	742.3	1.4	0.6
F41	M33	H25	4	0	4	1:2	1551.0	742.3	2.0	2.0
F41	M6	H3	6	6	0	1:1	1338.0	693.7	4.0	2.0
F42	M33	H10	6	3	3	1:1	1551.0	963.7	3.7	2.3

Note: Number of pollen is number of well-developed pollen. Expected number of offspring is calculated in relation to the relative number of well-developed pollen from hermaphrodites and males, respectively, in the pollen mixture used at pollinations.

DISCUSSION

Our results demonstrate that hermaphrodites and males produce pollen with the same ability to fertilize ovules. Males, however, potentially sire more seeds on a per flower basis than hermaphrodites because they produce more well-developed pollen grains per flower. However, this does not translate into higher male fitness for males versus hermaphrodites because this difference is counteracted by other fitness parameters associated with the life history of the three morphs. In particular, hermaphrodite individuals are larger than males, and therefore contribute more to the total seed production, overall. We discuss these results in relation to the stability of the subdioecious reproductive system.

Gender plasticity

Individuals in the study population were visually assigned to the three morphs at the onset of the study based on their style length and the occurrence of capsules from previous years. The overall agreement between pollen fertility and morphological flower traits justifies the initial assignment of individuals to either the hermaphrodite or male morph. On the other hand, most of the males produced a few capsules from time to time, whereas the hermaphrodites had capsules almost every year. At the time when we carried out the experiment, the plants were at peak flowering and, because of the bagging of the pollen donors to prevent pollen from disappearing, we could not get information on the number of capsules produced in that year. Gender plasticity in *S. acaulis* has also been studied in northernmost Sweden. Here, two of ten marked putative females changed to become hermaphroditic (Alatalo and Molau, 1995). In the present population, we found that females did not change sex. Pollen-producing plants were composed of some individuals that rarely or never produced capsules being mostly males, and others that produced capsules most years. The average size of the cushions may influence sex expression, as was found in *Wurmbea biglandulosa*, where larger plants had more female function than smaller individuals (Ramsay and Vaughton, 2002). In our study population, males were on average smaller than hermaphrodites, but whether the size of the cushion induces the sex expression or the sex of the cushion influences the size of the plant is not known.

Functional flower traits

Morphological measurements of the flowers confirmed that females have smaller corollas and longer styles than males and hermaphrodites (Philipp *et al.*, 1990; Hermanutz and Innes, 1994; Delph and Carroll, 2001). The total number of pollen grains per anther was close to that reported by Jürgens *et al.* (2002). The only trait separating hermaphrodites and males was the higher number of well-developed pollen grains, on average, in males (Table 1). A similar significant difference was found in northern Sweden, although the ratio of well-developed pollen grains there was much lower (Alatalo and Molau, 2001). In studies of *S. acaulis* on Baffin Island, no difference in pollen fertility between males and hermaphrodites was observed, which could be a consequence of the low number of hermaphrodites sampled (Hermanutz and Innes, 1994).

Pollen-competition experiment

The purpose of the pollen-competition experiment was to determine whether males have a greater ability than hermaphrodites to sire ovules, such that it compensates for their lack

of seed production. Pollen competition between self- and outcross-pollen has been shown to occur in *Silene nutans*, resulting in selection for outcross-pollen (Hauser and Siegismund, 2000). The present analysis indicates that pollen grains from hermaphrodites are on average slightly more efficient than those from males, although the difference is not statistically significant (Fig. 1). If we consider pollen from the males and hermaphrodites to have an equal chance to fertilize ovules, then males are likely to have higher siring success per flower because they produce more well-developed pollen grains per anther than the hermaphrodites.

Comparison of allocation to male and female

The above-mentioned results in combination with the results of flower and seed production indicate that, as expected, the different morphs acquire their fitness through different functions. Females allocated more resources into seeds per flower than hermaphrodites (they had significantly higher fruit set), resulting in 27 times more seed production compared with hermaphrodites on a per flower basis. Males produced more well-developed pollen grains than hermaphrodites on a per flower basis. Hence, hermaphrodites allocated resources into both male and female functions, but less into each on a per flower basis than the other two morphs. A similar situation was found in a study on *Wurmbea dioeca*, where the number of seeds produced by females was higher than the amount produced in hermaphrodites and pollen production in males was higher than in hermaphrodites (Ramsay and Vaughton, 2001).

