

# Effects of population size on performance and inbreeding depression in *Lupinus perennis*

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**Abstract** We investigated the relationships among population size, offspring performance, and inbreeding depression ( $\delta$ ) in *Lupinus perennis* by examining the effect of population size category (large vs. small) on seed production and offspring performance for three pollination treatments (open pollination, hand crossing and hand selfing). In each of our four pairs of populations, one member of the pair was substantially larger than the other. We then grew seeds from this factorial design (2 sizes  $\times$  4 pairs  $\times$  3 pollination treatments) in the greenhouse to investigate whether population size affects offspring performance in a common environment, and how small size affects purging of the inbreeding load. Multiplicative performance across four early life-stage components (seed production, seedling emergence, seedling survival and seedling growth) of smaller populations was not significantly lower, although biomass of seedlings declined in smaller populations. Self-pollination reduced seed production, seedling emergence and seedling growth, reflecting substantial inbreeding depression ( $\delta = 0.404 \pm 0.043$ ). Population size categories did not consistently differ in levels of inbreeding depression, suggesting that purging of genetic load in smaller populations has been limited, and that all populations still

harbor inbreeding load. We also found a significant decrease in log performance with increases in the population inbreeding coefficient. These results indicate that even in seemingly large populations, lupines are susceptible to considerable fitness declines through both inbreeding load within populations, and drift load via genetic erosion and fixation of deleterious alleles between populations.

**Keywords** *Lupinus perennis* · Plant population size · Inbreeding depression · Multiplicative performance · Purging

## Introduction

Anthropogenic destruction and fragmentation of natural populations often lowers population mean fitness (e.g., Young et al. 1996; Ellstrand and Elam 1993; Menges 1991; Heschel and Paige 1995; Leimu et al. 2006). Although ecological factors such as habitat degradation and isolation contribute to this decline, two genetic factors may also play a role (Keller and Waller 2002). First, small populations experience more genetic drift (Wright 1943; Eckert and Barrett 1992), which increases the frequency and likelihood of fixation of recessive deleterious alleles (Ellstrand and Elam 1993; Paland and Schmid 2003). Over time, this genetic erosion should lead to the divergence of populations, and the accumulation of “drift load” (Bataillon and Kirkpatrick 2000; Hedrick 2001). Second, plants in small populations may more often mate with close relatives (e.g., Jennersten 1988), increasing the frequency of inbred matings, and allowing expression of “inbreeding load” (e.g., Charlesworth and Charlesworth 1987; Carr and Dudash 1996; Whitlock 2000; Keller and Waller 2002). The inbreeding and drift components of genetic load contribute

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to inbreeding depression ( $\delta$ ), the decrease in fitness due to mating between close relatives (Byers and Waller 1999; Charlesworth and Charlesworth 1987).

Inbreeding depression is an important selective agent in many populations, and a major concern for the conservation of endangered species (Byers and Waller 1999; Frankham et al. 2002). The magnitude of inbreeding depression varies among populations and species (Byers and Waller 1999; Hedrick and Kalinowski 2000; Keller and Waller 2002; Reed and Frankham 2003). In particular, the relationship between population size and level of inbreeding depression should depend on population history, demographic structure, mating system, inbreeding level and habitat. Theoretical studies indicate that one reason for these dependencies may be that shrinking populations can purge recessive deleterious alleles, reducing the genetic load (Wang et al. 1999; Bataillon and Kirkpatrick 2000; Kirkpatrick and Jarne 2000). However, Byers and Waller (1999) found that less than half of published studies provide evidence for purging in plants, suggesting that the occurrence and extent of purging may vary among species and populations and that purging is likely to be effective only under specific genetic and demographic conditions (Keller and Waller 2002).

Even severe inbreeding depression will not affect realized population fitness if plants always outcross (Keller and Waller 2002). Thus the traditional measure of  $\delta$ ,  $[(W_{\text{cross}} - W_{\text{self}})/W_{\text{cross}}]$ , where  $W$  is the estimate of the fitness trait, reflects the potential, not realized cost of inbreeding in a particular population (Hedrick et al. 1999). The potential cost is typically measured by comparing progeny fitness from hand crossing and hand selfing (e.g., Dudash 1990; Husband and Schemske 1995; Kittelson and Maron 2000), which are two extreme alternatives along the mating system spectrum. Because many plants have a mixed mating system (Goodwillie et al. 2005), this comparison will generally overestimate the amount of inbreeding depression actually expressed in the field.

The purpose of this study is to investigate the relationships among population size, fitness, and inbreeding depression in large and small populations of *Lupinus perennis*. We first compared seed production in response to open pollination, hand crossing and hand selfing treatments in eight paired populations (four large, and four small). We then grew the resulting seeds in the greenhouse to investigate whether population size affects offspring performance and inbreeding depression in *L. perennis*. We ask the following questions:

1. Does population size affect seed production and offspring performance?
2. Does selfing affect seed production and offspring performance?
3. Does  $\delta$  vary between small and large populations?

