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Effects of self-pollination and maternal resources on reproduction and offspring performance in the wild lupine, *Lupinus perennis* (Fabaceae)

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Abstract We examined the effects of self-pollination and resource addition to maternal plants of Wild Lupine on seed production in the field, and on offspring performance in the greenhouse. Although 24% of flowers set fruits when open-pollinated, only 11% of flowers set fruits when self-pollinated. Self-pollination significantly reduced fruit and seed production per inflorescence and increased aborted seeds per fruit. Resource addition to maternal plants significantly increased fruit and seed number in the field. Moreover, selfed plants exhibited greater variability in seed production in the absence of resource addition to the maternal plant. We planted a total of 1,306 of the seeds from this experiment in the greenhouse. While self-pollination did not affect the proportion of seeds emerging, it slowed seedling emergence by 5–10%, and reduced offspring biomass by 25–35%. Interestingly, resource addition to the maternal plants significantly decreased proportion of seedlings surviving after 5 months. Moreover, offspring from maternal plants with resource addition expressed more inbreeding depression in the seedling stage compared to offspring from maternal plants without resource addition, for which more inbreeding depression occurred during seed maturation and emergence. These results indicate that conservation efforts using benign environments to increase number of seeds or offspring may face compensating reductions in survivorship at other life stages.

Keywords *Lupinus perennis* · Inbreeding depression · Selfing · Resource addition · Seed production · Survival · Biomass

Introduction

Inbreeding depression, the decrease in performance resulting from mating between close relatives, is thought to be an important selective agent in the evolution of selfing (Charlesworth and Charlesworth 1987; Byers and Waller 1999). It is also a major concern in the conservation of endangered species, especially those in small or recently fragmented populations (Byers and Waller 1999; Hedrick and Kalinowski 2000). In such populations, recessive or partially recessive deleterious alleles that would be masked as heterozygotes in large outbreeding populations are exposed by the increased homozygosity that follows from the increased rate of selfing and biparental inbreeding that often occurs in small populations (Byers and Waller 1999; Hedrick and Kalinowski 2000; Keller and Waller 2002). This might increase the expression of inbreeding depression in small or recently fragmented populations.

The expression of inbreeding depression reflects not only population genetic attributes, but also potential environmental effects (Uyenoyama et al. 1993; Jimenez et al. 1994; Hedrick and Kalinowski 2000). Environmental and genetic factors may interact in the expression of inbreeding depression in three ways. First, inbreeding effects could be more severe under environmental stresses such as resource limitation, pathogens, drought, humidity, or strong competition (Schemske 1983; Dudash 1990; Schmitt and Ehrhardt 1990; Hoffman and Parsons 1991; Jimenez et al. 1994; Miller 1994; Dahlggaard and Loeschcke 1997; Levri and Real 1998; Bijlsma et al. 1999; Dahlggaard and Hoffmann 2000; but see Waller 1984; Mitchell-Olds and Waller 1985; Heywood 1993; Norman et al. 1995; Hauser and Loeschcke 1994; Cheptou et al. 2000). Second, in a stressful environment,

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inbred offspring may be more variable in traits related to fitness than outbred offspring, for example, if heterozygotes are better able to buffer themselves against environmental variation and stress (Schemske 1983; Dudash 1990; Hoffman and Parsons 1991). Finally, environmental conditions may affect the magnitude and timing of expression of inbreeding depression. Resource enriched environments may reduce the manifestation of performance reductions due to deleterious alleles, artificially reducing selection in one life stage and delaying the expression of inbreeding depression (Montalvo 1994; Husband and Schemske 1996).

Most studies have focused on how different growth environments for offspring alter the expression of inbreeding depression (e.g. Pray et al. 1994; Jimenez et al. 1994; Dahlgaard and Loeschke 1997; Hauser and Loeschke 1994; Cheptou et al. 2000). However, the maternal environment may also be important: maternal plants in stressful environments might provision only the most vigorous seedlings, and this could potentially weed out inferior offspring at this early stage (i.e., during seed maturation, Melser and Klinkhamer 2001). A possible consequence of this would be that, when the maternal environment is more benign, the expression of inbreeding depression might be shifted to later life stages (e.g., seedling survival, Montalvo 1994). Resource levels in maternal plants can also affect seed mass, germination and other important fitness components in a number of species (e.g., Roach and Wulff 1986; Dolan 1994). We are interested in how maternal environment may change the magnitude and timing of the expression of inbreeding depression. More specifically, we investigated how resource conditions applied to maternal plants affect offspring performance.

In this study, we applied fertilizer and water to adult plants in the field, but only during the seed maturation stage. We grew the resulting seeds in the greenhouse, under a uniform resource environment, to investigate how resource addition during this critical point in seed provisioning affects seedling performance including seed emergence, seedling survival, and seedling biomass at 5 months in *Lupinus perennis*. We ask the following questions:

1. Does selfing affect seed production and offspring performance?
2. How does fertilizer/water addition during seed maturation affect offspring performance?

