Maternal sex and mate relatedness affect offspring quality in the gynodioecious *Silene acaulis*

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Abstract

In gynodioecious species, females sacrifice fitness by not producing pollen, and hence must have a fitness advantage over hermaphrodites. Because females are obligately outcrossed, they may derive a fitness advantage by avoiding selfing and inbreeding depression. However, both sexes are capable of biparental inbreeding, and there are currently few estimates of the independent effects of maternal sex and multiple levels of inbreeding on female advantage. To test these hypotheses, females and hermaphrodites from six Alaskan populations of *Silene acaulis* were crossed with pollen from self (hermaphrodites only), a sibling, a random plant within the same population, and a plant from a different population. Germination, survivorship and early growth revealed inbreeding depression for selfs and higher germination but reduced growth in sib-crosses, relative to outcrosses. Independent of mate relatedness, females germinated more seeds that grew faster than offspring from hermaphrodites. This indicates that inbreeding depression as well as maternal sex can influence breeding system evolution. The effect of maternal sex may be explained by higher performance of female genotypes and a greater abundance of female genotypes among the offspring of female mothers.

Introduction

The evolution of gender dimorphic breeding systems (i.e. the occurrence of more than one sexual morph) depends on costs and benefits associated with sex expression. Cosexual individuals must allocate limited resources to both male and female function; therefore, increased allocation to one sex function at the expense of another must achieve increased reproductive success to be evolutionarily stable (Fisher, 1930; Charnov *et al.*, 1976; Charnov, 1982; Charlesworth, 1999). This relationship implies an inherent trade-off between the physiological costs associated with male and female function, and the resulting gains in reproductive success through each. Such trade-offs associated with sex expression may lead to differences in the number and quality of offspring produced via each sex function, thereby determining the fitness of different sex morphs and the evolution of gender dimorphism.

Gynodioecy is an ideal system for assessing the evolution of gender dimorphism because it provides the co-occurrence of unisexual female and cosexual hermaphrodite individuals within populations (Darwin, 1877). In sexually reproducing gynodioecious plants, females contribute genes only through ovules, whereas hermaphrodites can contribute genes through both ovules and pollen. Faced with this 50% reduction in gene transmission, females must possess an advantage over hermaphrodites to persist in populations, either by

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producing more offspring or offspring of higher quality (Lewis, 1941; Lloyd, 1974, 1975; Charlesworth & Ganders, 1979). The magnitude of the advantage necessary for the maintenance of females is dependent on the genes determining sex expression. If nuclear genes determine sex, females require at least a twofold advantage in lifetime fitness as seed parents. If cytoplasmic genes are involved in sex expression (i.e. cytoplasmic male sterility genes, or CMS), as in many gynodioecious species, this condition is less restrictive. Females of many gynodioecious plants produce more offspring than hermaphrodites (review in Shykoff *et al.*, 2003), but differences between the sexes in offspring quality have been less well documented.

Inbreeding depression may be an important factor affecting offspring quality of females and hermaphrodites under several scenarios of sex inheritance (Lloyd, 1975; Sun & Ganders, 1986; Charlesworth & Charlesworth, 1987; Charlesworth, 1999). Hermaphrodites of many gynodioecious species are self-compatible and may undergo self-fertilization. In contrast, the ovules of females necessarily receive pollen from another individual, and thereby avoid selfing and the potential for inbreeding depression to reduce offspring quality. However, inbreeding depression can also arise through biparental inbreeding, especially when gene flow occurs locally within spatially structured populations (Levin, 1984; Uyenoyama, 1986). Importantly, both females and hermaphrodites are capable of biparental inbreeding, yet the sexes may differ in the frequency of biparental inbreeding and its effect on offspring quality. For example, local sex ratio bias may lead to greater biparental inbreeding in hermaphrodites vs. females, because a patch of related hermaphrodites can freely mate with one another whereas a patch of related females must receive pollen from a more distant source. Such spatial sex structure is expected to develop in gynodioecious species with CMS when offspring sex ratios are skewed towards the sex of the mother and seed dispersal is restricted (Manicacci *et al.*, 1996; Graff, 1999; Laporte *et al.*, 2001; Keller, 2002). Yet, few studies have experimentally addressed the impact of biparental inbreeding on offspring quality (Ashman, 1992; Mutikainen & Delph, 1998; Thompson & Tarayre, 2000; Delph, 2004).

Differences in offspring quality may also arise independently of inbreeding, as a consequence of factors intrinsic to each sex. Females and hermaphrodites may vary in the quality of seed offspring they produce because of differential opportunity for gametic selection (Wilson & Burley, 1983; Shykoff, 1992), because they provision their seeds differently (Eckhart & Chapin, 1997; Poot, 1997; Shykoff *et al.*, 2003), or through pleiotropic effects of the sex-determining genes themselves (de Haan *et al.*, 1997; Bailey, 2002). For these reasons, studies that compare inbred to outbred offspring from both hermaphrodites and females, thereby separating effects of inbreeding from intrinsic effects of maternal sex, are critical for understanding how these two factors act independently in determining offspring quality.