## Materials and methods

### The study organism

The wild lupine, *L. perennis* (Fabaceae), is a long-lived perennial growing on nutrient-poor, sandy soils throughout eastern North America (Gleason and Cronquist 1991). Reproductive plants, which flower from early May to mid June, may have up to 60 inflorescences (mean = 6.7) with 30–50 flowers on each inflorescence, and each flower typically has from five to six ovules (X. J. Shi, H. J. Michaels and R. J. Mitchell, unpublished data). *L. perennis* flowers are highly protandrous, and pollen is viable when flowers first open, remaining viable for the next 5 days (Hevner 2001). Stigmas are receptive when flowers are between 2 and 8 days of age (X. J. Shi, S. Hevner and H. J. Michaels, unpublished data), and the outcrossing rate is high ( $t \sim 0.85$ ; Shi 2004). Wild Lupine is pollinated primarily by *Bombus* spp. (Bernhardt 2000). The ballistically dehiscent fruits mature from mid June to early July.

*L. perennis* is a species of conservation interest. This oak savanna herb is the main larval food for the federally endangered Karner blue butterfly, *Lycaeides melissa samuelis* (Haack 1993). This butterfly is believed to have decreased in numbers because of a decline in lupine populations due to fire suppression and the resulting degradation and fragmentation of lupine habitat (Grigore and Windus 1994; Grigore and Tramer 1996). High-quality midwestern oak savannas currently occupy 0.02% of their formerly widespread pre-settlement distribution, in some areas disappearing within the past 100 years (Nuzzo 1985). In northwest Ohio's Oak Openings, *L. perennis* is described in Moseley's 1928 *Flora of the Oak Openings* as "abundant," indicating that its designation under state "potentially threatened" status by 1984 (Ohio Division of Natural Areas and Preserves 1984) was achieved following a relatively recent decline.

### Experimental design

We identified four pairs of relatively large and small populations in the Oak Openings region of northwest Ohio and southern Michigan (Table 1). Two requirements of our design constrained our choice of populations for this threatened species. First, populations had to be large enough to allow a statistically valid sample of the plants in our experiment, but leave a viable population of unused plants and avoid already compromised, much smaller populations. Thus we chose populations with at least 120 individuals. Although still relatively small (Cartwright 1997), these moderate-size populations may be less subject to drift and

**Table 1** The four pairs of populations of *Lupinus perennis* sampled in this study

Pair location (code)	Population name (code)	Population size <sup>a</sup>
Meilke Road Savanna (ME)	Blowout (BLO)	2,554
	Gap (GAP)	550
Lou Campbell Preserve (LC)	Central (CEN)	1,298
	Turnpike (TUR)	149
The Nature Conservancy (TNC)	South Piel (PIE)	1,346
	Sweet Fern (SWE)	713
Michigan (MI)	Petersburg (PET)	3,264
	Quarry (QUA)	1,262

<sup>a</sup> Population size was estimated as the number of reproductive individuals. Note there were two populations (large vs. small) within a pair

pollinator limitation than smaller populations would be, so that finding significant effects of population size will be more difficult. Second, our study populations had to have a nearby, paired population of a clearly differing population size within 5 km, to minimize the potential effects of geographic variation in environmental factors. In our Michigan pairing, this forced us to use a small population somewhat larger than our planned limit of 800 plants (based on data in Cartwright 1997). In spring 1999, we estimated the size of each population based on 1 × 1-m quadrats (H. J. Michaels and R. J. Mitchell, unpublished data;  $n = 26\text{--}355$  regularly spaced quadrats/population; quadrats were on 5-m spacing). We estimated the number of plants in each 1 × 1-m quadrat because individuals can be difficult to distinguish. For each population we counted inflorescence number per quadrat, and separately counted inflorescence number per plant in a different sample of plants that were clearly distinct individuals, then divided the quadrat inflorescence count by mean inflorescences per plant. We also counted total number of quadrats containing flowering lupine plants in each population, and derived the population size from those values (total number of plants = number of quadrats × number of plants/quadrat × area of the population).

In spring 2000, before flowers opened, we randomly chose 25 maternal plants of similar size, phenology, and number of inflorescences in each population. On each plant we selected four matched inflorescences and randomly assigned each to a treatment, either open pollination, hand crossing or hand selfing. Based on prior work suggesting higher levels of seed abortion following selfing (Shi et al. 2005), we used two inflorescences for the hand-selfing treatment to ensure production of enough seeds for later study. Having all treatments represented on the same plant might, in principle, confound these results if resources are reallocated among different inflorescences receiving different treatments (Zimmerman and Pyke 1988). However, a

1999 pilot study revealed no resource reallocation between inflorescences experiencing selfing versus open pollination (H. J. Michaels, R. J. Mitchell and X. J. Shi., unpublished data).

