

# THE ROLE OF POLLINATION LEVEL ON THE REPRODUCTION OF FEMALES AND HERMAPHRODITES IN THE GYNODIOECIOUS PLANT *GYPSOPHILA REPENS* (CARYOPHYLLACEAE)<sup>1</sup>

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In gynodioecious plant species, females are expected to have more resources available for maturing seeds because pistillate flowers are smaller, do not produce pollen, and are thus less costly than perfect flowers. The potential female advantage arising from more abundant resources is, however, likely to vary depending on whether seed production is limited by resource or pollen availability. Here we experimentally investigated the influence of pollen and resource limitation on female advantage in a gynodioecious species using two levels of pollination. Total seed production of females was always greater than that of hermaphrodites: females produced more flowers and more fruits that contained similar numbers of seeds of similar mass. Under low pollination, female and hermaphrodite plants allocated resources to increased flower production rather than to increased seed size or quality. We did not detect any influence of pollen or resource limitation on female advantage, which remained similar under low (= abundant resources) and full pollination. Outcrossed fruits performed better than selfed fruits when the same plant received both selfed and outcrossed pollen on different flowers. These differences were not greater under high pollination, possibly because resources available for each fruit did not differ between our pollen intensity treatments.

**Key words:** female advantage; gynodioecy; *Gypsophila*; inbreeding depression; pollen limitation; resource limitation.

Gynodioecy, the plant mating system where male-sterile (female) individuals coexist with hermaphrodite individuals, has attracted the attention of evolutionary biologists since Darwin (1877). In gynodioecious species, females contribute to the next generation via seeds only, while hermaphrodites produce seeds and pollen. Theoretical studies have shown that the persistence of females in a population therefore requires a compensatory reproductive advantage, mainly attributable to (i) a reallocation of resources not used for pollen production and pollinator attraction to seed production (Lewis, 1941; Charlesworth and Charlesworth, 1978) and/or (ii) avoidance of inbreeding depression that can affect the selfed offspring of hermaphrodites in self-compatible species (Charlesworth and Charlesworth, 1978). Many empirical studies have found a reproductive female advantage in several gynodioecious species, with females producing more flowers, more fruits, and heavier or better provisioned seeds with higher germination rates and higher seedling survival (reviewed in Shykoff et al., 2003).

The production of seeds is frequently limited by pollen quantity or quality (Ashman et al., 2004), but pollen limitation varies greatly among years and populations (Burd, 1994; Baker et al., 2000). When plants are not pollen limited, seed and fruit production are commonly limited by resource availability (Primack and Hall, 1990; Calvo, 1993; Ehrlén and Eriksson,

1995). Hence, most populations probably shift between reproductive effort being limited by pollen and resource availability over time. In gynodioecious populations, females and hermaphrodites may not be affected similarly by pollen and resource limitation, which likely influence female advantage and the maintenance of females in natural populations.

In pollen-limited gynodioecious populations, females may suffer a higher decrease in seed production because they bear pistillate flowers that are usually smaller than perfect ones (Delph, 1996), receive less pollen than do hermaphrodites in some natural gynodioecious populations (Philipp, 1980; Uno, 1982; Williams et al., 2000), and have no opportunity for selfing, as opposed to hermaphrodites of self-compatible gynodioecious species (Maurice and Fleming, 1995). Nevertheless, the actual evidence for increased pollen limitation in females of gynodioecious populations is still inconclusive (Shykoff et al., 2003).

When reproduction is resource-limited, females and hermaphrodites should differ in their investment of resources available for reproduction. Hermaphrodites bear higher fixed costs associated with the production of male and female floral structures (Lloyd, 1976), but may respond plastically by varying the relative amount of resources invested to female and male functions, reducing ovule production under conditions of resource limitation (Lloyd, 1974, 1976). Resource availability may therefore influence functional gender in gynodioecious populations by rendering hermaphrodite individuals relatively more male.