Silene acaulis L., a long-lived, gynodioecious species, provides an excellent opportunity to investigate differences in offspring quality between females and hermaphrodites. Previous work on *S. acaulis* has shown that both selfing and having a hermaphrodite mother reduces offspring survivorship (Shykoff, 1988). Several subsequent experiments focusing on the same initial study population as Shykoff (1988) have conclusively demonstrated survivorship differences among offspring from females and hermaphrodites (Delph & Mutikainen, 2003; Delph, 2004). However, seeds from both sexes are not provisioned differently in *S. acaulis* (Delph *et al.*, 1999), nor do gameotphytic selection or mitochondrial cytotype explain differences in seedling survival (Delph & Mutikainen, 2003). Thus, offspring quality seems to be under genetic control by an unknown mechanism in *S. acaulis*. However, it is unclear if these differences in offspring quality are unique to a single population or are broadly associated with the breeding system in this species. This is an important issue, as populations of *S. acaulis* often differ in factors that may influence offspring quality, such as sex ratio (Hermanutz & Innes, 1994; Maurice *et al.*, 1998; Delph & Carroll, 2001), the fecundity of the sexes (Maurice *et al.*, 1998; Shykoff, 1988; Morris & Doak, 1998; Delph & Carroll, 2001), and history of inbreeding (Abbot *et al.*, 1995; Philipp, 1997; Gehring & Delph, 1999).

In this paper, we provide independent estimates of the effects of inbreeding and maternal sex on offspring quality using families derived from six Alaskan populations of *S. acaulis*. We compared offspring quality among seven mating types. These included five within-population mating types: (1) self-fertilized hermaphrodites, (2) sib-crossed hermaphrodites, (3) sib-crossed females, (4) outcrossed hermaphrodites and (5) outcrossed females; as well as two among-population mating types: (6) among-population outcrossed hermaphrodites and (7) among-population outcrossed females. Crossing both females and hermaphrodites at varying levels of relatedness provides a powerful way to evaluate contributions to offspring quality, and has been used by similar studies of other gynodioecious species (e.g., *Sidalcea oregana*: Ashman, 1992; *Thymus vulgaris*: Thompson & Tarayre, 2000; *S. vulgaris*: Emery & McCauley, 2002). Among these studies, only Thompson & Tarayre (2000) have addressed multiple levels of both inbreeding and outcrossing within and among populations, but offspring quality in this study was only measured through the seed viability stage. We build upon this work by using multiple levels of inbreeding and outcrossing while extending the measurement of offspring quality to include not only seed viability, but also offspring survivorship and early growth. Further, we explore a novel mechanism to explain maternal sex effects by correlating offspring quality with the sex ratio. We discuss the role of

two distinct processes at work: the expression of deleterious alleles following inbreeding, and the potential for both direct and indirect effects of maternal sex to influence juvenile fitness traits.

Methods

Study species

Silene acaulis L. Jacq. (Caryophyllaceae) is a long-lived perennial distributed widely throughout arctic and alpine environments in North America and Europe (Hultén, 1968). The breeding system in *S. acaulis* is highly variable, with populations described as dioecious (Desfeux *et al.*, 1996), trioecious (Hermanutz & Innes, 1994; Philipp, 1997; Maurice *et al.*, 1998), and gynodioecious (Shykoff, 1988; Morris & Doak, 1998; Delph & Carroll, 2001). Previous work suggests sex in *S. acaulis* is determined by the joint influence of CMS genes that inhibit pollen production and nuclear genes that restore pollen production. Namely, maternal plants give rise to offspring sex ratios that are significantly biased towards the sex of the mother (Delph & Mutikainen, 2003), populations vary widely in their sex ratio, with female bias not uncommon (Hermanutz & Innes, 1994; Maurice *et al.*, 1998; Keller, 2002), and experimental studies in a congener (*S. vulgaris*) have shown sex determination to be under cytonuclear control (Charlesworth & Laporte, 1998; Taylor *et al.*, 2001).

Hermaphrodites are self-compatible and undergo significant selfing in the field, with multi-locus selfing rates ranging from 0.09 to 0.45 (Marr, 1997). The seeds are small, generally weighing less than 0.5 mg (Shykoff, 1988; Delph *et al.*, 1999), and possess no special dispersal mechanism.

Experimental design

Six study populations of *S. acaulis* located in interior and southcentral Alaska were chosen for inclusion in a greenhouse experiment (Fig. 1). During late August 2000, we selected 8–16 fruiting plants per population. Plants were selected at a minimum separation of three metres to minimize relatedness in the sample that may result from fine-scale genetic structure (Gehring & Delph, 1999). Five mature fruits from each individual were collected and bulked together, representing a single maternal sibship. Beginning in January 2001, seeds (1495 total from 78 maternal sibships) were germinated on wetted filter paper in Petri dishes under a 24 h photoperiod. Seeds were monitored daily, and germination was scored when the radicle penetrated the seed coat. Upon germinating, the seedling was transplanted into a 284 mL cup filled with a potting medium (1 : 1 : 1 Coco-Grow:perlite:vermiculite) and placed on a greenhouse bench under a 24 h photoperiod of natural and artificial light. Plants were watered and fertilized on a regular basis. Previous work has shown this technique rapidly induces flowering. As plants flowered, they were sexed as female if all flowers were pistillate, or as hermaphrodite if plants produced either all perfect flowers or a combination of pistillate and perfect flowers.
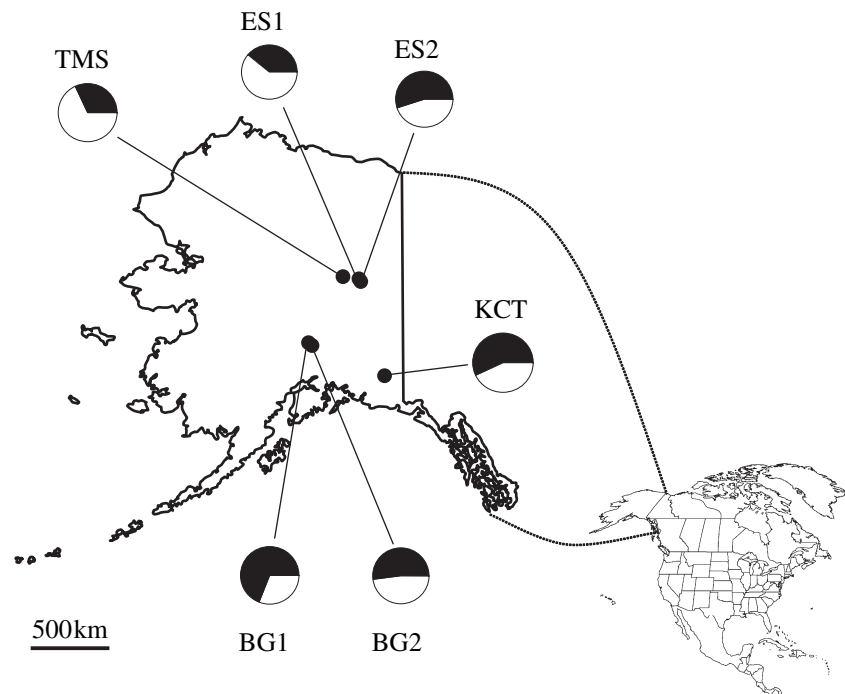