For the selfing treatment, we bagged the two inflorescences per plant before anthesis and self-pollinated the flowers (approximately 30 flowers per inflorescence). Bags for pollinator exclusion prior to selfing were constructed of white bridal veil mesh. Each day of pollination, we selfed each flower by “pumping” it 3 times, using a fresh 1.5-ml Eppendorf tube for each inflorescence. To pump flowers, the mouth of the Eppendorf tube was slipped over the keel and rotated downward about 45°, causing flowers to extrude pollen onto the side of the tube. During this pumping, the stigma contacted the side of the tube, which was saturated with self pollen grains. For hand outcrossing, we bagged one inflorescence before anthesis and pollinated ten to 15 flowers 3 times with outcross pollen on the same days selfing was performed. Each flower was pollinated 3 times with the appropriate pollen type (approximately when flowers were 3, 5 and 7 days old). Each day of pollination, we first removed self-pollen by pumping the flower once using a fresh 1.5-ml Eppendorf tube (emasculation is not effective in reducing self pollen deposition for *L. perennis*). Then outcross pollen from three to five plants (at least 4–5 m from the maternal plant) was collected in clean tubes. We applied the collected pollen to each outcross inflorescence by pumping the flowers so that the stigma contacted the outcross pollen on the tube wall. We also rotated the tube after pollinating a flower to ensure that the stigma from the next flower contacted outcross pollen. For open pollination, we did not manipulate the inflorescence and flowers received natural pollination by bees.

We bagged all inflorescences after the third pollination (or after open-pollinated flowers finished exposure and had senesced) to control for any effect of bagging on fruit development, and to collect seeds. Once fruit matured, we scored flower, fruit, and seed production. To better understand seed and ovule abortion, we scored each ovule in each pod as either mature or aborted/unfertilized. Because this was very labor-intensive, we were only able to score five of the populations in this manner.

To determine the effect of pollination treatment and population size on seedling emergence and offspring performance, in April 2001 we scarified and planted a total of 3,713 seeds from the eight populations in a greenhouse (Hirzel Farms, Perrysburg, Ohio). We combined all seeds from the two hand-selfed inflorescences on each maternal plant. All seeds were scarified by nicking the seed coat with a razor blade and then stored in moist sand in petri dishes at 4°C for 48 h. Seeds were individually planted in 4-inch pots filled with Farfard 52 bark mix (Farfard, Agawam, Mass.). Pots were fully randomized and placed in flats, and

rearranged every 2 weeks to minimize positional differences in levels of light, humidity and temperature.

Four weeks after planting we scored seedling emergence. At 15 weeks we scored seedling survival and harvested aboveground biomass, so the experiment approximately spans a typical lupine growing season from seed germination in May up to and including senescence after 2 months of growth. Belowground biomass was not harvested in order to return the resprouted plants to local management agencies for use in restoration. Tissues were dried at 75°C for 48 h before weighing.

#### Data analysis

We used ANOVA (SAS procedure GLM; SAS Institute 2000) to analyze the effects of population size category and pollination type on seed production, seedling emergence, seedling survival and seedling biomass. Plants with zero seed production were excluded in the seedling emergence analysis. Similarly, seeds that did not germinate or survive to 15 weeks were excluded from seedling survival or seedling biomass analyses. Seedling biomass was averaged for all seedlings within a maternal plant and treatment combination. To meet assumptions of ANOVA, percent seedling emergence was arcsine-square-root transformed and percent seedling survival was arcsine transformed. We used residual plots to confirm that the data were normally distributed. All models include main effects of population pair, size and pollination, with maternal plant as a random factor nested within the combination of pair and population size. We treated population pair as a random effect, and adjusted denominator MS accordingly (Steele and Torrie 1980). We therefore tested for effects of population size grouping over variation among populations (size  $\times$  pair). To account for the effect of flower number on total seed production per inflorescence, we used number of flowers per inflorescence as a covariate. We compared the effects of three pollination treatments on seed production and offspring performance using a Tukey test.

#### Multiplicative performance across four life stages

For each maternal plant and pollination treatment combination we estimated performance for each life stage: seed production, seedling emergence, seedling survival and seedling size in the first year, calculated as number of seeds per inflorescence, the proportion that produced seedlings, the proportion of seedlings surviving to the 15th week and biomass at the 15th week. For each maternal plant, we calculated cumulative performance by multiplying performance across the four life stages (Dudash 1990). Hence, there are three estimates of multiplicative performance for each maternal plant, corresponding to the three pollination

treatments. All maternal plant and pollination treatment combinations with zero seed production, zero seedling emergence, zero seedling survival or zero biomass, ultimately had a zero multiplicative performance across the four life stages. We applied ANOVA (procedure GLM; SAS Institute 2000) to test the effect of population size and pollination type on multiplicative performance. The models include main effects of population pair (a random factor), size category, and pollination, with maternal plant as a random factor nested within the combination of pair and population size.

#### Inbreeding depression

We estimated the magnitude of inbreeding depression based on multiplicative performance across the growing season, and for the component life stages. We estimated inbreeding depression for each family as  $\delta = 1 - (w_s/w_o)$ , where  $w_s$  is the mean performance of selfed progeny and  $w_o$  is the mean performance of outcrossed progeny (Charlesworth and Charlesworth 1987). This measure is biased downward when  $w_o$  is very small for a particular family (Ågren and Schemske 1993), as occasionally happened in our study. To avoid this bias we excluded as outliers values from two families that were more than 3 SD below the overall mean. This did not affect patterns of significance. To evaluate the effects of population size category on magnitude of stage-specific and multiplicative  $\delta$ , we performed ANOVA (procedure GLM; SAS Institute 2000) on  $\delta$ , with population size and population location as main factors, and maternal plant as the replication unit.

