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# NATURAL SELECTION ON FLORAL TRAITS IN TWO SPECIES OF LOBELIA WITH DIFFERENT POLLINATORS

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Abstract.-Using both multivariate and univariate regression techniques, I measured selection acting through female reproductive success in two hermaphroditic species with precise pollen placement but different pollinators: hummingbird-pollinated Lobelia cardinalis and bumblebeepollinated L. siphilitica. Six traits were analyzed in two populations of L. cardinalis and one population of L. siphilitica: flower number, mean number of flowers open per day, inflorescence height, number of days in flower, median-flower date and nectary-stigma distance. In another study it was found that female reproductive success in one population of L. cardinalis was much less pollen limited than in the other two populations, and it was therefore expected that selection of female reproductive traits in this population would be weaker. In the univariate analyses correlations caused nearly all traits to have significant directional selection coefficients. However, in the multivariate analyses no traits in L. siphilitica experienced directional or quadratic selection. Selection acted differently in the two L. cardinalis populations. The less pollen-limited population experienced positive directional selection on flower number and median-flower date, while in the other L. cardinalis population there was positive directional selection on flower number and nectarstigma distance and both positive directional and positive quadratic selection on height. The functional significance of floral traits in these two species and the probable effect of increased sample sizes are discussed.

*Key words.*—Bumblebee, directional selection, hummingbird, inflorescence traits, *Lobelia*, natural selection, phenology, phenotypic correlations, pollen limitation, pollen placement, seed-set, selection coefficient, selection gradient.

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One of the most striking patterns in the angiosperms is the general correspondence of many floral characteristics with the body shape and foraging behavior of their pollinators. These characters include flower size. shape, color and phenology; type and chemistry of reward; and position and temporal function of sex organs. Because pollinator morphologies and behaviors clearly must interact with floral characters to influence the proficiency of pollen receipt and dissemination, an appreciation of the functional role for these characters has a long history (Sprengel, 1793; Müller, 1871, 1883; Darwin, 1876, 1877a, 1877b; Grant and Grant, 1965; Procter and Yeo, 1973; Stebbins, 1974; Faegri and van der Pijl, 1979; Baker, 1983). Despite this appreciation, however, the way natural selection acts on floral traits. through their effects on fertility, remains

largely unknown (see Waser, 1983; Campbell, 1989).

There are two important reasons for understanding the way selection operates in natural populations. First, the intensity and form of selection are critical to evolutionary models investigating gene-frequency change and the balance of evolutionary factors (Fisher, 1930; Wright, 1931; Haldane, 1932), selection and random genetic drift in phenotypic evolution (Lande, 1976a) and the maintenance of heritable genetic variation (Lande, 1976b; Turelli, 1984). Second, discovery of selection on a trait suggests that there may be a functional relationship between that trait and fitness (Johnson, 1976; Lande and Arnold, 1983; Arnold, 1983; Wade and Kalisz, 1990). Only an experimental analysis can then determine how a change in the trait value affects fitness independently of any correlated traits that may not have been included in the selection analysis (Lande and Arnold, 1983; Endler, 1986; Mitchell-Olds and Shaw, 1987; Schluter, 1988; Wade and Kalisz,

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1990). The measurement of natural selection is thus a first step in the analysis of adaptation.

Although several studies have shown a relationship between seed or fruit production and some floral or inflorescence trait (Willson and Rathcke, 1974; Willson and Price, 1977; Willson et al., 1979; Stephenson, 1979; Schemske, 1980a, 1980b, 1984; Waser and Price, 1981, 1983; Bertin, 1982; Galen, 1985; Galen et al., 1987; Bell, 1985; Nilsson, 1988; Gori, 1989; Stanton et al.,1989; Robertson and Wyatt, 1990), only a few have explicitly attempted to measure selection (Stewart and Schoen, 1987; Campbell, 1989; Galen, 1989; Schemske and Horvitz, 1989; Brassard and Schoen, 1990). While each of these studies supports an adaptive role for floral traits, none of them describes how fertility changes for a trait independent of the effects of other floral traits. A multivariate regression determines how fitness changes with trait values, holding the effects of all other included traits constant (Lande and Arnold, 1983; Manly, 1985; Endler, 1986; Mitchell-Olds and Shaw, 1987). When conducted on the natural phenotypic distribution, this technique permits estimation of both directional and nonlinear (quadratic) selection coefficients and provides a direct estimate of the strength of selection on each measured trait, thus suggesting adaptive hypotheses (Wade and Kalisz, 1990).

