EFFECT OF CROSSING DISTANCE IN TWO SPECIES OF *LOBELIA*: THE FIRST GENERATION

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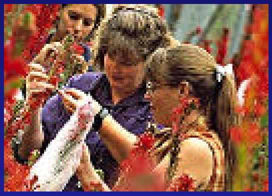
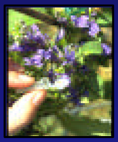
Abstract:

The concept of outbreeding depression is of interest to restorationists because of its implications for the choice of genetic stock in reintroduction projects. Outbreeding depression is a reduction in fitness that occurs when individuals that are very different genetically, such as plants from widely separated populations, are crossed. Our study is testing for outbreeding depression over three generations in two species of *Lobelia* that vary in neighborhood size. In 1998, two populations each of *Lobelia siphilitica* and *Lobelia cardinalis* were chosen, one population for each species near Chicago Botanic Garden (CBG) and one on or near Midewin National Tallgrass Prairie (MNTP) land. Fifteen plants received three pollination treatments; crossing with the nearest neighbor, crossing within the same population but with a plant 100-200 meters away, and crossing with a plant from the other population (about 100 km. away). Every plant received all three types of crosses, but the order of the crosses was rotated to avoid position effects. Common gardens were created at both the Chicago Botanic Garden and at Midewin National Tallgrass Prairie. Fruits from each of the 12 lines (2 species x 2 locations x 3 cross types) were pooled. Five hundred F1 seedlings (40+ from each line) were planted out in each common garden in August of 1999, allowed to overwinter, and were harvested in the fall of 2000 after seed set. Harvested plants were scored for flower number, fruit number and dry weight of above ground parts. There were very few significant differences between treatments in the F1 generation.





Introduction:

The concept of outbreeding depression is of interest to restorationists because of its implications for the choice of genetic stock in reintroduction projects. Outbreeding depression is a reduction in fitness that occurs when individuals that are very different genetically, such as plants from widely separated populations, are crossed. Outbreeding depression may be a consequence of local adaptation in the sense that crosses between plants adapted to different local conditions may yield offspring that are poorly adapted to both of the parental environments (Price and Waser, 1979; Schierup and Christiansen, 1996). Another cause of outbreeding depression is the disruption of coadapted gene complexes. When this occurs, first generation offspring may demonstrate hybrid vigor, and the major effect of outbreeding depression is not observed until the second or later generations (Templeton, 1986; Schierup and Christiansen, 1996). Evidence for outbreeding depression in plants is relatively scant although it has been documented in a few species (Waser and Price, 1989; Parker, 1992; Fenster and Galloway, 2000). Despite the lack of evidence, the danger of outbreeding is often a concern of restorationists. More empirical studies are needed to determine whether this is a common phenomenon in plants and at what spatial scales it occurs.

We chose to examine outbreeding depression in two species of *Lobelia*, *L. cardinalis* (cardinal flower) and *L. siphilitica* (great blue lobelia). These species were chosen for several reasons. They are native species that occur in the Chicago region at several sites (Swink and Wilhelm, 1994). The taxa are of restoration interest, especially in wetland mitigation projects. A substantial amount of research has been done on the breeding systems of *L. cardinalis* and *L. siphilitica*. *Lobelia cardinalis* is hummingbird-pollinated and has quite a large neighborhood size while *L. siphilitica* is primarily pollinated by bumble bees and has a somewhat smaller neighborhood size (Johnston, 1992). The taxon's breeding system and its historical patterns of gene flow and neighborhood size (the distance an individual typically disperses its genes, through pollen and seed movement) may affect the likelihood of outbreeding depression.

Progress to date:

Year One (1998). Two large (>20 plants) populations of each *Lobelia* species were selected, one near Chicago Botanic Garden in Glencoe, IL and one about 100 km. away, near Midewin National Tallgrass Prairie in Wilmington, IL. Fifteen plants of flowering size were marked at each site. A leaf was taken from each and frozen for future genetic analysis using ISSRs (inter simple sequence repeats) in order to determine genetic differentiation within and between the populations. When plants began to flower, fifteen plants received three pollination treatments on at least two flowers each (a total of six flowers were hand-pollinated on every plant). The three pollination treatments were crossing with the nearest neighbor; crossing within the same population but with a plant 50-200 meters away; and crossing with a plant from the other population (about 100 km. away).

Flowers were emasculated and bagged to prevent illegitimate pollinations. Every plant received all three types of crosses, but the order of the crosses was rotated to avoid position effects. Mature seeds were collected in October 1998 and weighed.

Year Two (1999). Common gardens were created at both the Chicago Botanic Garden CBG) and at Midewin National Tallgrass Prairie (MNTP). Fruits from each of the 12 lines (2 species x 2 locations x 3 cross types) were pooled. Seeds from each line were stratified and germinated in the spring. Seedlings were planted out in each common garden in August. Each garden received 480 seedlings; 180 for fitness measurement, 300 for next year's crosses. Not all plants flowered in their first year, so they were allowed to overwinter in the common gardens. All plants flowered in 2000.