Fig. 1 Map of Alaska, USA, showing the six study populations from which seeds were collected to generate the parent generation. Pie diagrams show the field sex ratio for each population during 2001 (hermaphrodites open; females shaded).

We assigned female and hermaphrodite maternal plants to one of seven crosstypes reflecting different levels of mate relatedness: (1) hermaphrodites received self-pollen from another flower in male phase on the same plant (i.e. geitonogamous selfing), (2) hermaphrodites, (3) females received pollen from a sibling hermaphrodite belonging to the same maternal sibship and population, (4) hermaphrodites, (5) females received outcross pollen from an unrelated hermaphrodite belonging to a different maternal sibship from the same population (6) hermaphrodites and (7) females received outcross pollen from an unrelated hermaphrodite originating from a different population (Fig. 2). Because field collected maternal sibships were open pollinated and of unknown paternity, they may consist of a mixture of half and full sibs. Assuming a noninbred maternal parent, the probability of two alleles in the offspring of a cross being identical by descent (IBD) is between 0.125 and 0.25 for the sib treatment, and 0.5 for the selfing treatment (Hartl & Clark, 1989). Because even 'random' outcrosses within populations may be among related individuals (i.e. IBD > 0), we included the among-population outcrosses to help ensure a treatment where mates shared no immediate common ancestry. The selection of parents for the among-population crosses proceeded primarily on the basis of plants being at the correct phonological stage for crossing on a given day, and was done haphazardly with regard to their source. By assigning individuals to only one breeding treatment level, we controlled for any within-plant bias in resource provisioning to developing seeds with different levels of inbreeding (Wilson & Burley, 1983; Ashman, 1992).

Hand pollinations were performed on open flowers by brushing pollen from freshly dehisced anthers across the receptive stigmatic surface of a flowering maternal plant. Hermaphrodite maternal plants in the sib-cross and both outcross pollination groups were emasculated before pollination to minimize the potential for selfing. We weighed all filled seeds in each fruit individually to the

nearest 0.001 mg. A total of 4641 seeds were collected and weighed, representing 246 maternal parents. The number of replicate maternal parents per crosstype for hermaphrodites was: self = 24, sib = 17, within-population outcross = 30, among-population outcross = 31; and for females: sib = 48, within-population outcross = 30, among-population outcross = 66. Each maternal parent was represented by a mean (SE) of 19 (1.5) seeds.

Seeds were planted in a completely randomized design among six trays filled with medium-fine vermiculite loosely packed into 1 cm^3 plastic lighting grid (number of seeds per tray: 764, 997, 756, 976, 751, 397). Trays were misted and placed into a growth chamber set to 16 °C and a 19:5 h (L : D) photoperiod. Seeds were observed daily for germination (when the radicle had penetrated the seed coat) for a total of six weeks, after which germination had largely ceased. Upon germinating, each seedling was transferred to an individual 30 mL plastic pot filled with wetted vermiculite, watered with 5 mL dilute liquid fertilizer, and placed in a flat into one of two additional growth chambers under identical environmental conditions. Flats were rotated in the growth chambers on a daily basis to reduce position effects. Plants were misted with water daily and seedlings were individually scored for survivorship every 7 days following the day they germinated. If a seedling was still alive at the end of each seven-day period, it received an additional 5 mL of fertilizer and remained in the growth chamber for another week. Three weeks after germination, each seedling was harvested, its roots washed free of vermiculite, and the entire plant dried to constant mass and weighed to the nearest 0.01 mg. A total of 1749 seedlings from 192 maternal plants were represented at harvest.

We characterized five aspects of offspring quality: seed mass, seed germination, emergence time, survivorship, and dry mass 3 weeks post-emergence. We chose to measure offspring dry mass for two reasons: (1) dry mass should reflect physiological performance (and hence offspring quality) during the juvenile life stage and (2) to facilitate comparison with previous results for *S. acaulis* (Shykoff, 1988). We also computed a multiplicative measure of the cumulative offspring quality produced by each maternal plant formulated as (proportion germination) × (proportion seedling survival) × (mean seedling dry mass (mg)).