If selection on floral characteristics is mediated by pollinator abundance and proficiency, then closely related plant species with similar life histories but different pollinators should experience different kinds of selection on floral traits. Furthermore, those traits most important to proficiency of pollen receipt should experience the strongest selection in populations where seed production is limited by pollen availability. In this study I analyze selection through seed production on six floral and inflorescence traits in two sympatric congeners that have similar life histories, complete separation of male and female phases within a flower, and precise pollen placement, but are specialized to different pollinators: hummingbird-pollinated Lobelia cardinalis L. and bumblebee-pollinated L. siphilitica L. (Lobeliaceae). The goal of this study was to measure selection

on floral traits acting through seed number with the hope that the results would suggest functional relationships between traits and female reproductive success.

## Study Species and Populations

Lobelia cardinalis and L. siphilitica are herbaceous, short-lived perennials inhabiting river banks, low woods and lake edges primarily in eastern North America (McVaugh, 1936, 1940a, 1940b; Bowden, 1959, 1960). In the northeastern portion of their range, these species produce flowers on a single racemose inflorescence from the end of July to late September, fruit from late August to mid-October and overwinter as rosettes. The large (4-cm long), deep-red flowers of L. cardinalis are visited only by hummingbirds (Meehan, 1902; Devlin and Stephenson, 1984, 1985, 1987). Thus, east of the Mississippi River successful pollinations depend upon one species, Archilochus colubris (Trochilidae), the ruby-throated hummingbird. The somewhat smaller (3-cm long), azure-blue flowers of L. si*philitica* are visited primarily by bumblebees (Bombus; Beaudoin Yetter, unpubl. data), and hummingbirds ignore them (Meehan, 1902; pers. obs.). Though unreported in the taxonomic literature, malesterile (=female) individuals occur in populations of L. siphilitica in Ohio (Beaudoin, 1984) and southwestern Michigan (pers. obs.).

Floral development and morphology are extremely similar in the two species, and several morphological features determine the location of pollen deposition and collection on the pollinator's body. The flowers are protandrous. Nectar is produced on the ovary at the corolla base, and pollen is shed from the end of a tube formed by the filaments and anthers. The pistillate phase begins as the style grows through the end of this stamen tube and the stigma opens. As a result, a flower's pollen deposition and receipt occur at similar distances from the nectary.

This study was carried out at two sites in southwestern Michigan, Wintergreen Lake in the Bird Sanctuary of Kellogg Biological Station (Kalamazoo Co.) and Glass Creek, a streamside forest 40 km to the north (Barry Co.). L. cardinalis occurs on the edge of Wintergreen Lake, while both species grow along Glass Creek. Compared to the Glass Creek site, the plants at Wintergreen Lake are much denser, less clumped, and less shaded. In addition, Wintergreen Lake has an extremely large and dense population of Impatiens capensis, which is in peak flower production at the beginning of September during the hummingbird migratory season. Seed number was pollen limited in all three populations in 1988 (Johnston, 1991). As suggested by the habitat differences, seed set in L. cardinalis is much less pollen limited at Wintergreen Lake than at Glass Creek. For both species at Glass Creek, naturally pollinated plants produce about 35% as many seeds as supplementally (hand-) pollinated plants, while at Wintergreen Lake, naturally pollinated L. cardinalis produce about 80% as many seeds as supplementally pollinated plants. Because this site difference in degree of pollen limitation follows expectations based on the habitat differences, it seems reasonable to assume that it persists. Therefore, any floral traits directly influencing the ability of L. cardinalis plants to acquire pollen are expected to be under stronger selection at Glass Creek than at Wintergreen Lake. Male-sterile plants are rare in this population of L. siphilitica and are not included in this analysis.