In addition, in self compatible species, resource limitation may strengthen the inbreeding depression affecting the selfed offspring of hermaphrodites. Smaller or fewer selfed vs. outcrossed seeds may result from differential competition among developing zygotes or from maternal choice. When resources

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available for reproduction are limited, differences in the ability to compete for maternal resources between embryos of different genetic quality may result in selective embryo abortion (reviewed in Korbecka et al., 2002) or production of smaller selfed seeds. This could also occur if maternal plants actively select among the available pollen or zygotes and invest preferentially in the fruits and seeds sired by high quality pollen (Janzen, 1977; Bertin, 1982; Haig and Westoby, 1988; Vaughton and Ramsey, 1997). Smaller (selfed) seeds may have lower germination rates and produce individuals of lower quality (Porcher et al., 2004) than do larger (outcrossed) seeds. Resource limitation can thus exacerbate differences in genetic quality.

Here we explore the relationship between pollen limitation, resource reallocation and female reproductive success of females and hermaphrodites of the gynodioecious species *Gypsophila repens* L. (Caryophyllaceae). We study female advantage under different levels of pollen limitation, with either full pollination or pollen limitation, induced by pollinating only 25% of flowers produced. In natural populations of *G. repens*, at least 50% of flowers commonly set seeds within a plant (López-Villavicencio et al., 2003), so that fruits developing from plants under 25% pollination should not be resource-limited. In contrast, when all flowers mature fruits, resource limitation for each fruit should be stronger. Under such conditions, differences in genetic quality are more likely to be revealed and mechanisms such as female choice or differential competitive ability among zygotes may come into play. We predict that under pollen limitation, hermaphrodites should have enough resources available to invest fully in pollen, fruit, and seed production. Thus, under low pollination, any differences in female reproductive success between females and hermaphrodites should not be due to the cost of pollen production, but rather to inbreeding depression or to some pleiotropic effects of genes restoring male fertility (Delph and Mutikainen, 2003). In contrast, when all flowers receive sufficient pollen for all ovules to be fertilized, we predict that hermaphrodites with dual sexual function should experience greater resource limitation and produce fewer or smaller seeds than do the females under similar conditions. Further, when selfed and outcrossed fruits compete for the same resources on the same maternal plant, competition between them should be stronger under full pollination, when resources are most likely to be limited.

We address the following questions: (1) What is the influence of pollen and resource limitation on female advantage? By comparing fruit and seed production of outcrossed hermaphrodites vs. females under two pollination levels, we tested whether this advantage was magnified under full pollination, when resources are likely to be reduced for each developing seed. (2) Is inbreeding depression exacerbated when resources rather than pollen limit seed production? This was investigated by comparing fruit and seed production of hermaphrodites that were either outcrossed or selfed under both pollination levels. (3) Is the difference between selfed and outcrossed offspring greater when they compete for the same common pool of limited resources on the same maternal plant?

## MATERIALS AND METHODS

**Study species and plant material**—*Gypsophila repens* is a long-lived perennial species distributed in the mountains of southern and central Europe. This species is gynomonocious-gynodioecious, i.e., with female and her-

maphrodite plants (bearing pistillate and perfect flowers respectively) and some gynomonocious plants (with both types of flowers). In natural populations, individuals bloom from June to late October. The perfect flowers are protandrous and larger than pistillate flowers (López-Villavicencio et al., 2003). Hermaphrodites are self compatible, but require insect visits to be pollinated (M. López-Villavicencio, personal observation). The most common pollinators are syrphid flies and small solitary bees (J. Shykoff, personal observation). Pollinators visit several flowers per plant and most of the neighboring plants before moving on over longer distances (M. López-Villavicencio, unpublished data).

A total of 108 flowering individuals (30 females, 78 hermaphrodites) was used for the experiment. Plants were sown in a greenhouse in January 2001 from bulk seed collected from a wild population in the Italian Alps (Grosio, 46°17'24" N and 10°15'11" E) the previous summer. Some hermaphrodites produced a few pistillate flowers, but were still considered hermaphrodites because both sexual functions were present on the same plant. Plants of each sex category were randomly assigned to one of the two following pollination treatments: high pollination (hand pollination of 100% of the flowers produced) or low pollination (hand pollination of 25% of the flowers). In the low pollination treatment, the nonpollinated flowers were pinched off as the flowers bloomed to reduce costs of flower maintenance. All female plants (F) were outcrossed. Hermaphrodites received one of three "pollen source" treatments: all flowers outcrossed (HO), all flowers selfed (HS) and 50% flowers outcrossed, 50% flowers selfed (HOS).