Materials and methods

The study organism

The wild lupine, *L. perennis* (Fabaceae), is a perennial that grows on nutrient-poor, sandy soils throughout Eastern North America (Gleason and Cronquist 1991). This species requires open areas with abundant sunlight (Grigore and Tramer 1996). *L. perennis* is considered

potentially threatened in the state of Ohio (Ohio Division of Natural Areas and Preserves 1996) and is currently listed as vulnerable or extirpated in several states or provinces across its former range in the US and Canada (NatureServe 2005). *L. perennis* is also a primary target of restoration and conservation efforts because it is the only larval food source for the federally endangered Karner Blue Butterfly, *Lycaeides melissa samuelis* (US Fish and Wildlife Service 1992; Ohio Division of Natural Areas and Preserves 1996; Haack 1993) as well as a significant food source for two Ohio state-listed butterfly species, the Persius Dusky Wing, *Erynnis persius*, and the Frosted Elfin, *Incisalia irus* (Shapiro 1974, Shuey et al. 1987; Ohio Division of Natural Areas and Preserves 1996). Furthermore, *L. perennis* is also of conservation interest because it is considered an indicator of high quality oak savanna habitat (Grigore and Windus 1996). This imperiled habitat consists of a mosaic of black oak savanna, oak woodland, and wet prairie communities persisting on a series of post glacial beach ridges and swales, and harbors an unusually large number of rare species.

Flowering for *L. perennis* occurs from late April to June (peaking in early May), and reproductive plants may have up to 60 inflorescences, each with 30–50 papilionaceous flowers that mature acropetally. Individual flowers are highly protandrous, with dehiscent pollen present in flowers immediately before opening. Pollen is viable when flowers first open, and remains viable for the next 5 days (Hevner, unpublished data). Stigmas are receptive when flowers are between 2 days and 8 days of age (Shi, Hevner, and Michaels, unpublished data), and ovaries typically have five to six ovules (Shi, Michaels, and Mitchell, unpublished data). Wild Lupine is pollinated primarily by *Bombus* spp. (Bernhardt, Mitchell, and Michaels, in preparation), and the ballistically dehiscent fruits mature from mid-June to early July. Preliminary estimation of the mating system of *L. perennis* indicates that it is highly outcrossing ($t \sim 0.85$; Shi 2004; Shi, Michaels, and Mitchell, unpublished data).

Reproduction

We studied *L. perennis* in a large population (~1,500 individuals) on private land in the Oak Openings of northwest Ohio (Holland Township, Lucas County, OH, USA). In spring 1998, before flowers opened, we chose 64 maternal plants of similar size, phenology, and number of inflorescences. We assigned treatments to whole plants, rather than applying both treatments to each plant. We did this as a means to reduce the potential for resource reallocation from selfed offspring to fruits developing on other inflorescences (Zimmerman and Pyke 1988). Such reallocation would reduce the number of selfed seeds available for vigor trials (see below), and more importantly, would complicate interpretation of the results. By applying treatments to whole

plants, any reduction in seed production for selfed plants cannot be explained by resource shifts, and so most likely indicate an inability to self-pollinate or a lack of vigor for selfed seeds.

For this nested design, on each plant we selected two similar sized inflorescences to study, and removed all others (plants had a mean of seven inflorescences per plant before trimming, based on a survey of over 500 plants across ten populations in 2001). We randomly assigned each maternal plant to one of four treatment combinations [factorial design with two pollination levels (open-pollinated vs hand selfing) by two nutrient levels (control vs fertilizer and water addition)].

For pollination treatments, we either left plants exposed to natural bee visitation (open-pollination treatment), or hand-pollinated them with self-pollen (selfed treatment). For the selfing treatment, we bagged each of the two remaining inflorescences per plant before anthesis, and self-pollinated each flower three times (when flowers were approximately 3, 5 and 7 days old). Bags for pollinator exclusion prior to selfing were constructed of white bridal veil mesh to minimize shading, yet allow for natural ventilation. Each day of pollination, we pollinated each flower by “pumping” it three times, using a fresh 1.5-ml Eppendorf tube for each inflorescence. We simulated bumblebee movement on an inflorescence by pollinating bottom flowers first and then moving to the top. To pump a flower, the mouth of the Eppendorf tube was slipped over the keel and rotated downward about 45°, causing the flower to extrude pollen onto the side of the tube. In this process of pumping, the stigma contacted the side of the tube, which was saturated with self-pollen grains. To determine the effectiveness of this pollination technique, we harvested ten stigmas after Eppendorf tube pollination and stained them with Fuchsin solution (Kearns and Inouye 1993). The number of pollen grains on the stigma was 112 ± 27 (mean ± 1 SD) from Eppendorf tube pollination flowers, whereas, number of pollen grains on the stigma for open-pollinated flowers averaged 46 ± 39 (mean ± 1 SD; $n=10$). Thus, selfed flowers received abundant self-pollen, while open-pollinated flowers received natural pollen delivery by bumble bees (Bernhardt 2000). Other work indicates that open-pollination generates primarily outcrossed offspring (~85% outcrossed; Shi 2004).

For resource addition, 1300 ml of liquid N–P–K fertilizer was added to plants once a week for 3 weeks during fruit maturation, while the control plants received no additional water and nutrients. We bagged inflorescences receiving either selfing or open-pollination treatments after the third pollination day to control for the effect of bagging on fruit development and to allow collection of seeds. Once fruit matured, we scored number of flowers pollinated, fruit, and seed production. Based on the morphological features of seeds and dehiscent fruits, we categorized seeds from each fruit as mature and full or aborted/unfertilized, and weighed mature seeds individually to the nearest milligram.