Traits Analyzed.-In this study I measured maternal fitness as seed number per plant and analyzed the following six traits: total flower number, mean flowers per day (equals mean number of flowers concurrently open), plant height, flowering span (equals total number of days with at least one pistillate-phase flower), median-flower date (equals date that a plant's median flower entered the pistillate phase) and nectarystigma distance. The differences in type and availability of pollinator lead to general expectations about the way selection might act in these three populations. Flower number is expected to be the trait most closely related to seed number. When seed production is pollen limited, pollinators favor larger floral displays (Geber, 1985) or taller plants (Galen, 1989), then these traits will also be under positive directional selection.

In species such as these, where flower maturation occurs from the base upward along the inflorescence, the total number of days a plant is in flower (flowering span) will be correlated with flower number and therefore seed production. Devlin and Stephenson (1984) have shown, however, that increased stigmatic pollen loads in L. cardinalis reduced the pistillate phases of individual flowers and increased the pace of flower opening and closing. Therefore, all else (such as flower number) being equal, it is expected that the most fecund individuals will actually have a shorter total flowering period, resulting in negative directional selection. This effect should be more pronounced in more pollen-limited populations. The time that a plant flowers relative to the rest of the population is also expected to influence its reproductive success. The date that a plant's median flower enters the pistillate phase is one measure of that plant's location in the population flowering period. This trait could influence seed production if pollen is more available at a time other than the population's average median-flower date. Such a situation could arise if, for example, 1) pollinator abundance depends more upon another plant species that flowers at a different time, or 2) pollinators are most abundant or pollen is most available when the population flower number reaches its peak, and this peak does not correspond with the average median-flower date.

The distance from the nectary to the end of the stigma should also influence the efficiency of pollen receipt in pollen-limited populations. In both species of Lobelia, pollinators enter the floral tube for only a short time. Pollen is therefore confined to a strip on the hummingbird's head (L. cardinalis) or the bumblebee's dorsal surface (L. siphi*litica*). This in-and-out motion can be contrasted with pollinator behavior at less tubular flowers in which pollinators move around to a greater extent, resulting in pollen being placed on less specific regions of the body. For any individual pollinator, the length of this pollen patch will be determined by how far it continues to insert itself after first contacting the end of the stamen tube. The location of the patch, however, will be determined by the nectary-stigma distance. To the degree that an individual pollinator inserts itself in the same manner with each flower visit, there should be stabilizing selection on the nectary-stigma distance: flowers with extreme nectary-stigma

distances will contact the pollinator's pollen patch less efficiently. In addition, flowers with smaller-than-average nectary-stigma distances should suffer a greater decrement in pollen receipt than flowers with greaterthan-average distances, simply because no amount of insertion allows full contact with the pollen patch. Bumblebees use their legs to brush pollen from their backs and thus probably disperse the pollen over an increased area of their bodies. The location of this patch could therefore be less important to efficient pollen receipt in L. siphilitica than in L. cardinalis. As in the cases of mean flowers per day, plant height, total flowering span and median-flower date, if nectary-stigma distance affects the proficiency of pollen receipt, it will experience the strongest selection in the most pollenlimited seasons or populations within a species.

#### MATERIALS AND METHODS

Trait and Fitness Measures. - Each of the three populations was censused at three- to five-day intervals during the flowering period in 1987. At each census the sexual state of every flower was recorded as staminate, between sex phases, pistillate or post-anthesis. Due to the large number of individuals sampled, it was not always possible to obtain exact phenological information. Therefore, because flowers mature acropetally on a single raceme, I used linear regression of date on floral position to estimate the dates that the first and median flowers entered the pistillate phase, and the date the final flower terminated its pistillate phase. Number of pistillate days was the difference between these final and first dates. The number of open flowers was averaged across censuses to yield mean flowers per day. Total flower number was included because it was expected to be correlated with other traits and to explain most of the fitness variation. Plant height was the distance from the ground to the top of the highest flower produced by the plant. Nectary-stigma distance was measured to the nearest 0.1 mm with digital calipers as the straight-line distance from the nectary to the tip of the stigma. Nectary-stigma distance differs significantly among plants (unpubl. data, all flowers measured on nine plants in 1986,

one-way ANOVA, F = 16.9, P < 0.0001). The mean of up to eight measurements was used as the individual's value for this trait.