Pollinations were performed between May and July 2001 by brushing styles with three fresh anthers presenting ripe pollen. Outcrosses were performed with anthers from three different individuals. Each pollinated flower was marked with a small piece of labeled tape on the pedicel. All treatments were continued for almost 3 months until most individuals under high pollination stopped flower production.

To estimate the effect of pollen quantity and quality on fecundity, fruits were collected as they matured (ca. 15 d after pollination), and seeds were bulked and counted. Mean seed mass was assessed by weighing a random sample of 400 seeds per plant to the nearest 0.01 mg. A sample of seeds was then placed in petri dishes with 1% agar (25 seeds per dish, two petri dishes per treatment and plant) and seedling emergence was recorded daily for 1 wk.

**Statistical analyses**—All the statistical analyses were conducted using SAS (SAS, 2000). We tested the differences in flower production among the four types of plants (F, HO, HS, and HOS) and the two pollination levels (high and low) using a two-way factorial ANOVA (PROC GLM). For the other analyses, hermaphrodites that had half of the flowers were selfed and half outcrossed (HOS) were studied separately to avoid pseudoreplication.

We estimated the female reproductive advantage due to resource allocation alone by comparing fruit set (i.e., the fraction of pollinated flowers developing into fruits), total seed production, mean seed number per fruit, mean seed mass, and germination rate between (outcrossed) females and outcrossed hermaphrodites (F vs. HO), using linear models including sex and pollination level as explanatory variables. The effects of inbreeding depression under different pollination levels were tested by comparing the same reproductive traits of outcrossed and selfed hermaphrodites (HO vs. HS), with the same models described earlier. Total seed production, mean seed number, and seed mass followed a normal distribution and were analyzed with a two-way factorial ANOVA (PROC GLM). Total seed production and seed mass were transformed using natural logarithm to stabilize the variance of the residuals (Shapiro-Wilk's test). Fruit set and germination rate represented proportions and were not normally distributed; they were analyzed with ANODEVs (PROC GENMOD) using the logit link function. The effects of seed mass, pollen source, and pollination level on germination rates were analyzed with a minimal factor ANCODEV (PROC GENMOD) with seed mass as the covariate and stepwise elimination of nonsignificant interactions.

We examined whether resources were preferentially invested in outcrossed vs. selfed embryos using hermaphrodites on which half of their flowers were selfed and half outcrossed (HOS). These individuals were studied separately, but were included in some figures. We compared fruit set, mean seed number per fruit, seed mass, and germination rate between selfed and outcrossed flow-

ers using paired *t*-tests (Sokal and Rohlf, 1995). For each plant, we calculated the difference between outcrossed and selfed flowers ( $o - s$ ) for each variable and tested whether the mean difference differed from zero. To test whether the relative performance of selfed vs. outcrossed flowers differed with level of pollination (25% vs. 100%), we performed a second *t* test on these differences, comparing them between the two pollination levels. This effectively examined whether there was an interaction between pollen source and pollination level.

## RESULTS

**Effect of pollination level on flower production**—Plants on which all flowers were pollinated produced fewer flowers than plants with only 25% of flowers pollinated ( $F_{1,100} = 11.23$ ,  $P = 0.0011$ , Fig. 1), suggesting that resources allocated to fruit production limited future flower production. Flower production also differed significantly among the four types of plants ( $F_{3,100} = 3.77$ ,  $P = 0.0131$ ). Multiple comparisons using the Tukey-Kramer method showed that females produced significantly more flowers than did hermaphrodites from all treatments. Comparisons among hermaphrodites revealed that flower production was significantly greater for selfing vs. outcrossing individuals, while hermaphrodites that received both self and outcross pollen were intermediate. The mean number of flowers (nontransformed data, mean  $\pm$  SE) pooled over the two pollination levels was in population F:  $56.2 \pm 4.67$ ,  $N = 30$ ; in HS:  $41.8 \pm 4.06$ ,  $N = 20$ ; in HOS:  $35.7 \pm 3.13$ ,  $N = 38$ ; and in HO:  $26.1 \pm 2.43$ ,  $N = 20$  (Fig. 1). We detected no interaction between pollination treatment and plant sex ( $F_{3,100} = 0.1928$ ).