Fourteen of the 128 experimental inflorescences were lost to deer browsing.

We used analysis of variance (SAS Institute 2000), to test for treatment effects on fruit and seed production, with plant as a random effect nested within the combination of pollination and resource treatments, and inflorescences as the unit of sampling (nested within plants). To account for the effect of number of pollinated flowers on total seed production per inflorescence, we used number of flowers per inflorescence as a covariate. A different approach is required to analyze numbers of mature seeds and aborted/unfertilized seeds in a fruit, because they are not independent of each other. To account for the interdependency of fruit content data, we performed multivariate analysis of variance (MANOVA), to determine the overall effect of pollination, resource addition, and their interaction on seed contents in a fruit, with inflorescence as observation unit (Scheiner 1993). If the MANOVA was significant, we used ANOVA to test the effects of pollination and resource addition on the individual dependent variables (mature seeds per fruit or aborted/unfertilized per fruit).

We used the coefficient of variation as an estimate of the variability of fruit and seed production among maternal plants within a treatment combination. We considered two coefficients of variation to be significantly different if they were separated by more than two standard errors (at level of $P=0.05$) (Simpson et al. 1960; Dudash 1990).

Seedling performance

To determine the effect of self-pollination and resource addition on seed emergence and offspring performance, we scarified and individually planted a total of 1,306 mature seeds from 56 maternal plants in the greenhouse in winter of 1999. We combined all seeds from the two inflorescences on any one maternal plant, and scarified them by lightly grinding in a mortar and pestle with 5 g of sterile silica sand for 1 min. To insure uniform scarification across families, we compared the degree of scarification of a seed from each batch against a standard scarified seed (Kelley 1998). We placed the seeds in moist sand in 96-well microtiter plates covered with plastic wrap, and stored them at 4°C for 48 h. We individually planted all seeds from each maternal family ($n=7-40$ seeds per family) in treeband pots (7×7×14 cm) filled with a bark-based soilless mix, Fafard 52 (Fafard Inc., Anderson, SC, USA). Prior to planting each seed was dipped in *Rhizobium* Type H innoculum (Liphatech Corporation, Wisconsin). We randomly placed pots in flats and randomized the flats on benches. We re-randomized the location of the flats weekly. Note that, in contrast to the maternal plants, all seedlings experienced identical resource environments in the greenhouse.

We scored seedling emergence twice a day for 4 weeks. Five months after planting, we scored survival and number of leaves. We harvested above ground

biomass for all the seedlings and collected root tissue for one-third of them (rootstocks for the other two-thirds of plants were used by local agencies for restorations). We separated above and below ground tissue, which were dried at 75°C for 2 days before weighing to the nearest milligram.

We estimated proportions of seed emergence and seedling survival by combining the seeds from the two inflorescences on each maternal plant. We also estimated emergence speed by calculating the time required for 50% of the seeds to emerge for each maternal plant. We analyzed proportions of seeds emerged, emergence speed, proportion of seedlings surviving to the fifth month, and seedling biomass using ANOVA under PROC GLM (SAS Institute 2000). For proportion emerging, emergence speed, and proportion surviving, we used maternal plant as the unit of replication and therefore, we could not quantify maternal plant effect on these traits. To equalize variances and normalize residuals, proportion of seed emergence was arcsine square root transformed while proportion of survival was arcsine transformed. Leaf number, and above and below ground biomass were square root transformed. As above, we considered maternal plant as a random effect, and used appropriate denominator MS when testing these mixed models (Zar 1996).

Cumulative inbreeding depression

We estimated mean performance for maternal plants receiving each combination of pollination and resource treatments for each life stage: seed production (seeds per inflorescence), seed emergence, seedling survival and seedling size (measured by above ground biomass) at the age of 5 months. Here, cumulative performance is a multiplicative function of number of seeds per inflores-

cence, the proportion that produced seedlings, the proportion of seedlings surviving to the fifth month and biomass at the fifth month. For each maternal plant, we calculated cumulative performance by multiplying performance across four life stages (Dudash 1990; Dudash and Fenster 2001). Then, we averaged cumulative performance of maternal plants in each treatment combination to get mean performance for each pollination and resource combination. The inbreeding depression coefficient was calculated as $\delta = 1 - (w_s/w_o)$ (Husband and Schemske 1995), where w_s is the mean performance of selfed progeny and w_o is the mean performance of open-pollinated progeny.

Results

Reproduction

Seed and fruit production per inflorescence responded significantly to both pollination and maternal resource treatments (Tables 1 and 4, Fig. 1a). In neither case was the interaction significant. Both treatment types had strong and similar effects on reproduction. Although *L. perennis* is clearly at least partially self-compatible, self-pollination significantly reduced fruit and seed number per inflorescence by 40–60% (Tables 1 and 4, Fig. 1a). Likewise, resource addition significantly increased fruit and seed number per inflorescence by 40–50% (Tables 1 and 4, Fig. 1a). Overall, 24% of flowers receiving open-pollination set fruits, whereas, only 11% of flowers set fruits in the selfing treatment ($F_{1, 57} = 28.58, P < 0.0001$). Similarly, resource addition increased proportion of flowers setting fruit by 50% ($F_{1, 57} = 6.76, P = 0.01$). Also, no interaction was significant for proportion of flowers setting fruit ($F_{1, 57} = 0.55, P = 0.46$).

