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## EFFECTS OF CROSS AND SELF-FERTILIZATION ON PROGENY FITNESS IN *LOBELIA CARDINALIS* AND *L. SIPHILITICA*

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**Abstract.**—Inbreeding depression, or the decreased fitness of progeny derived from self-fertilization as compared to outcrossing, is thought to be the most general factor affecting the evolution of self-fertilization in plants. Nevertheless, data on inbreeding depression in fitness characters are almost nonexistent for perennials observed in their natural environments. In this study I measured inbreeding depression in both survival and fertility in two sympatric, short-lived, perennial herbs: hummingbird-pollinated *Lobelia cardinalis* (two populations) and bumblebee-pollinated *L. siphilitica* (one population). Crosses were performed by hand in the field, and seedlings germinated in the greenhouse. Levels of inbreeding depression were determined for one year in the greenhouse and for two to three years for seedlings transplanted back to the natural environment. Fertility was measured as flower number, which is highly correlated with seed production under natural conditions in these populations. Inbreeding depression was assessed in three ways: 1) survival and fertility within the different age intervals; 2) cumulative survival from the seed stage through each age interval; and 3) net fertility, or the expected fertility of a seed at different ages. Net fertility is a comprehensive measure of fitness combining survival and flower number. In all three populations, selfing had nonsignificant effects on the number and size of seeds. *Lobelia siphilitica* and one population of *L. cardinalis* exhibited significant levels of inbreeding depression between seed maturation and germination, excluding the consideration of possible differences in dormancy or longterm viability in the soil. There was no inbreeding depression in subsequent survival in the greenhouse in any population. In the field, significant survival differences between selfed and outcrossed progeny occurred only in two years and in only one population of *L. cardinalis*. For both survival and fertility there was little evidence for the expected differences among families in inbreeding depression. Compared to survival, inbreeding depression in fertility (flower number) tended to be much higher. By first-year flower production, the combined effects on survival and flower number caused inbreeding depression in net fertility to reach 54%, 34% and 71% for *L. siphilitica* and the two populations of *L. cardinalis*. By the end of the second year of flowering in the field, inbreeding depression in net fertility was 53% for *L. siphilitica* and 54% for one population of *L. cardinalis*. For the other population of *L. cardinalis*, these values were 76% through the second year of flowering and 83% through the third year. Such high levels of inbreeding depression should strongly influence selection on those characters affecting self-fertilization rates in these two species.

**Key words.**—Bumblebee, flower number, hummingbird, inbreeding depression, *Lobelia cardinalis*, *Lobelia siphilitica*, Michigan, perennial, seed size, self-fertilization, survival.

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From crossing experiments in 57 species of flowering plants, Darwin (1876) made two general observations. First, progeny of self-fertilization tended to be less vigorous than progeny of outcrossing (inbreeding depression). Second, crosses between inbred strains increased progeny vigor (heterosis). That high levels of inbreeding depression and heterosis exist in agricultural and silvicultural species is now well documented (e.g., Wright, 1977 Ch. 2; Franklin, 1970; Ericsson et al., 1973; Griffin and Cotterill,

1988), and data are accumulating from natural populations (e.g., Griffing and Langridge, 1963; Riley, 1956; Jain, 1978; Price and Waser, 1979; Schemske, 1983; Schoen, 1983; Waller, 1984; Kesseli and Jain, 1984; Levin, 1984, 1989, 1991; Harder et al., 1985; Ritland and Ganders, 1987; Kohn, 1988; Kalisz, 1989; Karron, 1989; Sakai et al., 1989; Wolfe, 1989; Dudash, 1990; Holtsford and Ellstrand, 1990; Schmitt and Gamble, 1990; Fenster, 1991; reviewed by Charlesworth and Charlesworth, 1987). Two genetic causes have been proposed. According to the overdominance hypothesis, outbred progeny are fitter because they have a larger proportion of loci heterozygous, and

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many of these loci are overdominant. According to the partial-dominance hypothesis, outbred progeny are fitter because their deleterious, largely recessive alleles tend to be masked in the heterozygous condition.

Because inbreeding depression is the most general selective disadvantage of self-fertilization, it is a potentially important force in the evolution of plant breeding systems, such as gynodioecy and dioecy (Lloyd, 1975; Charlesworth and Charlesworth, 1978, 1979; Charlesworth and Ganders, 1979). It is represented as the proportional decrement in fitness of selfed relative to outcrossed progeny, or  $\delta = 1 - w_s/w_o$ , where  $w_s$  and  $w_o$  are the expected fitnesses of selfed and outcrossed progeny, respectively. Without inbreeding depression, a mutant gene causing complete self-fertilization, but no loss in pollen output, will spread rapidly to fixation, owing to the 50 percent increase in the number of haploid genomes transmitted to the next generation (Fisher, 1941). In the presence of inbreeding depression, however, self-fertilization should be favored unless inbreeding depression exceeds one-half, all else being equal (Kimura, 1959; Maynard Smith, 1977, 1978). Furthermore, nuclear-based gynodioecy and dioecy can evolve from hermaphroditism whenever the product of inbreeding depression and the selfing rate exceeds one-half (Lloyd, 1975; Charlesworth and Charlesworth, 1978). This threshold condition is altered in the evolution of both gynodioecy (Lloyd, 1975) and self-fertilization (Lloyd, 1979; Holsinger et al., 1984) according to whether selfing precedes, follows or competes with outcrossing. In particular, Charlesworth (1980) and Holsinger et al. (1984) found that lower levels of inbreeding depression are required to prevent the evolution of selfing with greater reduction of pollen dispersal by selfing.