All fruits with seeds were collected from plants having 12 or fewer flowers. For plants with more flowers, 12 fruits spaced equally along the raceme were collected. Because of the large number of seeds per plant, seed counts were obtained by first determining the mass of a 50-seed sample to the nearest 0.00001 g for each individual. Total seed production per plant was then estimated by the product: (50 seeds/mass of 50 seeds) × (mass of all collected seeds/number of fruits collected) × (number of fruits matured).

Statistical Analyses. - The strength and form of natural selection acting on these six traits were analyzed using the multivariate regression techniques of Lande and Arnold (1983). There are three benefits to this approach: 1) it provides both directional and quadratic descriptors of natural selection; 2) it estimates each character's direct contribution to standardized fitness independently of all other included characters; and 3) the selection coefficients fit directly into equations of evolutionary change of the mean phenotype, and their magnitude can be compared across populations or species (Lande, 1979; Lande and Arnold, 1983). The directional selection gradient,  $\beta_i$ , is the average slope of the selection surface in the plane of character *i*, holding all other included characters constant.  $\beta_i$  is the partial regression coefficient in a linear regression of standardized fitness, w, on all character values,  $z_i$ :  $w = \alpha + \sum \beta_i z_i$ , where  $z_i$  has been arithmetically transformed to a mean of zero by subtracting the trait mean from each value (to simplify the nonlinear calculations; see below). The standardized fitness of an individual is its absolute fitness (seed number) divided by the population average absolute fitness. It is equivalent to relative fitness in Lande and Arnold (1983), Arnold and Wade (1984) and Endler (1986). Because a linear regression slope depends on the fitness and character distributions, this gradient does not necessarily describe the functional relationship between the character and fitness. Instead, the directional selection gradient describes how much standardized fitness changes for a unit change in character *i*, independent of all other characters, for the fitness and character distributions of the population (see Schluter, 1988).

The quadratic selection gradients,  $\gamma$ , were then obtained by regressing standardized fitness on the characters and all pairwise products of deviations from the mean:

$$w = \alpha + \sum_{i=1}^{n} \beta_i z_i + \left(\frac{1}{2}\right) \sum_{i=1}^{n} \gamma_{ii} z_i^2$$
$$+ \sum_{i=1}^{n} \sum_{i < i}^{n} \gamma_{ij} z_i z_j.$$

For  $\gamma_{ii}$  the sign of each coefficient indicates only whether curvature in the relation between  $z_i$  and fitness is concave downward (negative) or upward (positive), and not necessarily stabilizing or disruptive selection (Mitchell-Olds and Shaw, 1987; Schluter, 1988; Phillips and Arnold, 1989). For  $\gamma_{ii}$  ( $i \neq j$ ), the quadratic selection gradient indicates correlational selection (Lande and Arnold, 1983). The gradients  $\beta$  and  $\gamma$  were multiplied by  $\sigma_i$  (standard deviation of  $z_i$ before selection) and  $\sigma_i \sigma_i$ , respectively, to yield  $\beta'$  and  $\gamma'$ , the standardized selection gradients. This transformation is equivalent to expressing the original individual trait values in units of standard deviations and allows comparisons of the strength of selection on traits of different sizes, populations, or species (Lande and Arnold, 1983). The transformation was performed after the regression, rather than before, to minimize rounding errors (Endler, 1986 p. 184).