Table 1 Analysis of effects of pollination type and resource addition on seed production in the field

Response	Source	df	MS	F	P value
Fruits per inflorescence ($R^2 = 0.90$)	Pollination	1	250.73	16.71	0.0001
	Maternal resource	1	146.75	9.78	0.01
	Pollination × resource	1	4.36	0.29	0.59
	Plant (pollination × resource)	57	15.01	4.33	< 0.0001
	Flower number	1	36.73	10.58	0.002
	Error	52	3.47		
Seeds per inflorescence ($R^2 = 0.84$)	Pollination	1	4343.5	31.49	< 0.0001
	Maternal resource	1	1000.9	7.32	0.01
	Pollination × resource	1	0.34	0	0.96
	Plant (pollination × resource)	57	137.93	4.33	< 0.0001
	Flower number	1	21.04	0.36	0.55
	Error	52	31.84		
Aborted/unfertilized seeds per fruit ($R^2 = 0.84$)	Pollination	1	21.39	15.83	0.0002
	Maternal resource	1	0.51	0.38	0.54
	Pollination × resource	1	0.01	0.01	0.93
	Plant (pollination × resource)	50	1.35	2.46	0.002
	Error	41	0.55		

Number of flowers per inflorescence was modeled as a covariate. The significance of pollination, resource and their interaction was tested over plant-to-plant variation. *P* values in bold are significant

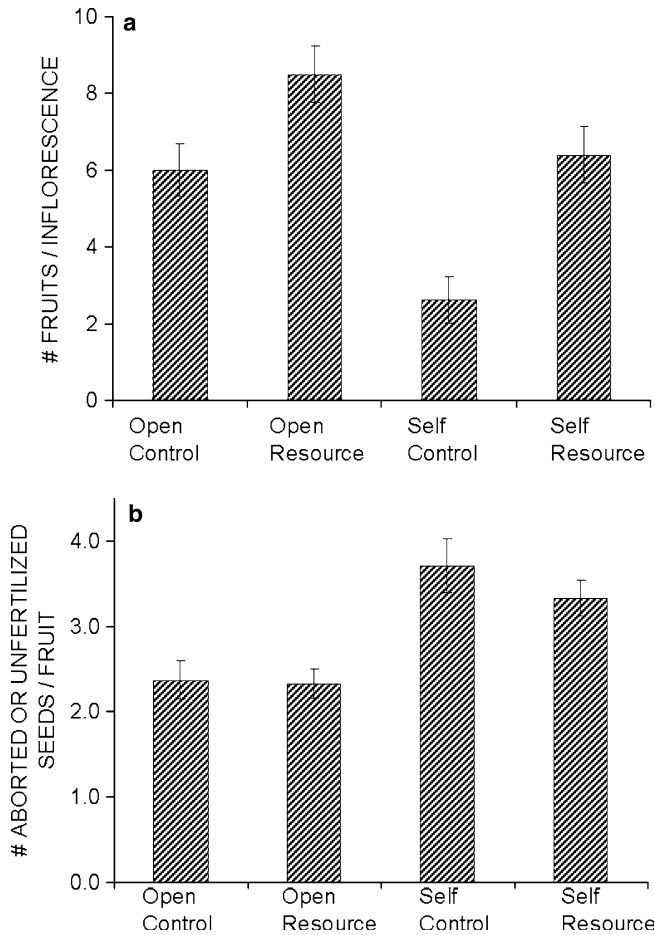


Fig. 1 Effects of pollination type and resource addition on aspects of reproduction: **a** number of fruits/inflorescence. **b** number of aborted/unfertilized seeds per fruit. Both panels show mean \pm 1 SE. *n* observations for open control, open resource, self control and self resource are 14, 15, 16 and 16, respectively. Analyses are in Table 1

Seed contents in a fruit also differed significantly among pollination treatments (MANOVA; Wilks' Lambda = 0.75, df 2, 49, $P = 0.0008$), while resource

addition and pollination–resource interaction had no effects on seed contents (Wilks' Lambda = 0.98, df 2, 49, $P = 0.68$ for resource; Wilks' Lambda = 0.99, df 2, 49, $P = 0.81$ for interaction). Individual ANOVAs indicated that self-pollination reduced the number of mature seeds ($F_{1, 50} = 15.51$, $P = 0.0003$) and significantly increased the number of aborted/unfertilized seeds per fruit by 43% (Fig. 1b; Table 1). However, resource addition had no significant effects on number of mature seeds ($F_{1, 50} = 0.75$, $P = 0.39$) or number of aborted/unfertilized seeds per fruit ($F_{1, 50} = 0.38$, $P = 0.54$). Individual seed mass did not vary significantly with pollination or resource treatment ($F_{1, 52} = 1.11$, $P = 0.29$ for self-pollination; $F_{1, 52} = 1.26$, $P = 0.27$ for resource addition; $F_{1, 52} = 1.51$, $P = 0.22$ for pollination–resource interaction).