**Differences between females and outcrossed hermaphrodites**—Females produced more fruits per pollinated flower (mean fruit set  $\pm$  SE, in population F:  $0.87 \pm 0.02$ ,  $N = 30$ ; HO:  $0.75 \pm 0.04$ ,  $N = 20$ ) and more seeds per plant than did outcrossed hermaphrodites (mean total seed production  $\pm$  SE, F:  $426.4 \pm 61.79$ ,  $N = 30$ ; HO:  $158.6 \pm 28.15$ ,  $N = 20$ ), but there were no differences for mean seed number per fruit or seed mass (Table 1, Fig. 1). Outcrossed seeds from hermaphrodites had a significantly higher germination rate than seeds from females when seed mass was included as a covariate (germination rate  $\pm$  SE, F:  $0.84 \pm 0.04$ ,  $N = 30$ ; HO:  $0.93 \pm 0.03$ ,  $N = 20$ ; Table 1).

Total seed production was, as expected, greater under high than under low pollination (high pollination treatment =  $471.5 \pm 69.1$ ,  $N = 25$ ; low pollination treatment =  $167.2 \pm 27.8$ ,  $N = 25$ ; Table 1, Fig. 1), but pollination level did not influence fruit set, mean seed number per fruit, seed mass, or germination rate. The interaction between pollination level and plant sex was never significant, so that differences between females and outcrossed hermaphrodites were not greater under high pollination level (Table 1).

**Differences between selfed and outcrossed hermaphrodites**—Germination of seeds produced by outcrossed hermaphrodites was higher than for those from selfed hermaphrodites (mean germination rate  $\pm$  SE, HO:  $0.93 \pm 0.03$ ,  $N = 20$ ; HS:  $0.79 \pm 0.04$ ,  $N = 20$ ; Table 2, Fig. 1). In addition, seeds produced under low pollination were more likely to germinate than seeds produced by plants under high pollination, but this difference was marginally significant (ANCODEV, Table 2). As before, total seed production was significantly greater under high than under low pollination (high pollination:  $270.6 \pm 27.1$ ,  $N = 20$ ; low pollination:  $89.0 \pm 10.4$ ,  $N =$

20). We observed no other significant difference between HO and HS or between pollination levels. No significant interaction was found between pollen source and pollination level. However, the mean seed number per fruit tended to be greater for outcrossed than selfed plants, and the interaction between pollen source and pollination level was almost significant, with self-pollinated plants producing fewer seeds per fruit when all available flowers were pollinated (Table 2, Fig. 1).

**Differences between selfed and outcrossed offspring from the same maternal plant**—Outcrossed fruits contained more seeds ( $t = 2.25$ ,  $P = 0.03$ ,  $N = 38$ ) that were more likely to germinate ( $t = 2.71$ ,  $P = 0.01$ ,  $N = 36$ ; Fig. 1) than did selfed fruits on the same plants. Fruit set and seed mass were similar regardless of the pollen source (fruit set,  $t = 0.66$ ,  $P = 0.51$ ,  $N = 38$ ; seed mass,  $t = 0.65$ ,  $P = 0.52$ ,  $N = 36$ ; Fig. 1). Finally, the differences between outcrossed and selfed flowers on the same plant were not larger under the high pollination level (fruit set,  $t = 1.14$ ,  $P = 0.26$ ,  $N = 38$ ; mean seed number,  $t = -0.87$ ,  $P = 0.39$ ,  $N = 38$ ; seed mass,  $t = -1.04$ ,  $P = 0.31$ ,  $N = 36$ ; germination rate,  $t = -0.62$ ,  $P = 0.54$ ,  $N = 36$ ).