Because self-fertilization exposes deleterious recessive alleles to selection, and outcrossing masks them, the level of inbreeding depression in a population should evolve with the selfing rate. Lande and Schemske (1985) modeled the joint evolution of selfing and inbreeding depression, in which 1) the selfing rate was controlled polygenically and evolved in small increments; and 2) inbreeding depression was caused by one locus under mutation-selection balance, the

model then being extended to multiple loci with multiplicative fitness effects and independence of genotypic frequencies at different loci. They concluded that plants in habitually large, outcrossing populations should exhibit large amounts of inbreeding depression and be selected to outcross, while populations having experienced recent bottlenecks or bouts of selfing should be selected to self-fertilize, though they can maintain moderate levels of inbreeding depression. Uyenoyama (1986) found that inbreeding between close relatives simultaneously favors both selfing and biparental reproduction, and that the evolutionary outcome of these opposing forces depends on the level of inbreeding depression.

Campbell (1986) and Holsinger (1988) emphasized that the dynamics of mating-system evolution may be complicated by nonrandom genotypic associations (identity disequilibrium) between loci affecting inbreeding depression and the selfing rate, and that therefore inbreeding depression has little predictive value. Holsinger (1988) found much support for this contention in a model where inbreeding depression was caused by deleterious recessives, but it is unclear to what extent the variability in simulation results was due to stochastic noise. Charlesworth et al. (1990) also modeled the coevolution of selfing and inbreeding depression, where inbreeding depression was caused by dominance, identity disequilibrium was allowed to accumulate, but there was no biparental inbreeding. They found, on the contrary, that inbreeding-depression values of less than one-half always led to increased selfing, while levels greater than one-half usually led to increased outcrossing, unless the selective disadvantage of mutations was very great. Furthermore, the model predicts that inbreeding depression should be around 50% in partially selfing populations. Results from homozygote-advantage models often contrast with those presented here (Charlesworth and Charlesworth, 1987, 1990; Holsinger, 1988), but this is not thought to be an important source of inbreeding effects. These theoretical investigations suggest that inbreeding depression can play a large role in shaping plant mating systems, and therefore those characters affecting them, such as heterostyly, separation of sexes and spatial

or temporal separation of sexual phases within flowers. There are at present very few comprehensive studies that analyze the fitness consequences of selfing under natural conditions, and there is almost no information for perennials at various life stages (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987).

In this paper I present results from experiments designed to determine the levels of inbreeding depression in hummingbird-pollinated *Lobelia cardinalis* L. and bumblebee-pollinated *L. siphilitica* L. (Lobeliaceae). These perennials inhabit water edges and low woods in North America and are thought to live for several years (McVaugh, 1936, 1940; Bowden, 1959). Both species flower from late July through September and overwinter as rosettes. They are highly, but not completely, outcrossing (Johnston, 1990) and have several features suggesting adaptations to avoid inbreeding. The flowers are protandrous, with no overlap in sexual phases (Trelease, 1879; Robertson, 1891; Meehan, 1902; Devlin and Stephenson, 1984, 1985). Flowers mature from the base upward along a single raceme, causing staminate-phase flowers to be borne above pistillate-phase flowers throughout the flowering season. In addition, the *L. siphilitica* population studied here has a low frequency of male-sterile individuals. It is therefore expected that these species would exhibit substantial inbreeding depression in fitness components upon selfing. The goal of this research was to measure inbreeding depression in survival and fertility in two populations of *L. cardinalis* and one population of *L. siphilitica*.

#### MATERIALS AND METHODS

This study was carried out on three populations at two sites in southwestern Michigan: Wintergreen Lake of the Kellogg Biological Station Bird Sanctuary (Kalamazoo Co.) and the forest of Glass Creek, 40 km to the north (Barry Co.). *Lobelia cardinalis* occurs at Wintergreen Lake, while both species occur at Glass Creek. The Wintergreen Lake site is sunnier and has a much higher density of *L. cardinalis*. (Full site descriptions are provided in Johnston, 1991a, 1991b.)

*Pollinations and Seed Stratification.*—

Hand pollinations were conducted in the field during the flowering season in 1987 for the Wintergreen Lake population of *L. cardinalis* and in 1988 for the two species at Glass Creek. To exclude pollinators, inflorescences of ovule parents were enclosed with bridal veil. Wire hoops laced through the netting and attached to a wooden stake minimized contact between the net and flowers. Mature stigmas were pollinated using a plastic cocktail spear bearing either self pollen or outcross pollen from a single sire. The outcrossed flowers on a plant received pollen from different sires. The two pollination treatments were alternated along the inflorescence to prevent correlations with floral position. There were fourteen seed parents (families) of *L. cardinalis* at Wintergreen Lake. A severe flood at Glass Creek during fruit maturation reduced the number of seed parents there to eleven for *L. cardinalis* and to eight for *L. siphilitica*.

Seeds were collected in September and October, after ripening but before capsule dehiscence, and were stored at room temperature in paper envelopes. I then randomly chose three fruits per maternal parent per cross type, with an occasional extra fruit. Twenty seeds per fruit were sown onto an equal mixture of moist sand and peat in flats, with one fruit per cell. There were 1,700 seeds sown from *L. cardinalis* of Wintergreen Lake, 1,320 from *L. cardinalis* of Glass Creek and 1,040 from *L. siphilitica* of Glass Creek. The flats were maintained at 5°C from mid-February to mid-April (eight weeks). Remaining, unsown seeds were weighed and counted. Mean seed mass per fruit was the mass of 50 seeds/50. Differences between selfed and outcrossed seeds in mass and number were assessed with Wilcoxon's signed rank test for paired values (Hollander and Wolfe, 1973), using the differences between parental means for each observation.