Statistical analyses

Because the experimental design contains a structurally empty cell (females are incapable of selfing), we combined sex and breeding treatment into a single explanatory variable (crosstype) with seven levels. The data were then analysed with mixed-model analysis of variance (PROC MIXED: SAS Institute Inc., 1999). This approach accommodates direct comparisons among all

Breeding treatment

Maternal sex	Self	Sibling	Within Pop.	Among Pop.
Hermaphrodite	$\mu_{h,self}$	$\mu_{h,sib}$	$\mu_{h,w}$	$\mu_{h,a}$
Female	✕	$\mu_{f,sib}$	$\mu_{f,w}$	$\mu_{f,a}$

Fig. 2 Experimental design showing the two levels of maternal sex and the four levels of breeding treatment. The structurally empty cell results from the inability of females to self. Breeding treatments are selfing (Self), sib mating (Sib), within-population outcrossing (W), and among-population outcrossing (A).

the levels of sex and breeding treatment, with the inclusion of selfs, in a complete design. Crosstype was designated as a fixed main effect. Because we consider the population from which a maternal plant originates to represent a random sample of the populations available, and because the number of maternal plants representing each population was variable, and not all populations were represented in each crosstype, we designated population and the crosstype × population interaction as random effects in the model. The crosstype × population effect did not approach significance for any variable ($P > 0.1$); therefore, it was dropped from the final model. For the variables seed mass, time to emergence, and seedling dry mass, maternal plant was also included as a random effect nested within population and crosstype. We also included a blocking factor for error control in the analysis of time to emergence (block = germination tray) and seedling dry mass (block = seedling flat). The remaining variables, proportion germination, proportion survivorship and cumulative offspring quality, are properties of maternal plants, so there was no nested random effect of maternal plant. For these three variables, the analyses were weighted by sample size (the number of offspring represented). We included the mass of each individual seed as a covariate when analysing time to emergence and seedling dry mass, and the mean seed mass produced by each maternal plant when analysing proportion germination, proportion survivorship and cumulative quality. This was done to partition the effects of seed provisioning from early life traits (Roach & Wulff, 1987). Prior to analysis, response variables were either natural-log transformed (time to emergence, seedling dry mass) or arcsine-square root transformed (proportion germination, proportion survivorship) to improve normality and homoscedasticity. Because of the presence of a large number of zero values, cumulative quality was not normal in its distribution, and could not be improved through transformation. Therefore, we analysed the frequency of families with zero ($n = 54$) vs. nonzero cumulative offspring quality with a G-test (Zar, 1999). The remaining families ($n = 192$) were analysed as above using ANOVA, with a square-root transformation to improve normality.

If the main effect of crosstype was significant in the ANOVA ($P < 0.05$), we conducted a set of *a priori* planned contrasts to test the following hypotheses: (1) no difference between females and hermaphrodites (excluding selfs), (2) no difference between selfs and sib-crosses, (3) no difference between selfs and within-population outcrosses, (4) no difference between selfs and among-population outcrosses, (5) no difference between sib-crosses and within-population outcrosses, (6) no difference between sib-crosses and among-population outcrosses, 7) no difference between within-population and among-population outcrosses and (8) no sex*breeding treatment interaction. Since there were three breeding treatments applied to two sexes, two degrees of freedom

were available to test the sex*breeding treatment interaction. All main effect and contrast hypotheses were tested using the appropriate degrees of freedom and mean square composition in the denominator, as formulated by PROC MIXED (SAS Institute Inc., 1999).

To estimate the relative performance of inbred vs. outcrossed offspring, we calculated inbreeding depression for cumulative offspring quality separately for each maternal plant as $\delta = (W_{WPOC} - W_I)/W_{MAX}$, where W_I is the cumulative offspring quality produced by an inbred maternal plant (for either the self or sib-crosses), W_{WPOC} is the mean cumulative offspring quality of within-population outcrossed plants (specific to each maternal sex), and W_{MAX} is the larger of the two numerator values. This formulation standardizes δ such that it varies from -1 to 1 (Thompson & Tarayre, 2000). Accordingly, $0 \leq \delta \leq 1$ indicates a reduction in fitness following inbreeding, while $-1 \leq \delta \leq 0$ indicates an enhancement of fitness following inbreeding.

Finally, we explored the possibility that maternal sex might affect offspring quality indirectly, as could occur if seed parents produce biased offspring sex ratios and offspring sex itself affects viability (Koelewijn & van Damme, 2005). A direct test of whether a female seed is more likely to germinate than a hermaphrodite seed is not possible, because there is currently no way to discriminate the sexes in *S. acaulis* prior to flowering. However, Emery & McCauley (2002) suggest that indirect evidence of gender-dependent fitness among pre-reproductive offspring would be a significant correlation between the fitness trait in question and the sex ratio among the flowering individuals of a sibship. Therefore, we recorded the proportion germination for each field-collected maternal family that formed the parental generation, as well as the sex of all plants in the sibship that flowered. We then used Spearman rank correlation (PROC CORR: SAS Institute Inc., 1999) to evaluate if the proportion of seeds that germinated is correlated across sibships with the frequency of female offspring.

Results

Seed mass and time to emergence varied significantly among maternal families but not among crosstypes (Table 1). Mean seed mass was 0.28 ± 0.02 (SE) g, and females tended to have smaller seeds than hermaphrodites (Fig. 2; $P = 0.1092$). Emergence time was significantly dependent on seed mass, with heavier seeds taking somewhat longer to germinate (Table 1).

Proportion of seeds that germinated varied among crosstypes (Table 1). Linear contrasts showed that, when averaged across treatments, seeds from females germinated 22% more than seeds from hermaphrodites (Table 2; Fig. 3). Independent of maternal sex, plants mated to a sib germinated a higher proportion of their seeds compared to plants that were selfed or outcrossed among populations (Table 2; Fig. 3). Selfed seeds may have somewhat

Table 1 Mixed-model analysis of variance for traits associated with offspring quality in *Silene acaulis*.