I measured fitness and the six characters on 133 individuals at Wintergreen Lake, 117 individuals of L. cardinalis at Glass Creek and 46 individuals of L. siphilitica. As no transformation of the original data could satisfy the assumptions of parametric significance testing (e.g., Sokal and Rohlf, 1981; Lande and Arnold, 1983; Mitchell-Olds and Shaw, 1987), the selection gradients were subjected to the delete-one jackknife procedure Free-Stat (by T. Mitchell-Olds) to test for statistical significance (see Miller, 1964, 1974; Efron, 1982; Wu, 1986). To explore whether nonsignificant selection gradients resulted from either extremely weak selection or merely insufficient sample sizes, I calculated the approximate minimum sample size required to achieve statistical significance at the  $\alpha = 0.05$  level as  $(st/\phi)^2$ , where  $\phi$  is the estimated selection gradient, s is the standard deviation of the jackknifed estimates of  $\phi$ , and t = 1.96. This method assumes that s will not change with a further increase in sample size.

Most selection estimates have been conducted on single characters (Endler, 1986) and therefore include the indirect effects of selection on any correlated characters. To investigate how omission of correlated characters would influence estimates of natural selection, I also conducted the analyses on each character individually. Linear and nonlinear gradients ( $\beta'$  and  $\gamma'$ ) were calculated as in the multivariate case. The directional selection differential,  $s_i$ , is the amount by which selection shifted the character mean between the actual and potential parents within a generation and was calculated as  $Cov(w, z_i)$ . The standardized differential,  $s_i'$ , was calculated as  $s_i/\sigma_i$  and is also known as the intensity of selection, i (Falconer, 1981 p. 174). The nonlinear selection differential,  $C_i$ , expresses the change in variance due to selection, excluding that portion resulting from directional selection.  $C_i$  was calculated as Cov(w,  $z_i^2$ ), and  $C_i'$  as  $C_i/\sigma_{i}^2$ .  $C_i'$  should not be confused with j, the standardized change in variance due to selection (Endler, 1986 p. 171), which includes the effects of directional selection. To help elucidate the source of any differences between the univariate and multivariate results, I used the CORR procedure of SAS to obtain the correlations among all traits and fitness for each population. Significance levels were determined with a delete-one jackknife.

#### RESULTS

Trait Means. — Means of trait and fitness measures for L. siphilitica and L. cardinalis are presented in Table 1. The values for L. cardinalis of Wintergreen Lake were greater than those of L. cardinalis of Glass Creek for seed number, flower number, fruit number per flower, mean flowers per day and nectary-stigma distance. Mean plant height, flowering span and median-flower date did not differ between the two populations. In both species, flower number, mean flowers per day, height and flowering span were all highly correlated among themselves and with seed number (Tables 2–4). In addition,

TABLE 1. Trait means and standard deviations of Lobelia cardinalis (two populations) and L. siphilitica in this
selection study. Means of L. cardinalis populations not sharing the same letter are significantly different at the
$\alpha = 0.05$ level. The t-test was used for mean nectary-stigma distance, and the Mann-Whitney U-test was used
for all other traits and seed number.

		Lobelia siphilitica				
	Wintergreen L	ake $(N = 133)$	Glass Creek	: ( <i>N</i> = 117)	Glass Cree	ek (N = 46)
	Mean	SD	Mean	SD	Mean	SD
Seed number	11,566 <sup>a</sup>	18,300	4,217 <sup>b</sup>	6,353	1,101	1.629
Fruits/flower	0.79 <sup>a</sup>	0.22	0.48 <sup>b</sup>	0.34	0.38	0.34
Flower number	22.3 <sup>a</sup>	21.0	12.5 <sup>b</sup>	10.4	9.6	8.9
Mean flowers/day	4.93 <sup>a</sup>	3.35	3.69 <sup>b</sup>	1.74	3.34	2.36
Height (cm)	64.9 <sup>a</sup>	27.8	55.7 <sup>a</sup>	27.7	30.6	21.6
Flowering span (days)	24.8 <sup>a</sup>	11.8	21.9 <sup>a</sup>	12.2	17.4	11.2
Median-flower date	8-12-87 <sup>a</sup>	10.1	8-13-87 <sup>a</sup>	8.63	9-4-87	6.34
Nectary-stigma distance (mm)	32.8 <sup>a</sup>	1.53	33.8 <sup>b</sup>	2.18	17.5	1.23

seed number of *L. cardinalis* was negatively correlated with median-flower date in both populations.