*Greenhouse: Survival and Flower Number.*—Seedlings germinated under natural light in the greenhouse. As germination in all three populations occurs by the third week following stratification, with almost no intervening seedling death, the number of seeds germinating from each fruit was scored during week three. In week six the seedling number was again determined, and from

each fruit up to five individuals were chosen randomly for subsequent growth in each of two environments, the greenhouse and the parental field site (see below). Progeny remaining in the greenhouse were at this time transferred individually to four-and-one-half-inch plastic pots and placed in standing water with weekly fertilization. At this stage there were therefore up to five offspring per fruit and three fruits per cross, or 15 inbred and 15 outbred progeny per seed parent. Progeny from Wintergreen Lake were grown inside the greenhouse on benches in 1988. Progeny from the two Glass Creek populations were grown outside the greenhouse in summer and fall 1989. As a result of shading by a building, these plants were cooler and also received approximately two hours less sunlight per day than the Wintergreen Lake plants. Tests for significant differences in survival between inbred and outbred progeny were made with *G*-tests (Sokal and Rohlf, 1981). Heterogeneity *G*-tests were used to ascertain whether maternal parents differed in the degree of inbreeding depression.

For both species progeny flowered in the greenhouse from August to October, at which time survival and flower number were recorded. In 1987 the correlation between flower number and seed number in natural populations was 0.85 at Wintergreen Lake, 0.86 for Glass Creek *L. cardinalis* and 0.79 for *L. siphilitica* (Johnston, 1991b;  $P < 0.001$  in each case). Flower number in these species can therefore be taken as a good estimate of at least the maternal component of fertility. ANOVAs were conducted on flower number (GLM procedure, SAS, 1988), with crossing treatment considered a fixed effect and family and interaction terms considered random effects. A significant interaction implies differences among families in the level of inbreeding depression. Unfortunately, nonlinear data transformations (to satisfy assumptions of parametric significance testing) can by themselves create or destroy interactions between treatments. Because the parametric assumptions were not satisfied in any population, I chose to jackknife the natural logarithm of the mean square terms from untransformed data, with each jackknife iteration omitting one family (Miller, 1986 p. 148). A *t*-test (TTEST pro-

cedure with Satterthwaite's approximation for degrees of freedom, SAS, 1988) was used to determine whether the appropriate jackknifed mean squares differed from one another. This method is conservative (Miller, 1968).

*Field: Survival and Flower Number.*—At week six an equal number of seedlings was also transferred to individual cells in flats. Transplanting to the field occurred in week 10 at the beginning of July. (No plants in flats died between weeks 6 and 10.) At transplanting to the parental field site, individuals were planted 20 cm apart in rectangular grids six rows deep and of various lengths. Transplanters were unaware of inbreeding status. In both Glass Creek populations, nearly all individuals producing an inflorescence were cropped by deer to a height of several centimeters near the beginning of flowering. As bigger plants flower earlier (Johnston, 1991b), it is probable that deer preferentially cropped larger plants, causing inbreeding depression to be underestimated.

Because the inbreeding depression study of *L. cardinalis* of Wintergreen Lake was initiated one year earlier than that for the Glass Creek populations, there are three years of information on survival and fertility there and two years at Glass Creek. Survival through the first winter was also determined in May 1989 for Wintergreen Lake *L. cardinalis*.

*Three Measures of Inbreeding Depression.*—Inbreeding depression involving the major fitness components of survival and flower number was calculated in three ways for each population in both the field and greenhouse. First, to determine the stages at which inbreeding depression occurs, I calculated the age-specific survival and flower production. Age-specific survival incorporates only those individuals alive at the previous census, while age-specific flower number includes all living individuals. Second, cumulative inbreeding depression in survival to age *X* was calculated as unity minus the product of the inbred-to-outbred ratios through age *X*. This cumulative measure thus represents the proportional decrease in survival of inbred relative to outbred seeds through age *X*. The third measure of fitness was net fertility, an analog of net reproduc-

TABLE 1. Comparison of number and mass of seeds resulting from outcrossed and selfed flowers in two populations of *Lobelia cardinalis* and one of *L. siphilitica*. Per-parent means are based on three fruits per cross. Two-tailed significance levels are presented for Wilcoxon's signed rank test for paired values.

Trait	Species and population	# Seed parents	Mean (SD) of per-parent:			P
			Outcross mean	Self mean	$\delta$	
Seed number per fruit	<i>L. cardinalis</i> , Wintergreen Lake	13	650 (319)	633 (324)	-0.024	>0.50
	<i>L. cardinalis</i> , Glass Creek	11	738 (231)	786 (312)	-0.054	>0.50
	<i>L. siphilitica</i> , Glass Creek	8	322 (108)	359 (153)	-0.095	>0.50
Seed mass (micrograms)	<i>L. cardinalis</i> , Wintergreen Lake	13	43.1 (9.9)	40.1 (10.0)	0.025	0.07
	<i>L. cardinalis</i> , Glass Creek	11	35.4 (7.3)	33.4 (6.8)	0.045	0.11
	<i>L. siphilitica</i> , Glass Creek	8	30.0 (5.3)	28.5 (4.9)	0.037	0.55

tive rate. Genotypes with the highest lifetime offspring production will increase in frequency in expanding populations (Haldane, 1927), in the absence of shifts toward earlier reproduction. Relative fitness, as measured by seed production is highly correlated with flower number (Johnston, 1991b). As a measure of overall fitness combining survival and fecundity, I calculated the average net fertilities of selfed and outcrossed progeny as  $\sum l_x b_x$ , where  $l_x$  is the proportion of seeds surviving to age  $X$  and  $b_x$  is flower number averaged over survivors at age  $X$ . Thus, net fertility at age  $X$  is the average number of flowers produced by a seed through age  $X$ .