Stability of the breeding system

To illustrate the dynamics in the studied population, the figures obtained from the study in 2002 and 2004 are combined in Fig. 2. The figures in box N (Fig. 2) indicate, as expected, that the females, by producing a relatively high number of seeds, deliver about 50% of the gametes to the seed pool, with the production of some seeds by hermaphrodites causing the contribution to fall slightly below 50%. In the focal population, hermaphrodites will, in spite of having few seeds per capsule and less-well-developed pollen grains per anther, out-perform the males if the model (Fig. 2) is extrapolated to 22 generations. This result is mainly a consequence of the larger average size of the hermaphrodites (2.7 times the median male size), resulting in greater flower production per individual. A balance in the frequency of males and hermaphrodites is obtained if hermaphrodites are 1.77 times larger than males with the present values for the reproductive units. If hermaphrodites exceed this threshold, the system will progress towards a gynodioecious system, and change to dioecism if the value is less than 1.77.

To assess the generality of this threshold value, data from a previous study on the genetic population structure in *S. acaulis* found in localities around the Arctic Station can be utilized. In that investigation, the size of 60 plants from each of 11 populations was measured (Philipp, 1997). Only a fraction of these cushions flowered and could be assigned to one of the three morphs. The median size of these flowering individuals was as follows: females, 78.5 cm^2 ($n = 276$); hermaphrodites, 104.1 cm^2 ($n = 40$); males, 63.6 cm^2 ($n = 97$). A Kruskal-Wallis test followed by a Dunn's test showed a significant difference between hermaphrodites and males. The hermaphrodites were thus on average 1.64 times larger than the male individuals, a value close to the above-mentioned calculated threshold separating
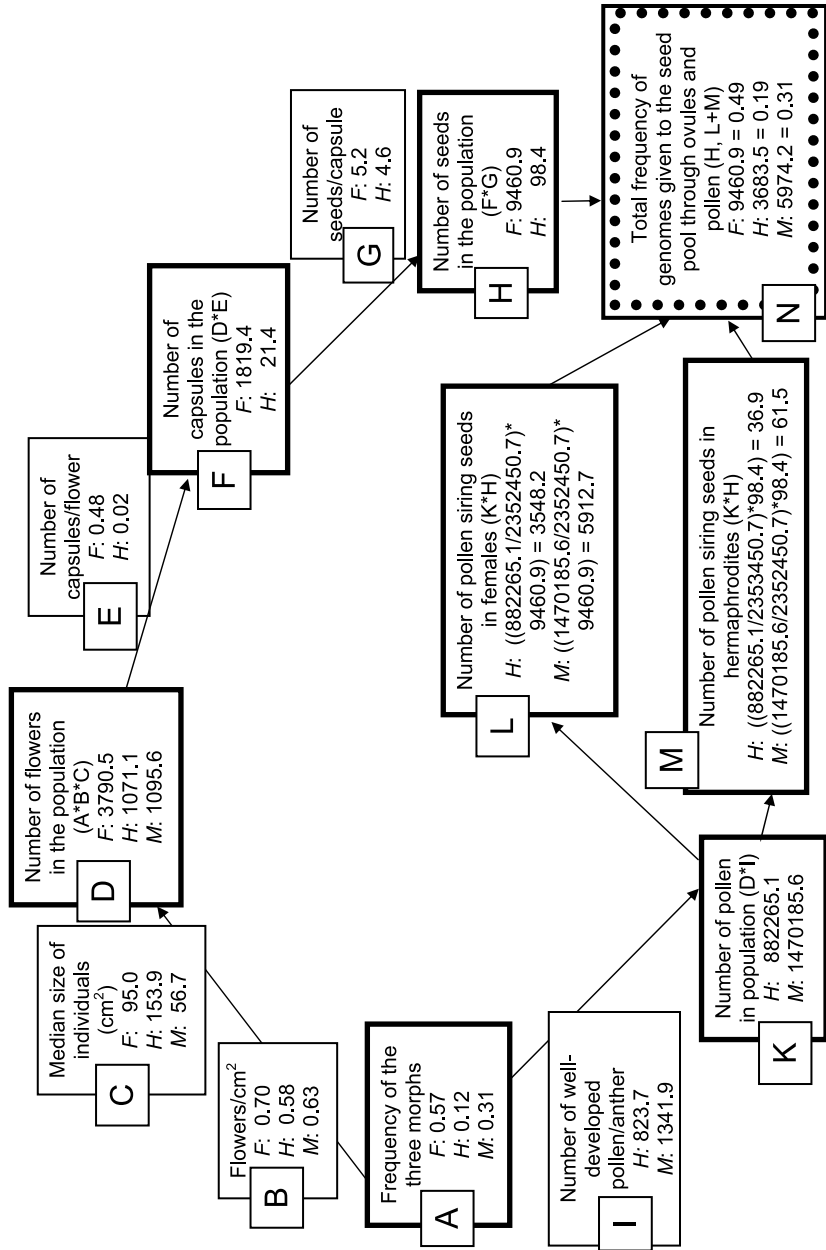
Fig. 2. A numerical model of how the three morphs [females (*F*), hermaphrodites (*H*), and males (*M*)] function in the studied population. The starting point is a population with 100 individuals distributed across the three morphs as in the studied population. The prerequisite for the model is that no selfing occurs in hermaphrodites and that the chances of siring ovules are the same for pollen grains from hermaphrodites and males.