Fixed effects	Seed mass		Time to emergence		Proportion germination		Proportion survivorship		Seedling dry mass		Cumulative quality	
	d.f.	F	d.f.	F	d.f.	F	d.f.	F	d.f.	F	d.f.	F
Crosstype	6,234	1.76	6,187	0.40	6,233	3.76**	6,186	3.01**	6,179	6.99***	6,179	4.17***
Seed mass			11,958	12.83**	1,233	63.98***	1,186	5.26*	11,519	355.05***	1,179	68.65***
Random effects	Estimate	Z	Estimate	Z	Estimate	Z	Estimate	Z	Estimate	Z	Estimate	Z
Population	5.6	1.27	1.8	1.46†	2.5	1.50†	0.1	0.56	0.1	0.72	0.3	1.45†
Maternal plant(Pop, Cross)	44.9	8.41***	2.1	5.27***					3.0	4.19***		
Block			1.9	1.49†					1.0	2.62**		
Residual	83.6		12.3		80.7		52.3		21.2		17.4	

$*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $†0.05 < P < 0.10$.
Crosstype was a fixed effect while population, maternal plant (population, crosstype), block, and the residual error variance were all random effects. Seed mass was included as a covariate to partition effects of seed provisioning on offspring quality. For time to emergence, blocks were germination trays, and for seedling dry mass, blocks were seedling flats.

lower germination than seeds outcrossed within populations ($P = 0.06$; Table 2). Seed mass significantly affected germination; plants with a heavier mean seed mass germinated a greater proportion of their seeds (Table 1).

Seedling survival varied significantly among crosstypes (Table 1), due to low survival of self-pollinated seedlings (Table 2). Survival of selfed progeny was reduced by more than 30% compared to the sib and outcross treatments (Fig. 3). No effect of maternal sex was observed, nor was there significant interaction between sex and breeding treatment (Table 2). Seed mass once again had a large effect, with seedlings from families of smaller seeds less likely to survive the first 3 weeks of growth compared to seedlings from families with a greater mean seed mass (Table 1).

After 3 weeks of growth, there were significant differences in seedling dry mass among the crosstypes (Table 1). Selfed offspring accumulated less biomass than sib-crosses and both within- and among-population outcrosses (Table 2; Fig. 3). There was also a significant effect of maternal sex, with offspring from females attaining 15% greater dry mass than those from hermaphrodites. This effect is after controlling for initial differences in seed size, indicating that offspring from females exhibit higher growth rates when compared to offspring from hermaphrodites. A trend was also present for reduced dry mass of sib-crossed plants relative to among-population outcrosses ($P = 0.0554$, Table 2). The dry mass of a seedling was also influenced by both its seed mass and its maternal parent (Table 1).

Table 2 Linear contrasts testing *a priori* hypotheses regarding inbreeding and maternal sex effects on offspring quality in *Silene acaulis*.

Contrast	Proportion germination	Proportion survivorship	Seedling dry mass	Cumulative quality
Hermaphrodite vs. female	$F_{1,233} = 6.36$	$F_{1,186} = 0.44$	$F_{1,179} = 10.81$	$F_{1,179} = 6.01$
$L = (\mu_{h,sib} + \mu_{h,w} + \mu_{h,a})/3 - (\mu_{f,sib} + \mu_{f,w} + \mu_{f,a})/3$	$P = 0.0124$	$P = 0.5098$	$P = 0.0012$	$P = 0.0152$
Self vs. sibs	$F_{1,233} = 8.84$	$F_{1,186} = 12.87$	$F_{1,179} = 13.70$	$F_{1,179} = 16.09$
$L = \mu_{h,self} - (\mu_{h,sib} + \mu_{f,sib})/2$	$P = 0.0033$	$P = 0.0004$	$P = 0.0003$	$P = <0.0001$
Self vs. within-population outcross	$F_{1,233} = 3.44$	$F_{1,186} = 13.81$	$F_{1,179} = 18.16$	$F_{1,179} = 13.52$
$L = \mu_{h,self} - (\mu_{h,w} + \mu_{f,w})/2$	$P = 0.0648$	$P = 0.0003$	$P < 0.0001$	$P = 0.0003$
Self vs. among-population outcross	$F_{1,233} = 1.43$	$F_{1,186} = 16.39$	$F_{1,179} = 23.49$	$F_{1,179} = 9.96$
$L = \mu_{h,self} - (\mu_{h,a} + \mu_{f,a})/2$	$P = 0.2322$	$P < 0.0001$	$P < 0.0001$	$P = 0.0019$
Sibs vs. within-population outcross	$F_{1,233} = 3.61$	$F_{1,186} = 0.05$	$F_{1,179} = 0.84$	$F_{1,179} = 0.49$
$L = (\mu_{h,sib} + \mu_{f,sib})/2 - (\mu_{h,w} + \mu_{f,w})/2$	$P = 0.0585$	$P = 0.8310$	$P = 0.3614$	$P = 0.4840$
Sibs vs. among-population outcross	$F_{1,233} = 9.64$	$F_{1,186} = 0.59$	$F_{1,179} = 3.72$	$F_{1,179} = 3.17$
$L = (\mu_{h,sib} + \mu_{f,sib})/2 - (\mu_{h,a} + \mu_{f,a})/2$	$P = 0.0021$	$P = 0.4417$	$P = 0.0554$	$P = 0.0766$
Within vs. among population outcross	$F_{1,233} = 1.48$	$F_{1,186} = 0.30$	$F_{1,179} = 0.95$	$F_{1,179} = 1.23$
$L = (\mu_{h,w} + \mu_{f,w})/2 - (\mu_{h,a} + \mu_{f,a})/2$	$P = 0.2251$	$P = 0.5856$	$P = 0.3307$	$P = 0.2696$
Sex* breeding treatment	$F_{2,233} = 0.54$	$F_{2,186} = 0.23$	$F_{2,179} = 1.05$	$F_{2,179} = 1.68$
$L = (\mu_{h,sib} + \mu_{f,w})/2 - (\mu_{f,sib} + \mu_{h,w})/2$; $(\mu_{h,sib} + \mu_{f,a})/2 - (\mu_{f,sib} + \mu_{h,a})/2$	$P = 0.5851$	$P = 0.7954$	$P = 0.3509$	$P = 0.1886$