## RESULTS

*Seed Number and Mass.*—Cross type did not significantly influence either seed number per fruit or average seed mass per fruit in any population (Table 1). The mass of outcrossed seeds, however, always exceeded that of selfed seeds, and this difference approached significance in the two *L. cardinalis* populations.

*Greenhouse: Survival.*—There was significant inbreeding depression in the proportion of seeds germinating in *Lobelia siphilitica* (12%) and in the Wintergreen Lake population of *L. cardinalis* (19%), but not in the Glass Creek population of *L. cardinalis* (8%; Table 2). Maternal parents differed in the degree of inbreeding depression in the Wintergreen Lake population of *L. cardinalis* (heterogeneity  $G = 32.2$ ,  $df = 13$ ,  $P < 0.005$ ), but not in either species at Glass Creek. Greenhouse survival in the six weeks following germination was high in all three populations. Thus, when progeny were

pooled across families, there was no significant inbreeding depression. In the Wintergreen Lake population of *L. cardinalis*, one family experienced very high inbreeding depression, creating significant total and heterogeneity  $G$ -values (Table 2). Nearly 100% of both inbred and outbred individuals that survived to six weeks (and were then transplanted to pots) also survived the ensuing two to three months to flowering. There was thus no significant (age-specific) inbreeding depression in survival between six weeks and flowering (Table 2). The nonsignificant survival differences in these two periods, however, always favored outbred progeny. By the time of flowering in the greenhouse, inbreeding depression in survival had accumulated to 13% in *L. siphilitica*, 10% in *L. cardinalis* of Glass Creek and 24% in *L. cardinalis* of Wintergreen Lake (Table 2).

*Greenhouse: Flower Number and Net Fertility.*—There was inbreeding depression in flower number in all three populations: 27% for Wintergreen Lake *L. cardinalis*, 64% for Glass Creek *L. cardinalis* ( $P \sim 0.06$ ) and 44% for *L. siphilitica* (Table 3). There was no indication of heterogeneity among families in the level of inbreeding depression (interaction terms nonsignificant, Table 3). Furthermore, while not statistically significant in any population, a greater proportion of outbred than inbred plants produced inflorescences (Table 4).

For Wintergreen Lake *L. cardinalis*, net fertility, or the average number of flowers of a sown seed, was 24.8 for outcrossed seeds, compared to 11.8 for selfed seeds (data not shown). Thus, inbreeding depression for net fertility through the first year in the greenhouse was 52%. For Glass Creek *L. cardi-*

TABLE 2. Age-specific and cumulative inbreeding depression in survival for individuals raised in the greenhouse and in the field. Proportions are means of family (maternal-parent) means. *N* is the total number of survivors at the beginning of each age interval. Inbreeding depression is the mean of per-family values. *P*-values for the total *G*-test represent the chance of obtaining the set of by-family *G*-values given no effect of cross type on progeny survivorship (*df* = family number). Pooled *G*-tests ignore families (*df* = 1). Heterogeneity *G*-tests indicate differences among families in the level of inbreeding depression (*df* = family number - 1). To aid reading, *P*-values less than 0.05 are in bold type.

Species and population	Environment and age interval	Cross type			Age-specific			Cumulative	
		Outcross Proportion ( <i>N</i> )	Self		Total	G-test: <i>P</i> < Pooled	Hetero.	Mean $\delta$	Mean $\delta$
			Proportion ( <i>N</i> )	Proportion ( <i>N</i> )					
<i>Lobelia cardinalis</i> , Wintergreen Lake	Greenhouse								
	Seed to germination	0.699 (860)	0.527 (840)	<b>0.001</b>	<b>0.001</b>	<b>0.005</b>	0.186	0.186	
	Germination to six weeks	0.873 (604)	0.814 (443)	<b>0.01</b>	0.5	<b>0.01</b>	0.066	0.240	
	Six weeks to flowering, Year 1	1.000 (181)	1.000 (135)	1	1	1	0.000	0.240	
	Field								
	Six weeks to flowering, Year 1	1.000 (186)	1.000 (143)	1	1	1	0.000	0.240	
<i>Lobelia cardinalis</i> , Glass Creek	Greenhouse								
	Seed to germination	0.930 (660)	0.858 (660)	0.98	0.5	1	0.0838	0.0838	
	Germination to six weeks	0.989 (614)	0.970 (566)	1	0.9	1	0.019	0.101	
	Six weeks to flowering, Year 1	0.988 (166)	0.988 (161)	1	1	1	-0.001	0.100	
<i>Lobelia siphilitica</i> , Glass Creek	Greenhouse								
	Seed to germination	0.912 (162)	0.853 (158)	1	0.9	1	0.061	0.155	
	Germination to six weeks	0.705 (147)	0.727 (135)	0.98	0.98	0.98	-0.173	0.009	
	Six weeks to flowering, Year 1	0.910 (540)	0.792 (500)	0.10	<b>0.05</b>	0.5	0.121	0.121	
	Field								
	Six weeks to flowering, Year 1	0.982 (493)	0.964 (393)	1	0.9	1	0.0183	0.137	
<i>Lobelia siphilitica</i> , Glass Creek	Greenhouse								
	Seed to germination	0.979 (129)	0.992 (119)	1	0.9	1	-0.0145	0.125	
<i>Lobelia siphilitica</i> , Glass Creek	Field								
	Six weeks to flowering, Year 1	0.939 (125)	0.965 (118)	1	0.9	1	-0.0328	0.096	
<i>Lobelia siphilitica</i> , Glass Creek	Greenhouse								
	Seed to germination	0.608 (104)	0.539 (99)	0.9	0.9	0.9	0.0777	0.166	

TABLE 3. Age-specific inbreeding depression in flower number of all survivors. Year 1 is 1988 for Wintergreen Lake and 1989 for Glass Creek. Flower number is the mean of family means. Inbreeding depression ( $\delta$ ) is the mean of per-family values. Significance levels of treatment effects were determined by jackknifing mean squares. For clarity, mean squares are not presented and  $P$ -values less than 0.05 are in bold type.