Multivariate Analyses. - In both populations of L. cardinalis, flower number was found to be under positive directional selection, and each standard-deviation increase in flower number added slightly more than one unit of relative seed production, holding all other included characters constant (Table 5). Median-flower date experienced positive directional selection in L. cardinalis at Wintergreen Lake but not at Glass Creek. On the other hand, both plant height and nectary-stigma distance experienced positive directional selection in this species at Glass Creek but not Wintergreen Lake. Plant height in L. cardinalis at Glass Creek was the only trait in any population to experience quadratic selection (positive). No correlational selection (nonzero  $\gamma_{ij}$ ,  $i \neq$ j) was detected in any population. With all six characters included, no directional or quadratic selection was detectable in L. siphilitica. However, the constant term (yintercept, equal to one) was significant at

the 0.001 level, and the directional and quadratic regression models accounted for 67% and 89% of the variance in standardized fitness, respectively. There was no evidence of ill conditioning from collinearity (Belsley et al., 1980) in any data set.

Univariate Analyses. - Compared to the multivariate analyses, the univariate analyses provided quite different selection coefficients and levels of significance (Table 6). In all three populations every trait experiencing significant selection in the multivariate analyses was also significant in the univariate analyses. In addition, positive directional selection was detected for flower number, mean flowers per day, plant height. and flowering span in all populations. Of these four characters, only plant height in Glass Creek L. cardinalis was found to experience directional selection in the multivariate analysis (Table 5). Two characters, flowering span in Glass Creek L. cardinalis and plant height in L. siphilitica, had nonsignificant quadratic coefficients in the multivariate analyses but significant coefficients in the univariate analyses. The direction of

TABLE 2. Lobelia cardinalis of Wintergreen Lake: Pearson product-moment correlation coefficients for seed production and six floral characters (N = 133). Significance levels were determined with a delete-one jackknife on untransformed data.

	Seed number	Flower number	Mean flowers per day	Height	Flowering span	Median- flower date
Flower number	0.85***					
Mean flowers per day	0.69***	0.82***				
Height	0.61***	0.71***	0.55***			
Flowering span	0.33***	0.44***	0.31***	0.30***		
Median-flower date	-0.21*	-0.35***	-0.39***	-0.47***	-0.26**	
Nectary-stigma distance	0.11	0.16	0.081	0.090	0.17*	-0.042

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001.

	Seed number	Flower number	Mean flowers per day	Height	Flowering span	Median- flower date
Flower number	0.86***					
Mean flowers per day	0.71***	0.85***				
Height	0.69***	0.66***	0.55***			
Flowering span	0.40***	0.52***	0.42***	0.40***		
Median-flower date	-0.29**	-0.34***	-0.30***	-0.25**	-0.38**	
Nectary-stigma distance	0.17	0.090	0.10	0.075	0.11	0.026

TABLE 3. Lobelia cardinalis of Glass Creek: Pearson product-moment correlation coefficients for seed production and six floral characters (N = 117). Significance levels were determined with a delete-one jackknife on untransformed data.

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001.

selection on median-flower date of L. cardinalis at Wintergreen Lake changed from positive in the multivariate analysis to negative in the univariate. Similarly, flowering span in Glass Creek L. cardinalis had a nearly significant (P < 0.10) negative directional coefficient in the multivariate case and a significant positive one in the univariate.