## DISCUSSION

The aim of this study was to investigate the relationship between pollen and resource limitation on female advantage in a gynodioecious species. We expected female advantage to be magnified under high pollination because hermaphrodites may not have sufficient resources to fully invest in pollen production as well as in the production of a large number of fruits and seeds. Besides, inbreeding depression may be stronger when resources are limited. However, we found little evidence for increased female advantage with increased pollination level, either through resource allocation or through avoidance of inbreeding depression, as revealed by nonsignificant interactions between pollination level and either plant sex or pollen source. Next, we discuss possible reasons why female advantage was not influenced by pollen and/or resource limitation.

**Limitation of resources at the plant level**—We assumed that manipulating pollination levels would vary the amount of resources available for each developing fruit. This methodology was preferred to the addition of fertilizer because fertilizer may alter the physiological status of plants, favoring vegetative growth over flower production (Eckhart and Chapin, 1997; Poot, 1997). The observed increased flower production under low pollination indicates that we succeeded in obtaining different resource levels across pollination treatments. In addition, flower production of plants under high pollination started to decline after 2 months of pollination and completely stopped after ca. 3 months, probably because fruit and seed development drained most resources from the plants (Colosi and Cavers, 1984; Meagher and Delph, 2001; Wright and Meagher, 2003). In contrast, plants with 25% pollinated flowers were still producing flowers at the end of the experiment. Finally, under full pollination female plants with presumable high-quality (outcrossed) seeds only matured about 85% of their flowers into fruits, suggesting that fruit production was resource limited, although other explanations for low seed/ovule ratios can be invoked (e.g., Melsner and Klinkhamer, 2001).