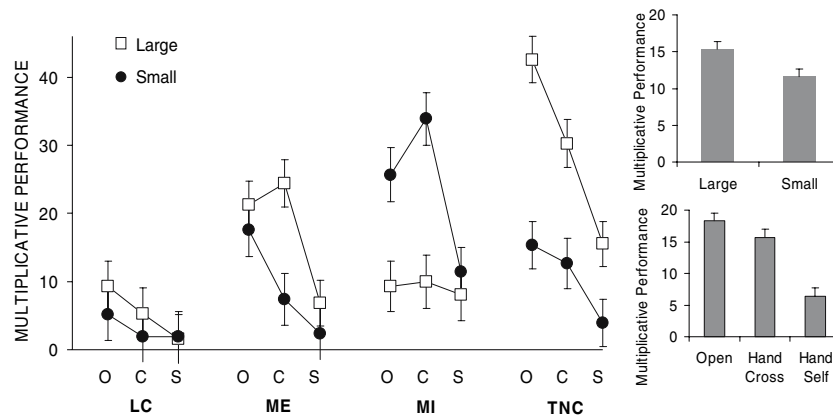
## Results

#### Multiplicative performance

ANOVA on overall performance of *L. perennis* across four life stages revealed strong effects of pollination type (open, hand crossing, and hand selfing), but no significant differences among population pairs or population size groups (Table 2; Fig. 1). The significant size  $\times$  pair interaction indicates that populations differed in overall performance, while the significant three-way interaction indicates that the effect of pollination treatment varied significantly among the individual populations. Analysis of covariance (ANCOVA) treating population size as continuous, rather than categorical, also revealed no significant effect of population size on performance (results not shown). Patterns of significance are identical for multivariate ANOVA (analysis not shown) on the vector of component response variables (seed production, seedling emergence, seedling survival, and aboveground biomass).

**Table 2** ANOVA effects of pair, population size and pollination treatments on multiplicative performance for seed production through seedling biomass. *P*-values in bold are significant. Model  $R^2 = 0.736$

Source	Denominator MS	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Pair	Pair × Size	3	140.95	1.62	0.35
Size group	Pair × Size	1	56.47	0.65	0.48
Pollination	Pair × Pollination	2	80.61	12.4	<b>0.0068</b>
Pair × Size	Pair × Size × Pollination	3	87.04	8.48	<b>0.014</b>
Pair × Pollination	Pair × Size × Pollination	6	6.28	0.61	0.72
Size × Pollination	Pair × Size × Pollination	2	0.18	0.02	0.98
Pair × Size × Pollination	Error	6	10.27	3.18	<b>0.005</b>
Plant (Pair × Size)	Error	175	10.66	3.30	<b>0.0001</b>
Error		326	3.23		



**Fig. 1** Multiplicative performance of *Lupinus perennis* as a function of pair, population size and pollination treatment. Values shown are least squares (LS) means  $\pm$  1 SE. Performance is calculated from four life stages (seed production, seedling emergence, seedling survival, aboveground biomass). Analysis in Table 2. The two panels to the

right present LS means  $\pm$  1 SE for main effects of population size (top), and pollination treatment (bottom). *O* Open pollination, *C* hand crossing, *S* selfing, *ME* Meilke Road Savanna, *LC* Lou Campbell Preserve, *TNC* The Nature Conservancy, *MI* Michigan

Inspection of the pattern of response for multiplicative performance helps interpret the patterns of significance (Fig. 1). In all cases selfing generated the poorest performance (Tukey test  $P < 0.0001$ ), while there was no overall difference between open pollination and hand crossing (Tukey test  $P = 0.39$ ). Although the significant three-way interaction indicates that the response to pollination treatment varied among populations and pairs, selfs always performed worst, and in five of the eight populations, open-pollination treatments performed better than hand cross-pollination treatments. In three of four pairs the large population performed better than the small population, while the reverse was true in the Michigan pairing (significant size  $\times$  pair interaction).

Analyses of the individual components of multiplicative performance also help to interpret these results. We discuss each component in turn. In all cases a regression analysis (treating population size estimates as continuous predictors) indicates no significant effect of population size (results not shown).

### Seed production

Seeds per inflorescence differed significantly among pollination treatments, but not among pairs or population size groups (Table 3; Fig. 2a). Interactions with pollination treatment were not significant, reflecting a very consistent ranking of the pollination treatments (open pollination > hand crossing > selfing, Tukey test  $P < 0.0001$  for all comparisons). Overall, 18% of flowers receiving open pollination and 11% of hand-outcrossed flowers set fruit, whereas only 8% of flowers set fruit in the selfed treatment. The reduction in seed production following selfing is most likely caused by early acting  $\delta$ , since self-pollination in *L. perennis* results in normal pollen tube growth (C. E. Bernhardt, R. J. Mitchell and H. J. Michaels, unpublished data). There was a non-significant tendency for greater seed production in large populations for all but the Michigan pairing. Furthermore, seed abortion per fruit was significantly greater for hand-selfed than for hand cross-pollination in two of five populations, and tended