Seedling performance

Germination rates did not respond to our experimental treatments, while speed to germinate did. Proportion of seeds emerging in the greenhouse was unaffected by pollination type or resource addition to the maternal plants (Table 2; overall mean = 0.79). However, the time required for 50% of the seeds to emerge was significantly longer for selfed seeds than for open-pollinated seeds (Fig. 2, $F_{1, 52} = 5.26$, $P = 0.03$ for pollination). This difference amounted to about 24 h, or 10% of the mean. However, resource addition and pollination–resource interaction did not affect the time for 50% emergence ($F_{1, 52} = 0.40$, $P = 0.53$ for resource addition; $F_{1, 52} = 0.43$, $P = 0.51$ for pollination–resource interaction).

Resource addition to maternal plants had the surprising and significant consequence of decreasing seedling survival rate by 5–10% (Table 2, Fig. 3). In contrast, the proportion of seedlings surviving to the fifth month did not vary significantly with pollination treatment (Table 2, Fig. 3). Neither pollination type nor resource addition significantly affected number of leaves at 5 months (data not shown). However, self-pollination significantly decreased above ground biomass, by

Table 2 Analysis of effects of pollination type and resource addition on seedling performance in the greenhouse

Response	Source	df	MS	<i>F</i>	<i>P</i> value
Proportion of seeds emerging ($R^2 = 0.03$)	Pollination	1	0.12	1.51	0.22
	Maternal resource	1	$< 10^{-5}$	0	0.99
	Pollination \times resource	1	$< 10^{-4}$	0	0.96
	Error	52	0.08		
Proportion of seedlings surviving ($R^2 = 0.12$)	Pollination	1	0.07	0.64	0.43
	Maternal resource	1	0.63	5.75	0.02
	Pollination \times resource	1	0.03	0.23	0.63
	Error	51	0.11		
Above ground biomass ($R^2 = 0.26$)	Pollination	1	3.82	6.28	0.02
	Maternal resource	1	0.46	0.76	0.39
	Pollination \times resource	1	0.86	1.41	0.24
	Plant (pollination \times resource)	50	0.61	3.58	< 0.0001
	Error	801	0.17		

The significance of pollination, resource and their interaction for above ground biomass was tested over plant-to-plant (maternal family) variation. *P* values in bold are significant

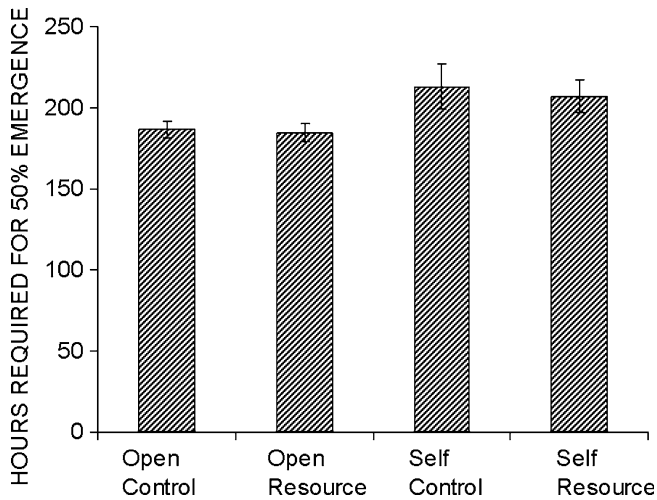


Fig. 2 Effects of pollination type and resource addition to the maternal plants on time required for 50% of the seeds to emerge. Mean \pm 1 SE. Analysis in Table 2

25–34%, whereas, resource addition to maternal plants had no effect on above ground biomass of their greenhouse grown offspring (Table 2, Fig. 4). Below ground biomass responses show the same patterns as do above ground (results not shown).

Variability in seed production

Under field conditions (no resource addition), variability in fruit and seed production was greater among selfed plants than among open-pollinated plants (Table 3). However, in the presence of resource addition, we did not detect a significant difference in CV between selfed and open-pollinated plants (Table 3). Moreover, seed and fruit production of selfed plants was more variable

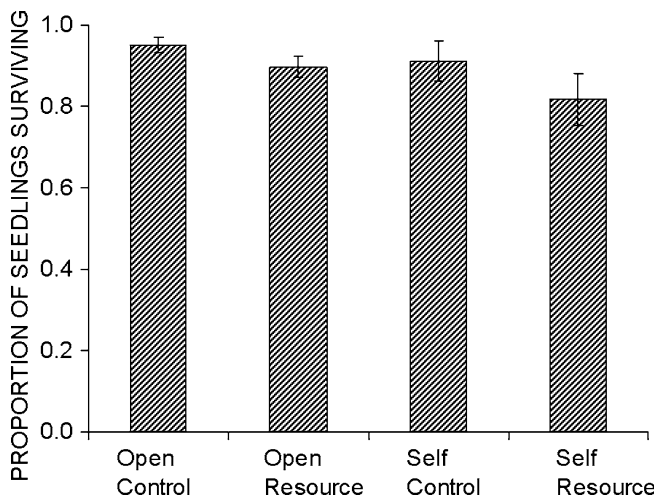


Fig. 3 Effects of pollination type and resource addition to maternal plants on proportion of seedlings surviving to the fifth month. Mean \pm 1 SE. Analysis in Table 2