Contrasts definitions are given in the table below each hypothesis (see also Fig. 2), and were performed only after a significant crosstype effect in the ANOVA model. Significant tests are in boldface.
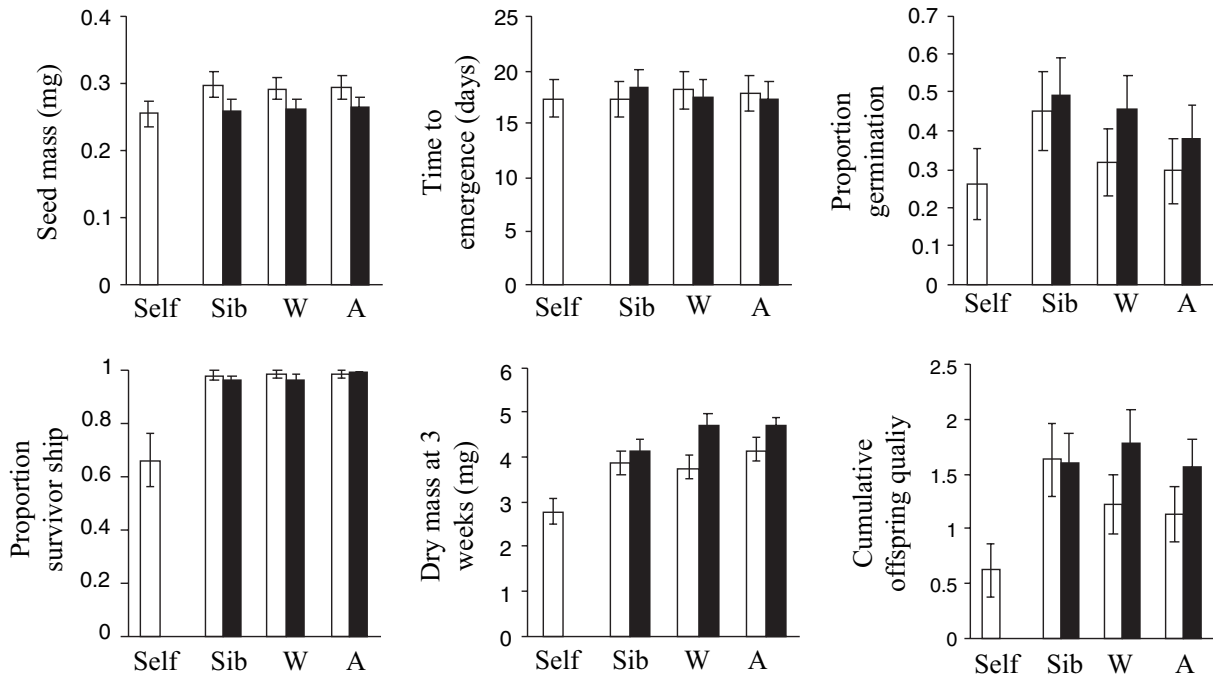
Fig. 3 Least-squares mean (SE) of offspring quality traits in *Silene acaulis*. Hermaphrodites are open bars, females are shaded bars. Breeding treatments are selfing (Self), sib mating (Sib), within-population outcrossing (W), and among-population outcrossing (A).

The multiplicative estimate of cumulative offspring quality showed strong differences among crosstypes. The frequency of families with zero cumulative offspring quality varied significantly with crosstype ($G = 21.56$, $P = 0.0014$), and was greatest for self-pollinated hermaphrodites (Table 3). When only families with offspring still present at harvest were analysed, offspring quality showed strong differences among crosstypes (Table 1). Selfed offspring suffered a reduction in quality compared to both outcross treatments (Table 2; Fig. 3). Independent of mate relatedness, females produced offspring of higher cumulative quality compared to hermaphrodites. Overall, the advantage to females due to offspring quality is 1.24, averaged across treatments (excluding selfs).

Table 3 Distribution of families with zero cumulative offspring quality.

	Hermaphrodite mothers				Female mothers			
	Self	Sib	W	A	Self	Sib	W	A
Families with zero cumulative offspring quality	12	1	7	7	–	7	14	6
Families with non-zero cumulative offspring quality	12	16	24	23	–	23	34	60

Breeding treatments are selfing (Self), sib mating (Sib), within-population outcrossing (W), and among-population outcrossing (A). *G*-test of independence between crosstype and presence/absence of families: $G = 21.56$; $P = 0.0014$.

There was no significant sex*breeding treatment interaction (Table 2). However, visual inspection of the means shows that female advantage was principally realized in the within- and among-population outcross treatments, as offspring from sib mating tended to be of similar quality between the sexes (Fig. 3).

As a measure of the relative fitness among treatments in cumulative offspring quality, inbreeding depression in selfed offspring had a mean of 0.67 and was significantly different from zero (Fig. 4). Values of sib-crossed hermaphrodites were variable, with δ ranging from −0.58 to 1.0, but the mean of −0.06 was not different from zero (Fig. 4). Sib-crossed females also showed a wide range in δ (−0.64 to 1.0), but averaged significantly above zero at 0.24 (Fig. 4).

There was a significant correlation between the proportion of seeds germinating in the parental generation and the frequency of females among the progeny ($r_s = 0.47$, $P < 0.0001$, $n = 75$). This indicates that field-collected sibships that germinated well also contained more female offspring.