**Table 3** Single-factor ANOVAs for effects of pair, population size and pollination treatments on several life stages<sup>a</sup>. *P*-values in **bold** are significant. *Poll.* Pollination treatment

Response	Source	Denominator <i>MS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Seeds per inflorescence, $R^2 = 0.73$	Pair	Pair × Size	3	1,157.69	0.944	0.52
	Size group	Pair × Size	1	0.01	0.00	0.99
	Pollination treatment	Pair × Poll.	2	2,770.58	18.84	<b>0.0026</b>
	Pair × Size	Pair × Size × Poll.	3	1,233.08	6.38	<b>0.027</b>
	Pair × Poll.	Pair × Size × Poll.	6	147.06	0.6	0.62
	Size × Poll.	Pair × Size × Poll.	2	10.53	0.05	0.97
	Pair × Size × Poll.	Error	6	193.18	2.06	0.06
	Plant (pair × Size)	Error	175	93.84	2.02	<b>0.0001</b>
	Number of flowers	Error	1	1,832.95	39.37	<b>0.0001</b>
	Error		325	46.56		
Proportion of seedling emergence, $R^2 = 0.60$	Pair	Pair × Size	3	0.40	2.22	0.26
	Size group	Pair × Size	1	0.83	4.66	0.12
	Pollination treatment	Pair × Poll.	2	0.38	8.34	<b>0.018</b>
	Pair × Size	Pair × Size × Poll.	3	0.18	1.87	0.24
	Pair × Poll.	Pair × Size × Poll.	6	0.05	0.48	0.81
	Size × Poll.	Pair × Size × Poll.	2	0.02	0.25	0.79
	Pair × Size × Poll.	Error	6	0.10	0.34	0.92
	Plant (pair × Size)	Error	116	0.28	2.28	<b>0.0001</b>
	Error		204	0.12		
	Proportion of seedlings surviving to the 15th week, $R^2 = 0.41$	Pair	Pair × Size	3	0.07	0.25
Size group		Pair × Size	1	0.00	0.01	0.90
Pollination treatment		Pair × Poll.	2	0.14	1.52	0.29
Pair × Size		Pair × Size × Poll.	3	0.27	2.45	0.17
Pair × Poll.		Pair × Size × Poll.	6	0.09	0.84	0.58
Size × Poll.		Pair × Size × Poll.	2	0.22	2.05	0.21
Pair × Size × Poll.		Error	6	0.11	0.98	0.44
Plant (pair × Size)		Error	113	0.11	0.92	0.69
Error			186	0.12		
Seedling biomass at 15 weeks, $R^2 = 0.63$		Pair	Pair × Size	3	4.16	30.21
	Size group	Pair × Size	1	1.98	14.40	<b>0.0032</b>
	Pollination treatment	Pair × Poll.	2	5.75	16.86	<b>0.003</b>
	Pair × Size	Pair × Size × Poll.	3	0.14	0.68	0.60
	Pair × Poll.	Pair × Size × Poll.	6	0.34	1.67	0.27
	Size × Poll.	Pair × Size × Poll.	2	0.43	2.10	0.20
	Pair × Size × Poll.	Error	6	0.20	0.26	0.95
	Plant (pair × Size)	Error	113	0.78	2.02	<b>0.0001</b>
	Error		179	0.39		

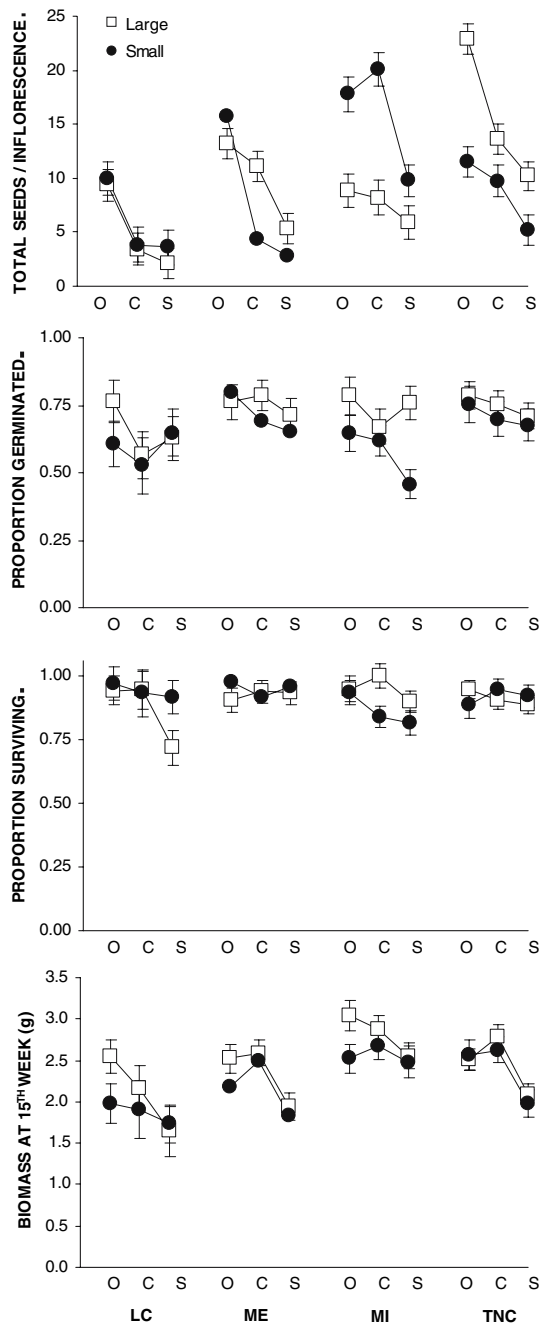
<sup>a</sup> For seed production, number of flowers on maternal plants was modeled as a covariate