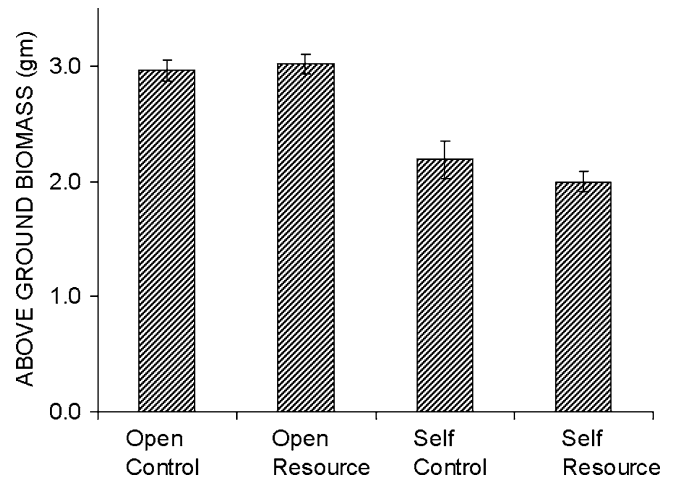


Fig. 4 The effects of pollination type and resource addition on aboveground biomass of greenhouse grown offspring. Mean \pm 1 SE. Analysis in Table 2

under field conditions than under resource addition (Table 3).

Cumulative inbreeding depression

Across all life stages (seed production per inflorescence, seed emergence, seedling survival to the fifth month and biomass at 5 months), cumulative inbreeding depression (δ) was 0.88 and 0.72 for maternal plants without and with resource addition, respectively (Table 4). Ignoring seed production in the field, cumulative inbreeding depression (δ) was 0.32 and 0.41 for maternal plants without and with resource addition, respectively. The magnitude and timing of inbreeding depression appeared to shift with resource addition (Table 4, Fig. 5). Adding resource to the maternal

Table 3 Coefficient of variation ($CV = 100 \times SD / \text{mean}$) of fruit (a) and seed production (b) of selfed and open-pollinated plants at different resource levels

Environment	Selfing	Open-pollinated
(a) Fruit production		
Control	117.16 ^{a, c} \pm 22.14 (16)	51.37 ^{b, c} \pm 10.07 (14)
With resource	51.28 ^{a, d} \pm 10.05 (16)	43.54 ^{a, d} \pm 8.23 (15)
(b) Seed production		
Control	119.71 ^{a, c} \pm 22.62 (16)	75.11 ^{b, c} \pm 14.73 (14)
With resource	62.08 ^{a, d} \pm 12.17 (16)	52.91 ^{a, c} \pm 10.00 (15)

CV \pm 1 SE was calculated (Simpson et al. 1960) to compare the variability of untransformed fruit and seed production for selfed and open-pollinated plants at different resource levels. We considered two coefficients of variation to be significantly different ($P \leq 0.05$) if they were separated by more than two standard errors (Simpson et al. 1960; Dudash 1990). Number of observations for each treatment combination is in parentheses. Entries with different letters ^a or ^b indicate a significant ($P \leq 0.05$) difference in CV between selfed and open-pollinated plants within a resource level. ^c or ^d indicate a significant ($P \leq 0.05$) difference in CV between control and resource addition within a pollination level

Table 4 Cumulative inbreeding depression and its components for *L. perennis*

Life stage	Selfed	Open-pollinated	δ
(a) Without resource addition to the maternal plants			
Seeds per inflorescence	3.53 ± 1.02 (16)	15.83 ± 3.05 (14)	0.78
Seedling emergence (proportion)	0.74 ± 0.08 (10)	0.84 ± 0.04 (14)	0.11
Survival to 5 months (proportion)	0.92 ± 0.06 (9)	0.95 ± 0.02 (14)	0.04
Biomass at 5 months (gm)	2.63 ± 0.44 (9)	2.96 ± 0.12 (14)	0.11
Cumulative means	4.83 ± 1.7 (16)	39.32 ± 9.98 (14)	0.88
(b) With resource addition to the maternal plants			
Seeds per inflorescence	12.13 ± 2.06 (16)	23.53 ± 3.08 (15)	0.48
Seedling emergence (proportion)	0.74 ± 0.06 (16)	0.83 ± 0.04 (16)	0.11
Survival to 5 months (proportion)	0.82 ± 0.06 (16)	0.90 ± 0.03 (16)	0.08
Biomass at 5 months (gm)	2.05 ± 0.17 (15)	3.07 ± 0.14 (16)	0.33
Cumulative means	15.04 ± 3.32 (16)	54.2 ± 7.71 (15)	0.72

Mean performance ± 1 SE at each stage was calculated by averaging the performance of the maternal plants in selfed or open-pollinated treatments. We calculated cumulative performance for each maternal plant by multiplying performance across its four life stages. Cumulative means are the average cumulative performance of maternal plants in each treatment combination. Number of maternal plants is given in parentheses. The inbreeding depression coefficient δ was calculated by subtracting the ratio of selfed mean performance over open-pollinated mean performance from one

plants was associated with a delay in the expression of inbreeding depression from early (seed maturation) to later life stages.