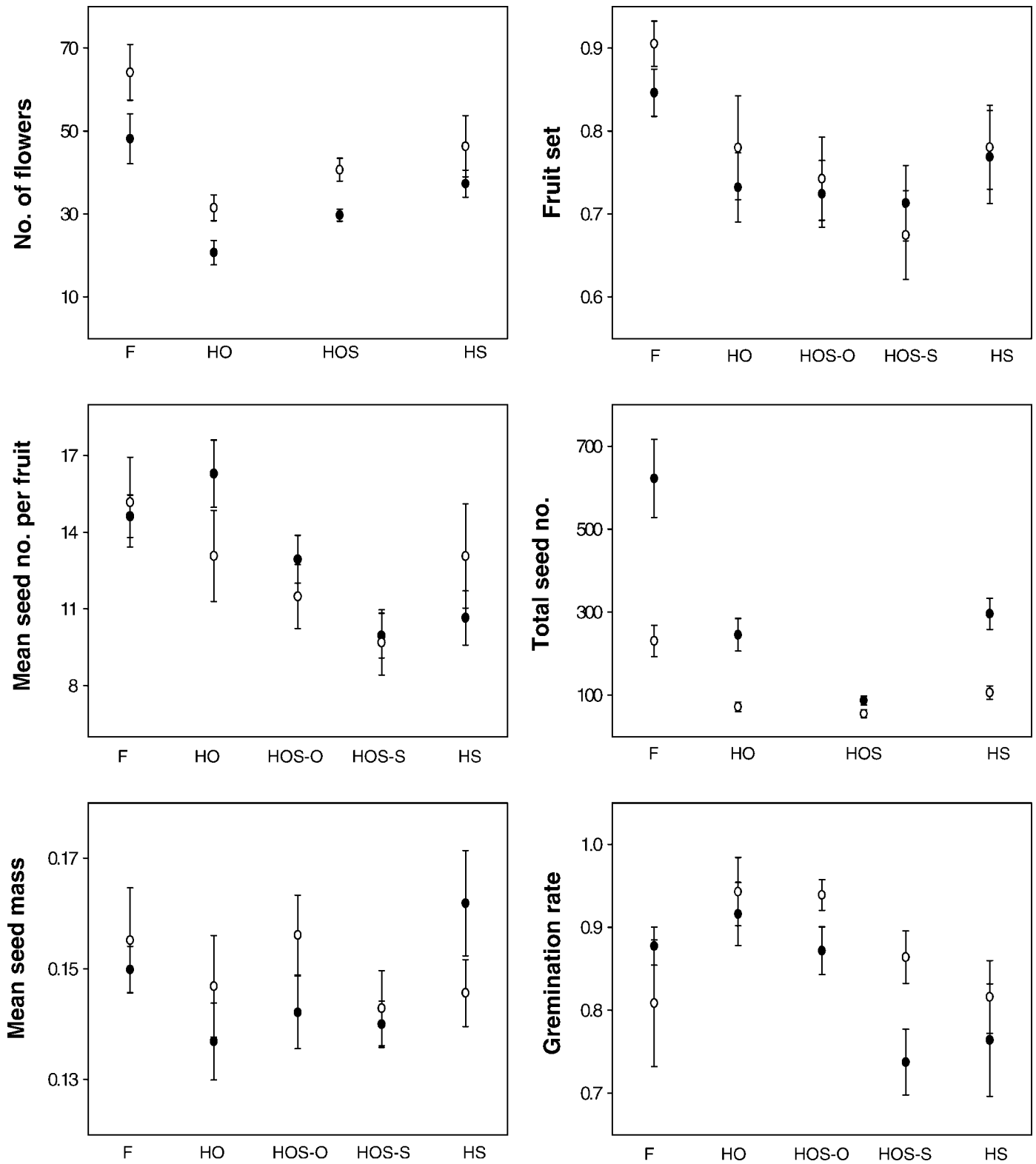


Fig. 1. Mean and standard error (1 SE) of reproductive characters for females (F), outcrossed hermaphrodites (HO), hermaphrodites receiving both treatments (HOS) (crossed HOS-O and selfed pollen HOS-S), and selfed hermaphrodites (HS). Plants were pollinated following two pollination levels: 25% (open circles) and 100% (solid circles) of the flowers produced.

TABLE 1. Differences for reproductive characters between female and outcrossed hermaphrodite plants under two pollination levels; 25% and 100% of the flowers produced. (A) Fruit set and germination rate were analyzed using ANODEV with a binomial error distribution (PROC GENMOD in SAS, 2000). Differences in germination rate with mass as a covariate were analyzed with an ANCODEV (PROC GENMOD). (B) Differences in total seed number, mean seed number per fruit and seed mass were tested using an ANOVA (PROC GLM). Significant values are in bold.

A)	Variable and source of variation	df	Pearson's $\chi^2$		P	
Fruit set ( $N = 50$ observations)						
	Sex	1	9.01		0.0027	
	Pollination level	1	1.31		0.25	
	Sex $\times$ Pollination level	1	0.83		0.36	
Germination rate ( $N = 49$ observations)						
	Sex	1	8.19		0.0042	
	Pollination level	1	0.02		0.9018	
	Sex $\times$ Pollination level	1	0.77		0.3792	
	Seed mass	1	9.59		0.0020	
B)	Variable and source of variation	$R^2$	df	MS	F	P
Ln total seed production						
		0.57				
	Sex		1	12.84	28.24	<0.0001
	Pollination level		1	14.03	30.85	<0.0001
	Sex $\times$ Pollination level		1	0.299	0.658	0.42
	Error		45	0.4548		
Mean seed no.						
		0.04				
	Sex		1	0.571	0.021	0.88
	Pollination level		1	21.28	0.791	0.38
	Sex $\times$ Pollination level		1	42.79	1.591	0.21
	Error		46	28.89		
Ln seed mass						
		0.06				
	Sex		1	0.064	2.242	0.14
	Pollination level		1	0.022	0.768	0.39
	Sex $\times$ Pollination level		1	0.007	0.234	0.63
	Error		45	0.029		

TABLE 2. Differences for reproductive characters between outcrossed and selfed hermaphrodites under two pollination levels; 25% and 100% of the flowers produced. (A) Fruit set and germination rate were analyzed using ANODEV with a binomial error distribution (PROC GENMOD in SAS, 2000). Differences in germination rate with mass as a covariate were analyzed with an ANCODEV (PROC GENMOD) with seed mass as the covariate and stepwise elimination of non significant interactions. (B) Differences in mean seed number per fruit and seed mass were tested using an ANOVA (PROC GLM). Significant values are in bold.