non-significantly in the same direction in the other three populations (results not shown).

#### Seedling emergence

Seventy percent of the 3,713 seeds planted in the greenhouse emerged. Seedling emergence differed significantly

among pollination treatments and maternal plants, but other main effects and interactions were not significant (Table 3; Fig. 2b). Emergence was lowest for seeds from hand selfing (66%), and highest for those from open pollination (74%; Fig 2b). Emergence tended (non-significantly) to be higher for seeds from large populations (73%) than for those from small populations (65%; Fig. 2b).



**Fig. 2** Effects of pollination type on components of performance in paired large and small populations of *L. perennis*. Values shown are LS means  $\pm$  1 SE. **a** Seed production per *L. perennis* inflorescence. **b** Proportion of *L. perennis* seedling emergence. **c** Proportion of seedlings surviving to 15 weeks. **d** Biomass after 15 weeks of growth. For abbreviations see Fig. 1, analysis in Tables 2 and 3

Seedling performance

Biomass at the 15th week was strongly affected by pollination treatment, population size group, population pairing, and maternal plant. None of the interactions was significant. Self-pollination significantly decreased biomass when

compared to either open pollination or hand crossing (Table 3; Fig. 2d; Tukey test  $P < 0.0001$ ). The significant effect of population size grouping reflects the fact that greenhouse-grown seedlings from small populations were on average 7.7% smaller than those from large populations (Table 3; Fig. 2d). In contrast, seedling survival over 15 weeks in the greenhouse was unaffected by any factor in the analysis (Table 3; Fig. 2c; overall mean = 91% survival).

Effects of population size on inbreeding depression

Overall inbreeding depression for multiplicative performance across four life stages was strong and significant. Inbreeding depression was similar whether estimated by comparing hand-outcrossed and hand-selfed offspring ( $\delta = 0.404 \pm 0.043$ ; mean  $\pm$  SE), or by comparing open-pollinated and hand-selfed offspring ( $\delta = 0.443 \pm 0.046$ ).

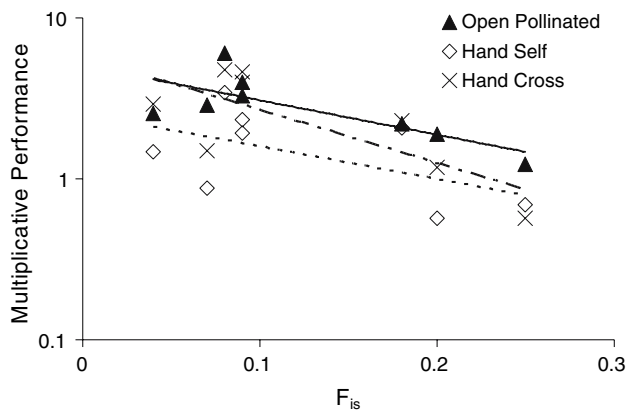
Population size categories did not differ in the strength of inbreeding depression ( $F_{1,3} = 1.92, P = 0.26, r^2 = 0.183$ ). This lack of an association also occurs when considering size as a continuous variable in an ANCOVA ( $F_{1,3} = 1.57, P = 0.30, r^2 = 0.183$ ). However, in the absence of purging, populations would be expected to show a linear, negative relationship between log performance and the inbreeding coefficient ( $F_{is}$ ) (Keller and Waller 2002). Because Shi (2004) estimated  $F_{is}$  from analysis of six microsatellite loci for 18–25 plants in each of ten *L. perennis* populations (including the eight studied here), we can also use this approach. This reveals (Fig. 3) that the log of multiplicative performance for each of our eight populations decreased strongly with  $F_{is}$  ( $F_{2,18} = 9.90, P < 0.005$ ), and also varied significantly among pollination treatments ( $F_{1,18} = 5.64, P < 0.012$ ). All three pollination treatments show a similar, linear decline in performance with  $F_{is}$  (interaction:  $F_{2,18} = 0.24, P > 0.7$ ), consistent with the expectation that more inbred populations have a larger drift load, and that there has been no purging in these populations.