Discussion

Self-pollination of *L. perennis* significantly decreased performance for most traits we studied, from seed production to offspring performance. In contrast, addition of resources to maternal plants had strong effects on seed and fruit production, but had little effect on seedling growth. Together, these results indicate that the magnitude of inbreeding depression not only varied across the life cycle, but also suggest that it varied with resource treatment. We consider each of these topics in turn.

Effects of selfing on seed production and offspring performance

Reduction in *L. perennis* seed production after self-pollination could in principle be due to either partial self-incompatibility or early acting inbreeding depression (Seavey and Bawa 1986; Krebs and Hancock 1990; Levri and Real 1998; Kittelson and Maron 2000). Self-incompatibility is a result of maternal tissue/pollen interactions and typically occurs at the surface of the stigma or in the style (Richards 1986). Since self-pollinations of *L. perennis* result in normal pollen tube growth (Bernhardt, Mitchell, and Michaels, unpublished data), self-incompatibility seems unlikely. Furthermore, the higher percentage of aborted ovules after self-pollination than that for open-pollination suggests that early-acting inbreeding depression causes the reduced seed production after self-pollination. Because we confirmed that our hand pollinations deposited abundant pollen,

we can eliminate the possibility that reduced reproductive success is a result of insufficient pollination

Beyond effects on seed production, self-pollination significantly decreased offspring performance: seeds from self-pollination took longer to emerge and offspring from self-pollinated plants were smaller than seeds from open-pollinated plants (Table 2, Figs. 2 and 3). Although bees visiting open-pollinated *L. perennis* make many intraplant moves that could cause geitonogamous selfing (Bernhardt 2000), the mating system is nonetheless $\sim 85\%$ outcrossing (Shi, Michaels and Mitchell unpublished results). Thus, our comparison of responses to self- and open-pollination is primarily a comparison between selfed and outcrossed offspring. Although other factors may contribute to differences in performance between these two groups, such effects of pollination treatment are most likely indications of inbreeding depression. However, apparent inbreeding depression at these later life stages (e.g., aboveground mass) might conceivably be the consequence of inbreeding depression that occurs only at early stages (such as seed provisioning or emergence speed). In this scenario, the differences in performance at later stages would be simple mechanical consequences of inbreeding depression in early traits. For seed mass, in other systems such indirect effects on later performance are common (e.g., Dolan 1994; Roach and Wulff 1986; Montalvo 1994), but are unlikely in our study because seed mass did not vary with pollination (or resource) treatment (see also Schaal 1980). Likewise, we found fairly subtle effects of pollination treatment on emergence speed. Although such variation can be an important determinant of offspring performance in the field because seedlings that emerge earlier may be at a competitive advantage over later emerging seedlings (Harper 1977), timing of emergence probably did not affect final seedling performance for our greenhouse-grown plants.

Effects of resource addition to maternal plants on offspring performance

Resource addition to maternal plants primarily affected seed and fruit production, not offspring growth of *L. perennis*. This result is most likely a logical consequence of the fact that seed and fruit traits are more directly under the control of maternal plant status than is offspring growth. However, a more surprising result is that resource addition to maternal plants significantly decreased the proportion of seedlings surviving to the fifth month (Table 2, Fig. 3). Moreover, resource addition to maternal plants seemed to delay expression of inbreeding depression (Fig. 5). Our results are consistent with Montalvo's (1994) finding that effects of selfing for *Aquilegia caerulea* increased each year for greenhouse-sown transplants, but not for field-sown plants. These patterns suggest that inbreeding depression is expressed later when conditions allow provisioning and survival of low quality offspring (see also Melser and Klinkhamer 2001). In our study, we observed that 14% of flowers set fruit under field conditions, while 21% of flowers set fruit when resources were added to the maternal plants. It is likely that abundant resources for maternal plants decreased the process of selective fruit and seed abortion (Martin and Lee 1993). Other studies have shown that with a decrease in abortion level, offspring quality decreased

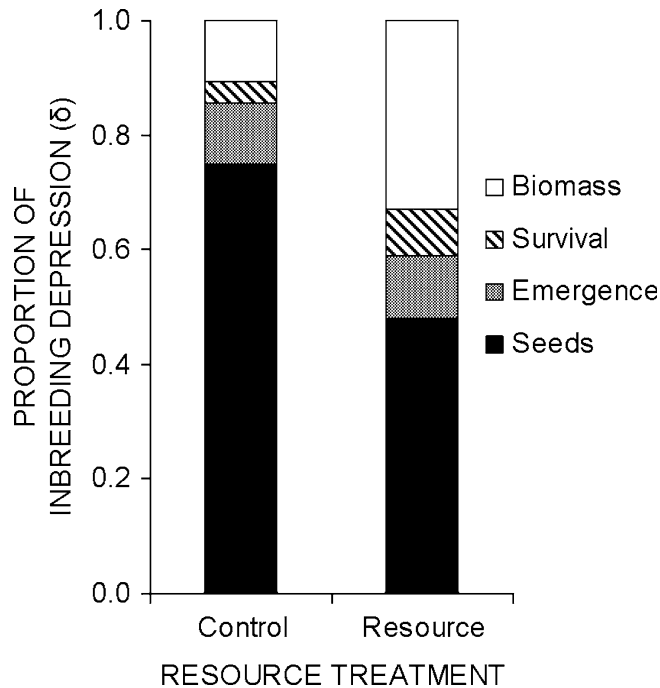


Fig. 5 Effects of resource addition to maternal plants on the expression of inbreeding depression across four life stages (seed production, seedling emergence, seedling survival to 5 months and seedling biomass at 5 months). Proportions are calculated based on the sum of the individual inbreeding depression estimates for these stages