A)	Variable and source of variation	df	Pearson's $\chi^2$		P	
Fruit set ( $N = 40$ observations)						
	Pollen source	1	0.04		0.9887	
	Pollination level	1	0.00		0.8365	
	Pollen source $\times$ Pollination intensity	1	0.00		0.9791	
Germination rate ( $N = 40$ observations)						
	Pollen source	1	27.82		<0.0001	
	Pollination level	1	3.72		0.0538	
	Seed mass	1	21.50		<0.0001	
B)	Variable and source of variation	$R^2$	df	MS	F	P
Ln total seed production						
		0.53				
	Pollen source		1	1.038	2.895	0.0975
	Pollination level		1	13.50	37.65	<0.0001
	Pollen source $\times$ Pollination level		1	0.075	0.208	0.6511
	Error		36	0.358		
Mean seed number per fruit						
		0.15				
	Pollen source		1	79.93	3.127	0.0855
	Pollination level		1	1.613	0.063	0.8031
	Pollen source $\times$ Pollination level		1	79.44	3.107	0.0864
	Error		36	25.57		
Ln seed mass						
		0.06				
	Pollen source		1	0.068	2.447	0.1265
	Pollination level		1	0.002	0.085	0.7728
	Pollen source $\times$ Pollination level		1	0.068	2.425	0.1282
	Error		36	0.028		

**Female advantage over outcrossed hermaphrodites**—Females outperformed hermaphrodites for a range of reproductive characters, producing more flowers and setting more fruits than did outcrossed hermaphrodites. This resulted in a larger total seed production, as frequently observed in gynodioecious species (Ashman, 1999; Shykoff et al., 2003). However, we found no evidence that females invested more or better resources per seed than did outcrossed hermaphrodites (Delph et al., 1999). In contrast, seeds from outcrossed hermaphrodites, although they did not differ in mass, had a higher germination rate under benign laboratory conditions than did seeds from females. Whether this reflects behavior in the wild remains open because germination rates can differ greatly between laboratory and natural conditions (Dudash, 1990; Wolfe, 1993; Ramsey and Vaughton, 1998). We observed high germination rates for all plant groups in the greenhouse, whereas in natural conditions seed germination and early establishment seem to be two of the most critical stages of the life cycle of *G. repens* (M. López-Villavicencio, unpublished data). The female advantage resulting from larger fruit and seed production was strong under both pollination levels, which contrasts with our expectation that female advantage should be magnified under high pollination, when female function of hermaphrodites is likely to suffer more strongly from resource limitation.

The absence of difference in female advantage between the two pollination regimes may result from an inaccurate simulation of high and low resource availability at the level of the developing fruit, although our treatment most likely affected resource availability at the plant level, as explained earlier. We found that plants with only a few pollinated flowers continued to produce flowers, ultimately producing many more flowers than did plants on which all flowers were pollinated. This suggests that plants allocated some of the released resources to additional flower production, and therefore may not have allocated them to developing fruits and seeds. We observed no differences between the two pollination treatments for fruit set, seed number per fruit or seed mass, confirming that fruit and seeds in the low pollination treatment did not benefit from additional resources. To date it is unclear how freely resources can be shunted within plants. Hand-pollinated flowers have been shown to usurp resources from control flowers on the same plants (McCall and Primack, 1987; Primack and Hall, 1990), suggesting a certain flexibility in resource allocation within individuals. However, the generality of this result has been questioned (Real and Rathcke, 1991; Ehrlén, 1992; Fox, 1992; Parker, 1997). Here we found that plants as a whole profited from not maturing all flowers into fruits, but this profit was turned into new flowers instead of being assigned to already developing fruits and seeds. Seed size is predicted to have an optimal value that depends on the relationship between juvenile size and juvenile mortality. Larger seeds have increased success; however, the relationship between seed size and success follows a diminishing returns function, so that at some optimal size, additional resources are best spent elsewhere rather than on further increasing the size of developing seeds (Lloyd, 1987).