None of the  $\delta$  values for the components of multiplicative performance varied significantly with population size (seed production  $F_{1,3} = 6.6, P = 0.08, r^2 = 0.15$ ; seedling emergence  $F_{1,3} = 0.77, P = 0.44, r^2 = 0.18$ ; seedling survival  $F_{1,3} = 0.13, P = 0.13, r^2 = 0.10$ ; seedling biomass ( $F_{1,3} = 2.04, P = 0.24, r^2 = 0.03$ ). The majority of inbreeding depression was expressed at the seed production stage ( $\delta = 0.67 \pm 0.033$ ), and to a lesser extent at final biomass ( $\delta = 0.21 \pm 0.034$ ).

Discussion

Population size and performance

Although fitness reductions in small populations have been found in many other studies (see Leimu et al. 2006 for a



**Fig. 3** Effects of pollination treatment on the relationship between multiplicative performance over four life stages and levels of inbreeding ( $F_{is}$ ) estimated from six microsatellite loci (Shi 2004) for eight populations. Lines are the separate regression estimates for the effect of inbreeding on performance for each pollination treatment: hand selfed (dotted line), hand crossed (dotted dashed line), open pollination (solid line)

recent review), overall performance of *L. perennis* across life stages and pollination treatments was not significantly lower in small populations than in large populations. This may have occurred for several reasons. First, populations varied greatly in their response. In particular, one larger population performed much worse than its paired small population (seeds per inflorescence for Michigan, Fig. 1). Because of this variation, we had low statistical power; despite a 31% overall reduction in performance for small populations (Fig. 1), power analysis indicates we would have needed to study about twice as many populations to have detected a significant effect of population size. Second, we did not expect strong effects of our population size groupings because our field manipulations were not feasible in the very small populations (<100) likely to show the strongest effects. Instead, we were forced to study more moderate-size populations, with several hundred plants.

Each of the components of multiplicative fitness (including offspring production, dispersal, and establishment) are also impacted by non-genetic factors and processes, including maternal environment and pollinator service. In our study we only found clear and consistent effects of population size on the last life stage measured, seedling biomass. Biomass at the end of the first year is an important benchmark for *L. perennis* success; in the field, larger first year seedlings are more likely to survive the winter and produce seed the following year (St. Mary 2007). Our greenhouse studies cannot rule out the possibility of size-correlated habitat effects of the maternal environment on seedling biomass, but these seem unlikely for two reasons. First, environmental factors did not vary consistently with population size in a previous study of 16 *L. perennis* populations (Cartwright 1997) ranging in size from 25–8,200, even

though performance increased with population size. Second, the lack of effects at earlier stages, which are probably under more direct maternal control, argues against the possibility that maternal environment would become more important later in the life cycle. In contrast, we found no evidence in these populations that population size or maternal habitat affected bumblebee pollination (Bernhardt 2000). Indeed, the absence of population size effects on seed production, and the generally greater seed production for open-pollinated inflorescences than for hand crosses suggest that pollination did not limit seed quantity. This is consistent with pollinator-visitation studies, which showed no effect of population size on visitation (Bernhardt 2000).

The strong association between multiplicative performance and population  $F_{is}$  suggests that drift in smaller populations has compromised their vigor. The contribution of reduced genetic variation to the poorer performance of small populations (Young et al. 1999; Ellstrand and Elam 1993) is supported by a concurrent survey of genetic variation for the same populations studied here, which revealed less allelic diversity in the small populations (Shi 2004). Although consistent with the scenario that the relationship between performance and inbreeding level is a consequence of genetic erosion, these are correlations that cannot account for how the association developed, nor whether these patterns also reflect some unquantified habitat differences. A recent meta-analysis (Leimu et al. 2006) found significant positive correlations among population size, fitness and genetic variation, which were independent of plant life span and the size range of the populations surveyed. Although theory predicts that inbreeding load should decline and drift load increase over time, and recent experiments using model systems have begun to explore the effects of inbreeding history and rates on purging (Reed et al. 2003; Swindell and Bouzat 2006a, b), data on the circumstances (Crnokrak and Barrett 2002) and relative rates for these processes in natural populations are needed in order to understand how changes in mating patterns arise as habitat loss, fragmentation, and degradation begin to place a population on the precipice of inevitable demographic decline.

#### Inferences from pollination-treatment responses

Comparison of self pollination to hand pollination is a standard way to assess the cost of inbreeding. Comparing those results to open pollination, where pollen is delivered by freely foraging pollinators, provides a more realistic and informative way to measure the effective cost of inbreeding for several reasons. First, open pollination reflects current reproductive performance, an important point of comparison for any hand-pollination experiments, and a measure of population vigor that is relevant to conservation. Second,

comparing the performance of open- and self-pollinated progeny suggests how much fitness may decline under further inbreeding and whether inbreeding depression occurs in populations experiencing natural pollinator service. Third, comparing these three treatments may provide insights regarding underlying mechanisms for decreased fitness in small populations. For example, if considerable inbreeding occurs and drives population fitness down, we expected to observe that  $W_{\text{cross}} > W_{\text{open}} > W_{\text{self}}$ . On the other hand, if low fitness was mainly due to the fixation of deleterious alleles due to drift load (Keller and Waller 2002), we might predict that  $W_{\text{cross}}$  and  $W_{\text{open}}$  approach  $W_{\text{self}}$ .