Discussion

Offspring quality in gynodioecious *S. acaulis* was influenced both by the sex of the maternal parent and by relatedness of the pollen donor. Traditional explanations for increased fitness of females compared to hermaphrodites involve either avoidance of inbreeding depression or the redistribution of resources from pollen to seeds. In
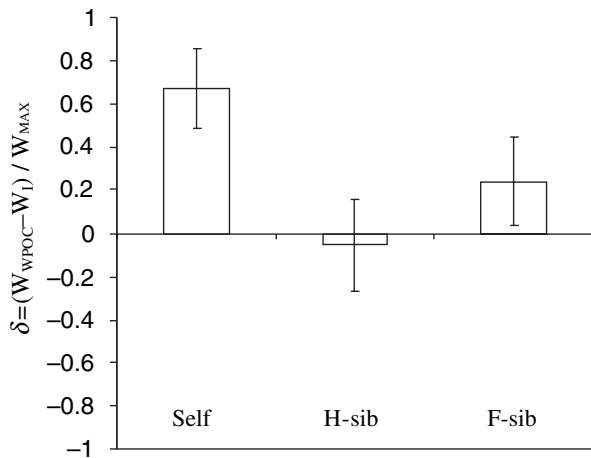
Fig. 4 Inbreeding depression for self and sib crosses based on cumulative offspring quality. Inbreeding depression was calculated for each maternal plant against the within-population outcross mean for each maternal sex. Values of $\delta > 0$ indicate reduced fitness in offspring of that crosstype relative to within-population outcrosses, while values of $\delta < 0$ indicate enhanced fitness. Error bars are 95% confidence intervals.

the current study, we controlled for inbreeding and found maternal sex was still significant when averaged across treatment levels (excluding selfing). Further, ours is one of few studies to include crosses *among* populations (the first in *S. acaulis*), thus controlling for the possibility of inbreeding when mating randomly within populations (see also Thompson & Tarayre, 2000; Emery & McCauley, 2002). Thus, even though females suffer a genetic cost by giving up gene transmission through pollen, they accrue the benefit of producing higher quality offspring. Below we propose mechanisms for the independent contributions of maternal sex and inbreeding to offspring quality, and discuss how they may influence evolution of this breeding system.

Maternal sex effects on offspring quality

Female mothers produced offspring of higher quality than hermaphrodite mothers.

Overall, offspring from female mothers germinated better and attained more biomass than hermaphrodites. This corroborates previous findings (Shykoff, 1988; Delph & Mutikainen, 2003; Delph, 2004), and supports the influence of maternal sex on offspring quality as a prominent feature across widely dispersed populations of *S. acaulis*.

The higher quality of offspring from female mothers does not appear to arise from greater maternal provisioning of seeds. Indeed, mass of seeds from female mothers may have been provisioned less than seeds from hermaphrodite mothers (Fig. 3). This result has arisen across multiple studies (Shykoff, 1988; Delph *et al.*, 1999; Delph

& Mutikainen, 2003; Delph, 2004). Because Delph *et al.* (1999) showed that the sexes do not differ for the quality of resources they allocate to seeds, sex-specific resource allocation does not seem an adequate explanation for the greater quality of offspring from female mothers.

We propose an alternative explanation for the higher quality of offspring from females of *S. acaulis*. For gynodioecious species with CMS, such as *S. acaulis*, female mothers may make more female offspring than hermaphrodite mothers. If female genotypes have inherently higher germination and growth rates than hermaphrodites, then the higher germination and growth observed among offspring from female mothers could arise from a surplus of female genotypes among their offspring. Skewed offspring sex ratios are common in gynodioecious plants with CMS, as maternally inherited genes often create sex ratios that are biased towards the sex of the mother (Gouyon & Couvet, 1987). Indeed, CMS is well documented in *Silene* (Desfeux *et al.*, 1996, Charlesworth & Laporte, 1998, Taylor *et al.*, 2001, Olson & McCauley, 2002), and biased sex ratios within sex morphs of *S. acaulis* have been previously observed (Delph & Mutikainen, 2003). While this mechanism has recently been proposed as a potential explanation for maternal sex effects in species with CMS (Koelewijn & van Damme, 2005), it has not yet received empirical support. We observed a significant correlation between the proportion of seeds germinating among field collected maternal sibships and the frequency of flowering females among the offspring. While this is not direct proof of a causal relationship between offspring sex and performance, especially since the sex of the maternal plants in the field were unknown, it is consistent with the hypothesis of an indirect effect of maternal sex, whereby female mothers make more female seeds which are more likely to germinate than hermaphrodite seeds. However, it is still possible that maternal sex itself has a direct and unknown genetic effect on offspring quality and sex ratio.

We could not perform the same correlation for juvenile growth rate, as we did not measure this trait on the parental generation. However, Caruso *et al.* (2003) recently demonstrated for *Lobelia siphilitica* (a gynodioecious species with CMS) that pre-reproductive females have higher photosynthetic rates than hermaphrodites. Further, male-sterile individuals of several species show clear differences from hermaphrodites in the production and metabolism of plant growth substances that affect germination and growth (Sawhney & Shukla, 1994).

An alternative explanation for differences in offspring quality between sex morphs is that sexes *per se* do not differ in quality, but that genes responsible for sex determination have pleiotropic effects on fitness traits. A cost to carrying alleles that restore male fertility in CMS lines is posited in several models of gynodioecy (Frank, 1989; Gouyon *et al.*, 1991; Bailey *et al.*, 2003). However, in most experiments (including the current study), a cost of male fertility restoration is impossible to separate from the 'cost' of being

a hermaphrodite. The distinction being that the former requires the direct action of restorer alleles on fitness, while the latter may be caused by other attributes indirectly associated with the sex of the plant, such as hormone regulation or photosynthetic rates. For example, in most well-studied systems, multiple CMS types exist which can be restored by alleles at independent restorer loci specific to the CMS types (e.g. Frank, 1989; Charlesworth & Laporte, 1998). Therefore, a plant may carry several restorer alleles but not possess one at the locus that restores its particular CMS type. Thus, it is genetically female even though it carries restorer alleles and thus may potentially still incur a cost of restoration. Future studies could separate a direct effect of offspring sex from a cost of restoration by correlating fitness traits with the number of restorer alleles carried by individuals (e.g. Bailey, 2002), and doing so for both sexes.