Species and population	Location	Year	Flower number		Mean $\delta$	ANOVA $P$ -values		
			Outcross	Self		Cross	Family	Interaction
			Mean ( $N$ )	Mean ( $N$ )				
<i>L. cardinalis</i> , Wintergreen Lake (14 families)	Greenhouse	1	40.6 (181)	27.6 (135)	0.27	<b>0.001</b>	<b>0.008</b>	0.948
		2	34.0 (92)	16.0 (42)	0.53	<b>0.001</b>	0.151	0.289
	Field	1	5.1 (184)	2.1 (140)	0.72	<b>0.011</b>	<b>0.041</b>	0.380
		3	45.8 (49)	11.9 (15)	0.73	<b>0.001</b>	0.550	0.204
<i>L. cardinalis</i> , Glass Creek (11 families)	Greenhouse	1	27.1 (195)	14.6 (159)	0.64	0.062	0.124	0.424
	Field	1	0.09 (163)	0.07 (126)	0.70	0.802	0.858	0.469
		2	10.1 (118)	5.3 (91)	0.45	<b>0.001</b>	0.652	0.613
<i>L. siphilitica</i> , Glass Creek (8 families)	Greenhouse	1	166.8 (169)	93.5 (118)	0.44	<b>0.001</b>	<b>0.012</b>	0.858
	Field	1	1.9 (121)	0.99 (91)	0.07	0.227	0.351	0.598
		2	15.3 (93)	9.2 (57)	0.29	<b>0.038</b>	0.280	0.651

*nalis*, the average outbred seed produced 24.6 flowers, while a selfed seed produced 12.0 flowers, resulting in an inbreeding depression of 51%. In *L. siphilitica* the average outcrossed seed produced 146 flowers, while the average selfed seed produced 70.8 flowers; inbreeding depression in net fertility was 51% (greenhouse data not shown).

**Field: Survival**—Cross type had no significant effect on survival between the six-week census and first-year flowering in the field in any population (Table 2). For both progeny types, survival from flowering in year one to flowering in year two was greater than 50% in *L. siphilitica* and 70% in *L. cardinalis* of Glass Creek; there was no significant inbreeding depression (Table 2). At Wintergreen Lake, in contrast, selfed progeny survived the first winter (census in May 1989) 28% less well than outcrossed, raising the cumulative survival ( $l_x$ ) inbreeding depression to 46% (Table 2). While not significant over such a short time period, the three-percent inbreeding depression in survival from May 1989 to flowering in late summer resulted in a cumulative inbreeding depression in survival alone of 48% by flowering in year two (Table 2). For the period between flowering in years two and three, selfed progeny at Wintergreen Lake also survived 42% less well than outcrossed, raising the cumulative inbreeding depression in survival alone to 70% (Table 2).

**Field: Flower Number and Net Fertility.**—

The proportion of plants flowering during their first season was lower in the field than in the greenhouse, especially for *L. cardinalis*. In *L. siphilitica* a greater proportion of outcrossed than selfed progeny produced an inflorescence in the field, and the same trend (though not significant) existed for both populations of *L. cardinalis* (Table 4). For both species at Glass Creek, flower number did not differ between selfed and outcrossed progeny in the first year. At Wintergreen Lake, however, there was 72% inbreeding depression in flower number for plants surviving to their first year (Table 3). In years two and three, nearly every surviving *L. cardinalis* at Wintergreen Lake flowered (Table 4), but inbred plants produced 53% fewer flowers in year two and 73% fewer in year three (Table 3). Similarly, over 97% of plants of both Glass Creek species flowered in their second year. Mean inbreeding depression in flower number there was 45% in *L. cardinalis* and 29% in *L. siphilitica* (Table 3). As in the greenhouse, there was no evidence that inbreeding depression varied among families.

By the end of flowering in year one, inbreeding depression in net fertility,  $\Sigma l_x b_x$ , was 54% for *L. siphilitica*, 34% for *L. cardinalis* at Glass Creek and 71% for *L. cardinalis* at Wintergreen Lake (Figs. 1, 2, and 3). In year two these values changed to 53%, 54%, and 76%, respectively. At Wintergreen Lake, inbreeding depression in net fertility



TABLE 4. Proportion of survivors flowering. There is one degree of freedom for each *G*-test.

Species and population	Location	Year	Cross type						<i>G</i>	<i>P</i> <	$\delta$
			Outcross			Self					
			#	Prop.	Total	#	Prop.	Total			
<i>L. cardinalis</i> , Wintergreen Lake	Greenhouse	1	138	0.762	181	98	0.726	135	0.138	0.9	0.048
	Field	1	41	0.220	186	23	0.161	143	1.50	0.5	0.270
		2	105	1.000	105	47	1.000	47	0	1	0
<i>L. cardinalis</i> , Glass Creek	Greenhouse	1	60	0.366	164	40	0.252	159	3.43	0.1	0.312
	Field	1	17	0.116	147	13	0.096	135	0.249	0.9	0.167
		2	103	0.972	106	97	0.990	98	0.017	0.9	-0.019
<i>L. siphilitica</i> , Glass Creek	Greenhouse	1	126	1.000	126	114	0.996	118	0.048	0.9	0.034
	Field	1	78	0.667	117	46	0.404	114	7.54	<b>0.01</b>	0.395
		2	65	0.985	66	60	1.000	60	0.007	0.98	-0.015

reached 83% by flowering in year three (Fig. 1).