dioecy and gynodioecy. However, several components that are likely to influence sex ratio are not included in this prediction. One of these is that hermaphrodites in years with a low level of pollinators might gain a further advantage with respect to reproductive assurance via selfing. Seed germination and seedling survival are also not included, but these have mostly been in favour of the females (Shykoff, 1988; Delph *et al.*, 1999; Keller and Schwaegerle, 2006). The frequency of females above 50% in our population points towards an advantage for seeds becoming female individuals in accordance with Delph and Mutikainen (2003). Finally, we do not know the segregation of sexes in offspring from females and hermaphrodites, a factor that could markedly influence the sex ratio.

Conclusion

In conclusion, *Silene acaulis* may be another example of a species where instability in sex expression could be a selective advantage (Delph and Wolf, 2005). Given that the three morphs are likely to have different advantages depending on conditions, this may result in a stable subdioecious reproductive system.

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REFERENCES

Alatalo, J.M. and Molau, U. 1995. Effect of altitude on the sex ratio in populations of *Silene acaulis* (Caryophyllaceae). *Nord. J. Bot.*, **15**: 251–256.

Alatalo, J.M. and Molau, U. 2001. Pollen viability and limitation of seed production in a population of the circumpolar cushion plant, *Silene acaulis* (Caryophyllaceae). *Nord. J. Bot.*, **21**: 365–372.

Alatalo, J.M. and Totland, Ø. 1997. Response to simulated climatic change in an alpine and subarctic pollen-risk strategist, *Silene acaulis*. *Global Change Biol.*, **3**: 74–79.

Barrett, S.C.H. 2003. Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. *Phil. Trans. R. Soc. Lond. B*, **358**: 991–1004.

Bay, C. 1992. A phytogeographical study of the vascular plants of northern Greenland – north of 74° northern latitude. *Meddr. Grønland Biosci.*, **36**: 1–102.

Böcher, T.W., Fredskild, B., Holmen, K. and Jakobsen, K. 1978. *Grønlands Flora*. Copenhagen: P. Haase & Sons Forlag.

Brown, E. and Kephart, S. 1999. Variability in pollen load: implications for reproduction and seedling vigor in a rare plant, *Silene douglasii* var. *oraria*. *Int. J. Plant Sci.*, **160**: 1145–1152.

Charlesworth, D. 1999. Theories of the evolution of dioecy. In *Gender and Sexual Dimorphism in Flowering Plants* (M.A. Geber, T.E. Dawson and L.E. Delph, eds.), pp. 33–60. Berlin: Springer-Verlag.

Charlesworth, D. 2006. Evolution of plant breeding systems. *Curr. Biol.*, **16**: R726–R735.

Delph, L.F. 2003. Sexual dimorphism in gender plasticity and its consequences for breeding-system evolution. *Evol. Devel.*, **5**: 34–39.

Delph, L.F. 2004. Testing for sex differences in biparental inbreeding and its consequences in a gynodioecious species. *Am. J. Bot.*, **91**: 45–51.

Delph, L.F., Bailey, M.F. and Marr, D.L. 1999. Seed provisioning in gynodioecious *Silene acaulis* (Caryophyllaceae). *Am. J. Bot.*, **86**: 140–144.

Delph, L.F. and Carroll, S.B. 2001. Factors affecting relative seed fitness and female frequency in a gynodioecious species, *Silene acaulis*. *Evol. Ecol. Res.*, **3**: 487–505.

Delph, L.F. and Mutikainen, P. 2003. Testing why the sex of the maternal parent affects seedling survival in a gynodioecious species. *Evol. Ecol. Res.*, **57**: 231–239.

Delph, L.F. and Wolf, D.E. 2005. Evolutionary consequences of gender plasticity in genetically dimorphic breeding systems. *New Phytol.*, **166**: 119–128.