Inbreeding effects on offspring quality

The reduced survival, growth and cumulative quality of selfed offspring in this experiment all suggest that avoidance of selfing can contribute to female advantage in *S. acaulis*. Our estimate of inbreeding depression following selfing ($\delta = 0.67$) is not as severe as the previously reported estimate ($\delta = 0.98$, calculated based on number of surviving seedlings/plant; Shykoff, 1988). However, in accordance with previous results (Shykoff, 1988; Delph, 2004), we found no significant effect of selfing on germination traits but significantly reduced survival of selfed offspring compared to outcrosses. The reduced dry mass of selfs in our study contrasts with Shykoff (1988) who found no differences in seedling growth rate. This suggests the amount of genetic load and the life stages where it is manifested shows variation among geographic regions and/or experimental conditions, and highlights the importance of studying multiple populations. Moreover, it should be stressed that our results provide a minimum estimate of inbreeding depression pooled across these six populations of *S. acaulis*, given that the establishment conditions were benign and observations limited to early life stages. Establishment conditions for seedlings in the field are probably quite strenuous, and other investigators have observed minimal and episodic recruitment of *S. acaulis* (Philipp *et al.*, 1990). Therefore, the reduction in fitness of selfed offspring in the field may be much greater than in the greenhouse, as well as potentially compounded across a plant's lifetime (Armbruster & Reed, 2005).

An interesting consequence of biparental inbreeding is that it increases the proportion of seeds germinating. One explanation is that inbreeding increases the proportion of females in a sibship and females have greater germination ability, as argued above. Most genetic models of gynodioecy assume that alleles restoring male fertility on a CMS background are dominant, such that RR and Rr genotypes are hermaphrodite and the rr genotype is

female (Frank, 1989; Gouyon *et al.*, 1991; Charlesworth & Laporte, 1998). Evidence supporting dominance of restorers has also been observed for *S. acaulis* (Delph & Mutikainen, 2003). Given this model of sex determination, inbreeding should increase the frequency of females in a cohort, relative to the outbred case. Recent work on a related species with CMS, *S. vulgaris*, has demonstrated this (Emery & McCauley, 2002; Bailey & McCauley, 2005). If female seeds are more likely to germinate than hermaphrodite seeds, as may be the case (Table 2), then the higher rate of germination in sib-crosses versus outcrosses could be explained by inbreeding increasing the frequency of females.

While biparental inbreeding may influence the quality of a maternal sibship by changing the sex ratio, this effect may not be exclusive of inbreeding depression. When male fertility restorer alleles are dominant on a given CMS background, then inbreeding may not only increase the frequency of females, but also increase the expression of deleterious alleles. The net effect of these two antagonistic forces on offspring quality will be determined by the CMS and restorer genotypes of the parents, the relationship between inbreeding and inbreeding depression, and extent to which female and hermaphrodite offspring differ in their performance. Thus, fitness traits that show an effect of maternal sex in *S. acaulis* (proportion germination and dry mass), and would thus be expected to increase under inbreeding, may also be reduced by the expression of deleterious recessive alleles.

Interestingly, the relative fitness of offspring from sib-crossing and outcrossing, expressed as biparental inbreeding depression, was different between the sexes (Fig. 4). Female mothers experienced significant biparental inbreeding depression, while sib-crossed hermaphrodite mothers did not differ from within-population outcrosses. One explanation is that female lineages contain a buildup of deleterious alleles, as outcrossing lines are expected to harbor substantially more genetic load compared to inbreeding lines (Charlesworth & Charlesworth, 1987; Husband & Schemske, 1996; but see Byers & Waller, 1999). Delph (2004) found biparental inbreeding diminished offspring survival in a different population of *S. acaulis*; although there was no indication that the sexes responded differently. Among other gynodioecious species with CMS, Mutakainen & Delph (1998) found no evidence for biparental inbreeding depression in *Lobelia siphilitica*, while in *Thymus vulgaris*, Thompson & Tarayre (2000) found inbreeding depression for sib-crossed female and hermaphrodite mothers, although this varied among populations. In *Sidalcea oregana* ssp. *spicata*, Ashman (1992) found reduced progeny performance of sib-crossed females, but less so for hermaphrodite mothers, consistent with our study. It thus appears that biparental inbreeding can have important fitness consequences that may differ for each sex, and that species and populations within species are likely to vary in their response to inbreeding.

Summary

Overall, female mothers possess a significant advantage in offspring quality over hermaphrodites due to effects of maternal sex and by avoiding selfing and inbreeding depression. The effect of maternal sex may relate to either differences in progeny sex ratio between females and hermaphrodites and a direct effect of offspring sex on performance, or to a cost of carrying male fertility restorers. Biparental inbreeding also impacts offspring quality in *S. acaulis*, perhaps by influencing the offspring sex ratio. Results from this study affirm that while females of *S. acaulis* give up fitness through male function by not producing pollen, they make higher quality seed progeny, possibly by making more female offspring. This tradeoff in fitness with sex expression can contribute to the maintenance of females in gynodioecious populations, and also suggests factors affecting offspring sex ratios, such as inbreeding, will have important consequences for the evolution of this breeding system.

Acknowledgments

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