## DISCUSSION

### *Life Stages Expressing Inbreeding Depression*

Inbreeding depression is a common feature of plant populations. In addition to studies using standard comparisons of progeny derived from selfing and outcrossing, there are at least two other kinds of study corroborating the prevalence of inbreeding depression. The first is the comparison of progeny produced by cleistogamous and chasmogamous flowers, especially of *Impatiens* (Schemske, 1978; Waller, 1984; Schmitt and Ehrhardt, 1987, 1990; McCall et al., 1989). These studies differ from a strict comparison of selfed and outcrossed progeny because chasmogamous flowers are not wholly outcrossing, and there are differences between the two fruit types, such as seed size, in addition to level of inbreeding. In studies where the seed-size differences have been statistically removed (Mitchell-Olds and Waller, 1985) or chasmogamous flowers are wholly outcrossed (Schmitt and Gamble, 1990), inbreeding depression is still found. The second type of study corroborating standard inbreeding depression results involves matings between plants at different distances, with nearby matings representing higher levels of inbreeding than distant matings (Price and Waser, 1979; Levin, 1981; Schemske and Pautler, 1984; Redmond et al., 1989; Waser and Price, 1989, 1991; Fenster, 1991;

McCall et al., 1991; but see Lee and Bazzaz, 1982; reviewed by Waser, 1992). Such studies often focus on "outbreeding depression," which presumably results from interactions among loci when dissimilar genomes are combined. In contrast, inbreeding depression results primarily from interactions within loci.

It is also evident from other studies that inbreeding depression can be expressed at any life stage from seed maturation through flower and seed production (Charlesworth and Charlesworth, 1987; Kalisz, 1989). For plants it is not yet clear whether inbreeding depression tends to increase through the life cycle. Such a pattern might be expected if selfing disrupts developmental canalization (Kalisz, 1989). Increasing inbreeding depression with age, however, could result simply from the way it is measured. For example, before reproductive age, selfed and outcrossed offspring may differ in size, but not survival. There will thus be inbreeding depression in the continuous character (size), but not in the threshold character (survival). Accordingly, in the two *Lobelia* species studied here, inbreeding depression tended to be greater in flower number than in survival.

*Seed Number and Size.*—In this study selfed and outcrossed flowers produced seeds of indistinguishable number. At least five other self-compatible species showed no effect of inbreeding on seed number: *Costus guanaiensis* (Schemske, 1983), *Gilia achilleifolia* (Schoen, 1983), *Astragalus linifolius*, *A. lonchocarpus* (Karron, 1989) and the highly selfing *Phlox cuspidata* (Levin, 1989).

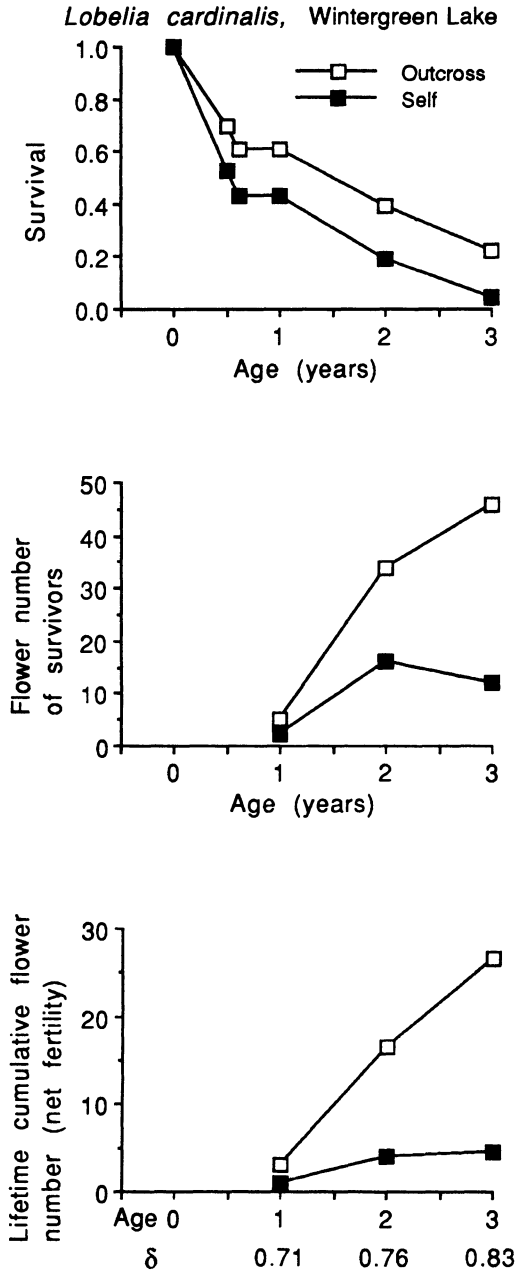


FIG. 1. *Lobelia cardinalis* of Wintergreen Lake: age and the performance of selfed and outcrossed progeny grown in the parental field sites. Age 0 is the mature seed, age 1 is flowering at year 1, etc. The top graph shows survival ( $l_x$ ) schedules, and the middle graph shows flower number of survivors including nonflowering plants ( $b_x$ ). The levels of inbreeding depression in survival and flower number are given in Tables 2 and 3, respectively. The bottom graph shows net fertility ( $\sum l_x b_x$ ), or the cumulative number of flowers produced by a seed through age  $X$ . Levels of inbreeding depression in net fertility are presented below the abscissa, and the greenhouse levels are presented in the text.