Desfeux, C., Maurice, S., Henry, J.-P., Lejeune, B. and Gouyon, P.-H. 1996. Evolution of reproductive systems in the genus *Silene*. *Proc. R. Soc. Lond. B*, **263**: 409–414.

Ehrlén, J. and Lehtila, K. 2002. How perennial are perennial plants? *Oikos*, **98**: 308–322.

Hauser, T.P. and Siegismund, H.R. 2000. Inbreeding and outbreeding effects on pollen fitness and zygote survival in *Silene nutans* (Caryophyllaceae). *J. Evol. Biol.*, **13**: 446–454.

Hermanutz, L.A. and Innes, D.J. 1994. Gender variation in *Silene acaulis* (Caryophyllaceae). *Plant Syst. Evol.*, **191**: 69–81.

Hosmer, D.W. and Lemeshow, S. 1989. *Applied Logistic Regression*. New York: Wiley.

Hultén, E. 1968. *Flora of Alaska and Neighboring Territories*. Stanford, CA: Stanford University Press.

Jürgens, A., Witt, T. and Gottsberger, G. 2002. Pollen grain numbers, ovule numbers and pollen–ovule ratios in Caryophylloideae: correlation with breeding system, pollination, life form, style number, and sexual system. *Sex. Plant Reprod.*, **14**: 279–289.

Keller, S.R. and Schwaegerle, K.E. 2006. Maternal sex and mate relatedness affect offspring quality in the gynodioecious *Silene acaulis*. *J. Evol. Biol.*, **19**: 1128–1138.

Maurice, S., Desfeux, C., Mignot, A. and Henry, J.-P. 1998. Is *Silene acaulis* (Caryophyllaceae) a trioecious species? Reproductive biology of two subspecies. *Can. J. Bot.*, **76**: 478–485.

McCauley, D.E. and Olson, M.S. 2003. Associations among cytoplasmatic molecular markers, gender, and components of fitness in *Silene vulgaris*, a gynodioecious plant. *Mol. Ecol.*, **12**: 777–787.

Morris, W.F. and Doak, D.F. 1998. Life history of the long-lived gynodioecious cushion plant *Silene acaulis* (Caryophyllaceae) inferred from size-based population projection matrices. *Am. J. Bot.*, **85**: 784–793.

Philipp, M. 1997. Genetic diversity, breeding system, and population structure in *Silene acaulis* (Caryophyllaceae) in West Greenland. *Opera Botanica*, **132**: 89–100.

Philipp, M., Böcher, J., Mattsson, O. and Woodell, S.R.J. 1990. A quantitative approach to the sexual reproductive biology and population structure in some arctic flowering plants: *Dryas integrifolia*, *Silene acaulis* and *Ranunculus nivalis*. *Meddr. Grønland Biosci.*, **34**: 1–60.

Ramsay, M. and Vaughton, G. 2001. Sex expression and sexual dimorphism in subdioecious *Wurmbea dioica* (Colchicaceae). *Int. J. Plant Sci.*, **162**: 589–597.

Ramsay, M. and Vaughton, G. 2002. Maintenance of gynodioecy in *Wurmbea biglandulosa* (Colchicaceae): gender differences in seed production and progeny success. *Plant Syst. Evol.*, **232**: 189–200.

Shykoff, J.A. 1988. Maintenance of gynodioecy in *Silene acaulis* (Caryophyllaceae): stage-specific fecundity and viability selection. *Am. J. Bot.*, **75**: 844–850.

Shykoff, J.A. 1992. Sex polymorphism in *Silene acaulis* (Caryophyllaceae) and the possible role of sexual selection in maintaining females. *Am. J. Bot.*, **79**: 138–143.

Sokal, R.R. and Rohlf, F.J. 1995. *Biometry*, 3rd edn. New York: Freeman.

Soltis, D.E., Haufler, C.H., Darrow, D.C. and Gastony, G.J. 1983. Starch gel electrophoresis of ferns:

a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Am. Fern J.*, **73**: 9–27.

Torres, A.M. and Berg, B.O. 1978. Isozymes as indicators of outcrossing among 'Pinkerton' seedlings. *Yrbk. Calif. Avocado Assoc.*, **62**: 103–110.

Weeden, N.F. and Wendel, J.F. 1989. Genetics of plant isozymes. In *Isozymes in Plant Biology* (D.E. Soltis and P.S. Soltis, ed.), pp. 46–72. Portland, OR: Dioscorides Press.