However, in this study  $W_{\text{open}}$  was usually similar to or greater than  $W_{\text{cross}}$ , while both were higher than  $W_{\text{self}}$ . This is consistent with the largely outcrossed mating system of this plant (Shi 2004), in that hand and open pollinations have comparable overall performance, while inbreeding depression is expressed only when plants are forced to self. Inbreeding experiments on *Lupinus arboreus* (Kittelson and Maron 2000), *Lupinus texensis* (Helenuum and Schaal 1996) and Kincaid's lupine (Severns 2003) reveal similar fitness declines when these outcrossing species are hand-self pollinated. However, in some populations (e.g., Turnpike population in Lou Campbell Preserve in Fig. 1, the smallest population in the study at  $n = 149$ ),  $W_{\text{cross}}$  and  $W_{\text{self}}$  were comparable and quite low. This absence of inbreeding load in this case suggests a loss of fitness due to fixation of the segregating load (Bataillon and Kirkpatrick 2000; Willi et al. 2005) that could be confirmed by interpopulation crosses (e.g., Paschke et al. 2005).

Open pollination always produced more seeds than did hand cross-pollination (Fig. 2a), suggesting that freely foraging bees deposit more pollen, or better-quality pollen, than did our hand pollinations. Previous studies of *L. perennis* indicate that natural bee pollinators visit multiple times over the life of a flower regardless of population size (Bernhardt 2000) and that bee visitation can promote stigmatic receptivity (Shenk 2005). This might increase the amount of pollen (Hevner 2001), or diversity of potential sires arriving on stigmas or achieving fertilization, compared to the four to five donors applied during the one to three hand cross-pollinations experienced by each flower. Low within-population donor diversity can reduce offspring growth, particularly in small populations (Paschke et al. 2002, 2005). Changes in pollinator behavior in response to declines in population density and size that alter pollen donor diversity could increase local inbreeding in the absence of pollinator limitation, even if populations have similar genetic diversity.

### Inbreeding depression

We found considerable inbreeding depression in *L. perennis* across four life stages of the first year (seed production,

seedling survival, and seedling growth). Even stronger differences may have been evident if seedlings were grown for longer, or in more challenging conditions (Fischer and Matthies 1998; Byers and Waller 1999; Kolb 2005; Paschke et al. 2005). Environmentally dependent expression of inbreeding depression does occur in *L. perennis* (Shi et al. 2005), so our estimates of inbreeding depression are probably lower than those experienced in the field.

Although inbreeding depression was strong overall, population size did not significantly affect the magnitude of inbreeding depression for overall multiplicative performance, or for the individual components. Our results and those of others (Ouborg and Van Treuren 1994; Hauser and Loeschcke 1994; Ouborg and Van Treuren 1995; Mavraganis and Eckert 2001) provide little evidence that deleterious alleles are purged from small populations (Byers and Waller 1999; Hedrick et al. 1999). This may reflect recency of population size reductions, or limited opportunities for inbreeding (Keller and Waller 2002). For long-lived, outbreeding perennials such as *L. perennis* (Shi 2004), purging following contemporary human habitat alterations is probably unlikely. Thus, although we documented considerable inbreeding depression in *L. perennis*, a significant portion of that may be due to the accumulation of drift load in our smaller populations. However, detecting performance deficits due to drift load requires comparisons to other populations, or among population crosses (Heschel and Paige 1995; Paschke et al. 2005; Willi et al. 2005). Although performance for small and large size categories was not significantly different, and we were unable to perform interpopulation crosses, we did find clear relationships between performance and natural population-level inbreeding values ( $F_{is}$ ). We found that multiplicative performance decreased approximately linearly with increases in population level inbreeding (measured by  $F_{is}$ ) regardless of pollination treatment. This is consistent with the inference that both drift and inbreeding load have contributed to  $\delta$  in these populations and that purging has been limited.

### Conservation implications

Most *L. perennis* populations in our study show fitness declines as inbreeding levels increase, suggesting that drift has affected smaller populations more than larger populations. Indeed, the smaller population at Lou Campbell Preserve, showed little difference in multiplicative performance for self and cross treatments, suggesting that it in particular suffers from drift load and that population persistence may be improved by bringing in pollen as a remediation strategy. Butterfly restoration programs often focus on availability and distribution of larval host and adult nectar sources (e.g., Schultz and Dlugosch 1999; Grundel et al. 2000; Schultz and Crone 2005). However, effective long-

term success may also require consideration of how spatial components of host plant ecology (population size and density; Morgan and Scacco 2006) influence long-term viability of host plant populations in order to create restorations with reliable host plant resources that are resistant to future demographic declines.

Given the recent and relatively high rate of habitat loss for *L. perennis*, inbreeding depression is likely to continue to reduce fitness in this species despite efforts to improve habitat through oak savanna restoration and management. Although populations of large to moderate size remain, it is particularly noteworthy that even though our smaller populations had  $n > 120$ , they still showed consistently smaller seedling size. This suggests that fitness declines can occur even in seemingly large lupine populations, and that restoration programs should allocate resources towards maximizing population size and genetic diversity of existing, moderate-size populations before seeding and transplanting new populations for habitat corridor development.

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