FIG. 2. *Lobelia cardinalis* of Glass Creek: age and the performance of selfed and outcrossed progeny grown in the parental field sites. See Figure 1 caption for other details.

In contrast, an effect has been found in *Limnanthes alba* (Jain, 1978); *Delphinium nelsonii* (Price and Waser, 1979); *Costus allenii* and *C. laevis* (Schemske, 1983); wild and cultivated *Phlox drummondii* (Levin, 1984, 1989, 1991); *Erythronium americanum*

(Harder et al., 1985); *Cucurbita foetidissima* (Kohn, 1988); *Allium schoenoprasum* (Stevens and Bougourd, 1988); *Sabatia angularis* (Dudash, 1990); *Crinum erubescens* (Manasse and Stanton, 1991; Manasse and Pinney, 1991); and *Amsinckia grandiflora* (Weller and Ornduff, 1991). Severe reductions in seed production following selfing occur in many gymnosperm species (e.g., Sorensen, 1969; Koski, 1973; Franklin, 1972). The lack of an effect of selfing on seed number in *L. cardinalis* and *L. siphilitica* means that these species are wholly self-compatible and that inbreeding depression is negligible or nonexistent between fertilization and maturation of seeds. In manually pollinating these flowers, I saturated stigmas with pollen, causing a large increase in seed number over that produced under open pollination (Johnston, 1991a). Such high levels of pollination are a requirement for testing inbreeding depression at the level of seed production: in cases where self pollen is less successful than outcross at reaching the ovules, seed number will be reduced in low-pollination levels, but there will be no reduction in zygote fitness, and therefore no inbreeding depression. It would be interesting to know whether cross type affects seed number with lower levels of pollination.

Inbreeding also had no significant effect on seed size in these two *Lobelia* species (Table 1). In all three populations, however, the selfed seeds were smaller than outcrossed by approximately three percent. Harder et al. (1985), Kohn (1988), Karron (1989) and Dudash (1990) similarly found no effect of inbreeding on mean seed mass, while Kalisz (1989) and Wolfe (1989, *Hydrophyllum appendiculatum*) found a significant decrease, and Stevens and Bougourd (1988) report effects in only some populations. Because seed size has been commonly found to be correlated with early plant growth and can influence later fitness (e.g., Schaal, 1984; Stanton, 1984; Waller, 1984; Wulff, 1986; Mazer, 1987; Kalisz, 1989), it would be especially interesting to know whether these small and nonsignificant seed-mass differences cause significant fitness differences following germination. This question could be answered using seeds with known inbreeding coefficients.

*Germination.*—There was significant in-

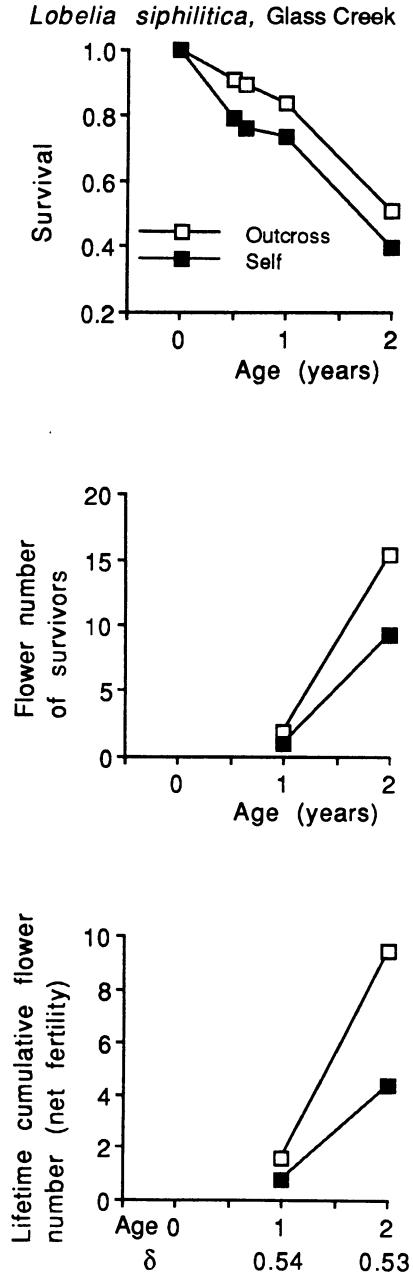


FIG. 3. *Lobelia siphilitica* of Glass Creek: age and the performance of selfed and outcrossed progeny grown in the parental field sites. See Figure 1 caption for other details.

breeding depression in germination rate in *L. siphilitica* and Wintergreen Lake *L. cardinalis*, but not in *L. cardinalis* from Glass Creek (Table 2). The lack of a significant effect in Glass Creek *L. cardinalis* could possibly be attributed to more-favorable ger-

mination conditions in 1989 as compared to 1988. The average germination rate, combined over selfed and outcrossed treatments for Glass Creek *L. cardinalis* in April 1989 (89%) was higher than that for Wintergreen Lake *L. cardinalis* in April 1988 (61%). Because I subjected all seeds to eight weeks of stratification, and these species are perennials, I assume that nongerminating seeds are dead, but there are no relevant data for these two species. Unfortunately, the proneness of water edges to ice and flood damage, combined with very small seed size, made planting seeds directly into the field impractical. Therefore, inbreeding depression in germination may err in the conservative direction. Inbreeding depression in germination has been found in *Thlaspi alpestre* (Riley, 1956), *Thymus vulgaris* (Valdeyron et al., 1977), *Silene vulgaris* (Jolls, 1984), and *Collinsia verna* (Kalisz, 1989), but not in *Sabatia angularis* (Dudash, 1990) or *Clarkia tembloriensis* (Holtsford and Ellstrand, 1990). There are very few studies examining inbreeding depression in germination rate under field conditions: a significant effect was found in *Delphinium nelsonii* (Waser, pers. comm.), in *Costus allenii* (12%) under treefall gaps but not elsewhere, and in neither microenvironment in *C. laevis* or *C. guanaiensis* (Schemske, 1983); similarly, no effect was found in *Gilia achilleifolia* (Schoen, 1983).