Year Three (2000). The plants used for fitness measurement were harvested at the end of their second growing season (October 2000). Space and personnel constraints precluded us from determining total flower number, fruit number, and above ground dry weight per plant so we took those measures for one randomly selected stalk per plant and counted the number of stalks. The remaining plants in the common gardens (F1's) were allowed to flower and two flowers on each of 90 plants of each species were sibling-pollinated at each common garden. The seed was harvested, weighed and divided as described above.

Future years. This F2 seed was germinated and seedlings will be planted in the same common gardens in September of Year 4 (2001). A portion of the seed (720 seedlings; 360 per site) will be used for fitness measurements in Year 5 (2002). Another portion (600 seedlings, 300/site) will be used to create the next generation (F3). The F3 generation seeds will be planted in Year 6 (2003) and harvested in Year 7 (2004).

Data analysis:

For both species, we analyzed a metric of flower number, fruit number, or dry weight of one randomly selected stalk multiplied by the number of stalks for each plant. For each species, the differences between treatments (site grown, growth in local vs. distant common garden, and cross length) for each variable (flower number, fruit number and dry weight) were analyzed using t-tests or one-way ANOVAs (Systat, 7.01). Because the location of the common garden did have a significant effect, cross length data was also analyzed with site grown as a covariate, but this did not affect the overall results.

Results:

For both species, plants in the shadier and wetter CBG common garden were larger and produced more flowers and fruits than plants in the MNTP common garden and in most cases these differences were significant (Table 1).

Table 1. The effect of site grown on flower number, fruit number and dry weight metrics in *Lobelia cardinalis* and *Lobelia siphilitica*.

	At CBG	At MNTP	t-test, *P* value
L. cardinalis flower number	1198.45	581.28	P<0.001
L. cardinalis fruit number	772.27	474.00	P<0.001
L. cardinalis dry weight (g.)	114.15	47.92	P<0.001
L. siphilitica flower number	952.16	856.27	P=0.36
L. siphilitica fruit number	829.64	814.97	P=0.88
L. siphilitica dry weight (g.)	93.36	67.71	P=0.01

Plants did not perform significantly better in the local common garden, than in the distant common garden. *Lobelia cardinalis* plants were smaller in the home site (dry weight= 76.36 g. vs. 84.79 g., *P*=0.321 by t-test), while *Lobelia siphilitica* plants were larger in the home site (dry weight= 84.14 g. vs. 76.56 g., *P*=0.449 by t-test) though neither difference was significant. Flower and fruit number showed similar trends (data not shown).

Flower number, fruit number, and dry weights were not significantly different between cross types, but in every case there was a trend for the offspring of long crosses to be smaller with fewer flowers and fruits (Figs. 1 and 2).

Figure 1. *Lobelia cardinalis* flower number, fruit number, and dry weights for the three cross lengths. Error bars = SE.

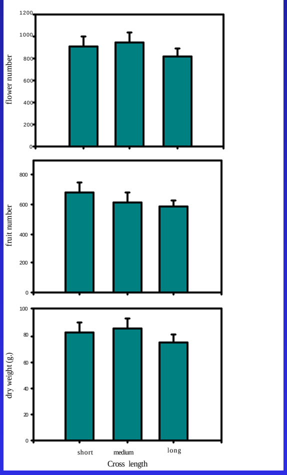
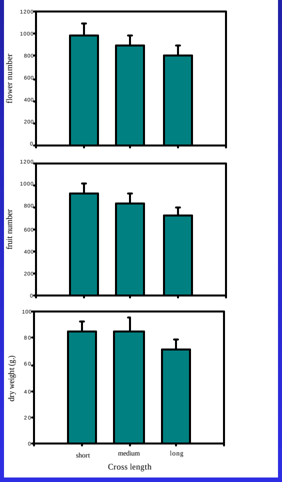


Figure 2. *Lobelia siphilitica* flower number, fruit number, and dry weights for the three cross lengths. Error bars = SE.



Conclusions:

We predicted that plants would perform better in the local common garden as opposed to the more distant common garden, but this was not the case. This could indicate that the common gardens were different enough (in soil, exposure and/or hydrology) from the natural sites that local adaptation was not a factor or it could indicate that these two *Lobelia* taxa are not strongly locally adapted.

We did not expect to see strong evidence of outbreeding depression in the F1 generation because much of the theoretical and empirical work to date suggests outbreeding depression may not become apparent until the F2 or later generations. We were intrigued by the trend we observed for offspring of long crosses to be less fit in the F1 generation. We are increasing sample sizes for the F2 and F3 generations, which should increase our power to detect differences between treatments.

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