significantly for *Lotus corniculatus* (Stephenson and Winsor 1986), *Crypthanta flava* (Casper 1984, 1988) and *Phaseolus coccineus* (Rocha and Stephenson 1991). Our work and the studies described above suggest that benign environments (e.g., ex situ propagation in the greenhouse, supplemental nutrients) might slow down the process of weeding out inferior offspring, and thus reduce inbreeding depression at early stages. However, the inferior offspring might then be exposed to harsh natural conditions after transplantation or reintroduction to their natural habitat in the field, leading to unexpectedly higher mortality.

Our experimental design is well suited to detect interactive effects of pollination treatment and resource addition on offspring performance, yet we found no significant interactions in any of our analyses (Tables 2 and 3, see text). The significant main effects in those analyses indicate that our manipulations of both pollination and resources were large enough to affect plant response. Likewise, Wild Lupine plants in our field population regularly showed evidence of water limitation (cupping of leaves) during fruit maturation in the field. Yet despite these stressful conditions, resource addition (including water) to maternal plants did not alter the effects of selfing on performance at the seed production or seedling growth stages (no significant pollination–resource interactions). Our results are consistent with previous studies, which found no interactions between inbreeding and stress (Waller 1984; Mitchell-Olds and Waller 1985; Heywood 1993; Martin and Lee 1993; Dahlgaard et al. 1995; Norman et al. 1995; Hauser and Loeschke 1994; Cheptou et al. 2000; but see Helenurm and Schaal 1996). The lack of inbreeding–stress interactions may be in part a result of the high variation in seed production after selfing that we observed in the absence of resource addition (Table 3; see Dahlgaard et al. 1995). Because most other studies of inbreeding–stress interaction applied stressful conditions to offspring in the greenhouse, while our study applied resource treatments to maternal plants in the field during the seed maturation stage, it is hard to make meaningful comparisons. However, studies of the effects of adding resources to offspring generally report more inbreeding depression under stressful offspring environments (e.g. Schemske 1983; Dudash 1990; Schmitt and Ehrhardt 1990; Hoffman and Parsons 1991; Byers and Waller 1999).

There was considerable inbreeding depression in *L. perennis* across the four life stages (seed production, seed emergence speed, seedling survival, and seedling growth; $\delta = 0.88$ without resource addition and $\delta = 0.72$ with resource addition). Overall inbreeding depression was lessened when maternal plants received supplemental resources. The magnitude and timing of stage-specific inbreeding depression in our study of *L. perennis* shows a bimodal distribution across life stages with strongest inbreeding depression in very early (seed set, δ from 0.48 to 0.78) and late life stages (seedling biomass, δ from

0.11 to 0.33) (Fig. 5). Our observations are consistent with other studies, which generally find that outcrossing species express the majority of inbreeding depression either early (during seed production) or late (during growth/reproduction), whereas, selfers express much of the inbreeding depression late (Husband and Schemske 1996).

Implications for conservation

Our results provide two important insights of relevance to conservation. First, understanding and evaluating inbreeding depression in endangered species requires consideration of the environmental variation experienced by both parents and offspring. The effects of inbreeding on endangered species have generally been studied in the greenhouse or other fairly benign conditions (Schemske et al. 1994; Olfelt et al. 1998). Estimates of inbreeding depression in such generous conditions may underestimate (or at least differ from) the effects of inbreeding in natural environments. For example, there may be no detectable inbreeding depression in the greenhouse, but a significant effect under field conditions (Hedrick and Kalinowski 2000). Our results demonstrate that this caution applies not only to postdispersal offspring environments but also to maternal environments during early offspring development. Second, the environmental dependency of the expression of inbreeding depression has important practical implications for the conservation of endangered species. In traditional restoration programs, substantial efforts are often expended on ex situ propagation programs aimed at increasing population size by release of captive-bred offspring (Hedrick and Kalinowski 2000). Because a population may suffer from inbreeding depression in one environment but not another (e.g., Montalvo 1994; Hayes et al. 2004), inbred offspring from parents maintained in the forgiving conditions of captivity or greenhouse environments may perform poorly when transferred to the harsher environment of the field. Hence, an initial demographic increase for endangered species in restoration programs raising offspring in resource-rich conditions might be canceled out when the organisms are reintroduced to their natural habitats. Therefore, propagation programs for restoration of endangered species that utilize benign growth conditions should be pursued with caution, balancing the potential early-life demographic gains of this approach with the possible increased loss of those offsprings in a more stressful field environment.

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