*Early Survival.*—While there was no significant difference between selfed and outbred seedlings in survival from germination to six weeks in any population, the differences always favored outbred progeny. Further survival before flowering in both the field and greenhouse was extremely high. This is no doubt a result of early growth in the greenhouse with ample resources, as well as transplanting to perpetually moist soil in the field. (The transplants at Wintergreen Lake in 1988 were thus unaffected by the severe drought that summer.) The annual *Clarkia tembloriensis* similarly suffers little or no inbreeding depression in survival (Holtsford and Ellstrand, 1990), while *Impatiens capensis* (Mitchell-Olds and Waller, 1985) and *Cucurbita foetidissima* (Kohn, 1988) do. Compared to the period from transplanting through flowering, Wintergreen Lake *L. cardinalis* had a much lower survival through winter (Table 2).

*Flower Production.*—When measured, inbreeding depression in progeny flower number, or a correlated indicator of size, has usually been found (Jain, 1978; Kesseli and Jain, 1984; Kalisz, 1989; Wolfe, 1989). There were high levels of inbreeding depression in first-year flower number for greenhouse plants in all populations studied here. Harsher environments probably increase levels of inbreeding depression (see Dudash, 1990), because selfed progeny do proportionally worse than outcrossed progeny. Thus, inbreeding depression in first-year flower number was greater in the field than in the greenhouse for *L. cardinalis*. The opposite was true for *L. siphilitica*. This is probably the result of deer preferentially cropping larger, more-outbred individuals, as well as the relatively shady field environment at Glass Creek causing much smaller plant size. Many field-grown plants did not flower, and the others were smaller than in the greenhouse. Thus, in the “harsher” environment, there was less opportunity for inbreeding depression to be expressed. In the greenhouse all but four (all selfed) of the 244 greenhouse *L. siphilitica* progeny flowered in year one (1989). Under natural conditions, *L. siphilitica* generally produces flowers in its first year, while *L. cardinalis* waits until year two (Bowden, 1959). In all three populations there was a nonsignificantly greater tendency for outcrossed than selfed plants to flower (Table 4). It is difficult to say whether the higher proportion of plants producing flowers in *L. cardinalis* from Wintergreen Lake (0.75) than from Glass Creek (0.31) is a population difference or results instead from the fact that the Wintergreen Lake plants grew in much warmer and somewhat sunnier greenhouse conditions (see Methods). Devlin (1988) found that light stress, applied after flower formation, reduces seed production in *L. cardinalis*.

*Net Fertility.*—In the greenhouse the differences between selfed and outcrossed progeny in survival were augmented by differences in propensity to flower (nonsignificant) as well as differences in flower number (significant). These factors combined to create an inbreeding depression in net fertility, by the end of the first reproductive bout, of 52% in Wintergreen Lake *L. cardinalis*, 51% in Glass Creek *L. cardinalis*

and 51% in Glass Creek *L. siphilitica*. A similar value was obtained for *L. siphilitica* grown in the field. The lower value (34%) obtained for field-grown Glass Creek *L. cardinalis* is probably unreliable due to the extremely small number of flowering plants. At the Wintergreen Lake field site, first-year inbreeding depression in net fertility (71%) was higher than in the greenhouse (52%) and increased to 76% by flowering in its second year and 83% in the third year. In the annual *Gilia achilleifolia*, germinated and grown in the field, inbreeding depression was 44% in net reproductive rate [ $\sum l_x b_x$ , where  $b_x$  was measured as seed production (Schoen, 1983)].

#### *Evolutionary Consequences*

The levels of inbreeding depression observed in this study suggest that avoidance of inbreeding may be under strong selection in these two species. The commonly stated critical value of 50% is the minimum level of inbreeding depression required to prevent the evolution of selfing when two special conditions hold. First, selfing does not reduce male outcrossing success. If selfing does reduce male success in these populations, then levels of inbreeding depression lower than 50% will prevent the evolution of selfing. The second condition is that selfing and outcrossing are concurrent [the "competing" mode of Lloyd (1979)]. With any level of inbreeding depression below 100%, increased levels of self-fertilization will be favored whenever selfing follows all possible outcrossing ("delayed selfing").

Another proposed factor mitigating the strength of selection against self-fertilization is pollination assurance in pollen-limited situations (Darwin, 1876 p. 441; Baker, 1955; Lloyd, 1979). Seed production is limited by pollen receipt in all three populations (Johnston, 1991a). In these two species staminate and pistillate floral phases do not overlap within flowers, and selfing does not occur without a vector (Trelease, 1879 for *L. siphilitica*; Johnston, unpubl. data for both species). All selfing is geitonogamous, so there can be no delayed self-pollination. The possibility of self-fertilization evolving as a means of increasing seed production is therefore difficult to assess. Delayed self-fertilization could occur if self-pollen tubes grow down the style more slowly than out-

cross, and therefore all outcrossing that can occur will occur. Such delayed selfing does not exist in Wintergreen Lake *L. cardinalis* (Johnston, 1990). In the absence of delayed self-fertilization, it may be impossible for *Lobelia* to increase the number of selfed seeds (to increase seed production), without decreasing the number of outcrossed seeds beyond the point where average offspring fitness has declined. The levels and causes of self-fertilization in these two species will be presented in a manuscript in preparation.

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