Our observation that plants tend to allocate extra resources to flower production rather than to seeds suggests that the evolution of resource allocation may be driven mainly by pollen, not resource, limitation in natural populations of *G. repens*. Increased flower production is known to generally result in increased pollinator attraction (Worley et al., 2000); this would result in a potentially increased seed set for pollen-limited fe-

males and increased male reproductive success as well as increased progeny quality (see next) for outcross pollen-limited hermaphrodites. Although pollen limitation is expected to be stronger for females in gynodioecious populations (Shykoff et al., 2003), similar behavior of females and hermaphrodites in response to pollen limitation indicate that it might not be the case in the present natural populations of *G. repens*. This is confirmed by a previous study showing no sex differential pollen limitation at flower level for *G. repens* (López-Villavicencio et al., 2003). Hence, although pollen (and resource) limitation are important mechanisms controlling the evolution of plant populations in general, they might not be fundamental in determining female advantage and female maintenance on gynodioecious populations, as already emphasized by Asikainen and Mutikainen (2005) because they affect both sexes similarly.

**Inbreeding depression and resource availability**—When inbreeding depression was measured as the relative decrease in fitness of offspring from experimentally selfed vs. experimentally outcrossed individuals, we found evidence of inbreeding depression only for seed germination (Table 2). None of the interactions between pollination level and selfed vs. outcrossed pollen was significant, showing that the effects of inbreeding depression were not stronger under resource limitation. Inbreeding depression measured in benign greenhouse or garden conditions can be less than inbreeding depression under stressful natural environments (Dudash, 1990; Johnston, 1992; Wolfe, 1993; Bijlsma et al., 1999). Fully pollinated selfed plants produced far fewer seeds per fruit than fully pollinated outcrossed plants, whereas no difference was observed under partial pollination (Table 2, Fig. 1). This single observation is in line with our predictions that inbreeding depression, in this case probably resulting in embryo abortion (Husband and Schemske, 1996), should be more pronounced under resource-limited conditions.

Selfed hermaphrodites produced significantly more flowers than outcrossed hermaphrodites. This increased flower production with self-pollination is difficult to explain, because it was not traded off against any of the other currencies that we measured, such as whole plant seed production, fruit set, or seed mass. Completely selfed, hermaphroditic plants produced almost as many flowers as females though these flowers contained pollen and the over-production was not compensated in any detectable way. Unfortunately, we did not measure flowers or estimate pollen production, so we cannot be sure that later flowers of these selfed hermaphrodites were not smaller or contained less pollen. Plants have been found to modify aspects of their floral biology in response to the nature of the pollen they receive, especially when they are highly selfed (Benjamin and Hainsworth, 1986). Conceivably, completely selfed hermaphrodites therefore invest in additional flower production to increase overall display, attract more pollinators, and increase the chance of receiving outcross pollen.

**Reproductive characters of selfed and outcrossed offspring on the same maternal plants**—When inbreeding depression was measured by comparing the fitness of selfed and outcrossed offspring on the same maternal plant, we observed significant inbreeding depression for seed production as well as for germination rates. That inbreeding depression is expressed more strongly within a single plant that receives both self and outcross pollen has been observed for other species,

such as *Blandfordia grandiflora* and *Banksia spinulosa* (Ramsey and Vaughton, 1996, 1998; Ramsey, 1997; Vaughton and Ramsey, 1997). Several authors suggest that such differences in fitness between outcrossed and selfed progeny result from maternal control and/or embryo competition (Korbecka et al., 2002), which may be stronger under resource limitation, as in *Banksia spinulosa* (Vaughton and Ramsey, 1997). In our study, we found no significant increase in the differences between outcrossed and selfed progeny at higher pollination level, which shows that these differences were not exacerbated when resources were rendered more limited. Furthermore, the difference in mean seed number and seed germination between selfed and outcrossed flowers in this treatment was similar to differences previously described between selfed and outcrossed hermaphrodites receiving only one type of pollen. This suggests that inbreeding depression in *G. repens* involves mainly deleterious mutations, whose expression depends little on resource availability, and is not affected by maternal control of resource allocation and/or competition between selfed and outcrossed embryos.

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