Minimum Sample Sizes. — Assuming that the statistically nonsignificant selection gradients were real, I estimated the minimum sample sizes required to bound these away from zero (Table 7). These values were less than 500 for all six directional gradients in L. siphilitica, for five in Glass Creek L. cardinalis and for three in Wintergreen Lake L. cardinalis. Significant negative quadratic selection on nectary-stigma distance and mean flowers per day, consistent with stabilizing selection, would be detected in Glass Creek L. cardinalis with 221 and 266 plants. respectively, while positive quadratic selection on median-flower date would be detected with 191 individuals. Minimum sample sizes for negative quadratic selection in Wintergreen Lake L. cardinalis were all large.

### DISCUSSION

Populations of L. cardinalis.-Positive directional selection on flower number occurred in both populations. Within seasons, flower number will probably always be under positive selection and so should be considered a major component of fitness. The date that a plant's median flower entered the pistillate phase was the only trait found to be under selection in Wintergreen Lake but not Glass Creek L. cardinalis. At Wintergreen Lake, plants whose median flowers became pistillate later in the season produced more seeds, all else being equal. This occurred despite bigger plants having earlier median-flower dates (Table 2). Positive directional selection on median-flower date at Wintergreen Lake could have resulted from greater hummingbird activity later in the season, when the much more abundant, hummingbird-pollinated Impatiens capensis is in peak flower production.

Two characters expected to influence pollen-receipt proficiency experienced positive directional selection in *L. cardinalis* at Glass Creek but not at Wintergreen Lake: nectary-

TABLE 4. Lobelia siphilitica of Glass Creek: Pearson product-moment correlation coefficients for seed production and six floral characters (N = 46). Significance levels were determined with a delete-one jackknife on untransformed data.

<u> </u>	Seed number	Flower number	Mean flowers per day	Height	Flowering span	Median- flower date
Flower number	0.79***					
Mean flowers per day	0.69***	0.81***				
Height	0.60***	0.62***	0.50***			
Flowering span	0.42**	0.57***	0.40**	0.42**		
Median-flower date	-0.066	-0.031	0.14	0.0064	-0.39**	
Nectary-stigma distance	0.089	0.26	0.24	0.069	0.29*	0.044

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001.

TABLE 5. Multivariate selection gradients (regression coefficients) for six floral and inflorescence characters in *Lobelia cardinalis* and *L. siphilitica.* Selection gradients express the rate of change of standardized fitness with the character ( $\beta'$ ) and with squared deviations from the character mean ( $\gamma'$ ), holding all other characters constant. All coefficients are in units of character standard deviations. Significance levels were determined with a delete-one jackknife procedure on untransformed data.

		Lobelia ca	rdinalis		Lobelia	siphilitica
	Winterg	reen Lake	Glass (	Creek	Glas	s Creek
	β'	γ'	β'	γ'	β'	γ'
Flower number	1.35***	-0.49	1.25***	-1.00	0.86	-0.27
Mean flowers per day	0.016	-0.063	-0.14	-0.59	0.30	0.58
Height	0.11	-0.098	0.33***	0.34*	0.27	0.75
Flowering span	-0.050	-0.13	-0.15	-0.075	-0.13	0.62
Median-flower date	0.19*	-0.00080	-0.031	0.19	-0.16	-0.54
Nectary-stigma distance	-0.046	0.015	0.15*	-0.20	-0.14	-0.13

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001.

stigma distance and plant height. Nectarystigma distances much shorter than the population average are expected to be more detrimental to pollen acquisition than those much larger than average, as discussed earlier. Therefore, if plants do not receive enough pollen for maximal seed set, then a premium is placed on proficiency of pollen receipt. The mechanics of pollen deposition and receipt also suggested that nectary-stigma distance should experience stabilizing selection. While this was not found, it is suggestive that the minimum sample size required for a significantly negative quadratic coefficient is 221 in Glass Creek L. cardinalis (Table 7). For both directional and quadratic selection gradients, this difference between populations could simply result from the trait being less variable at Wintergreen Lake (coefficient of variation = 4.7%) than at Glass Creek (6.4%). In the hermaphroditic perennial Ipomopsis aggregata Campbell (1989) found that flowers receiving more powdered dye (a pollen analog) tended to have more exserted stigmas. In a selection analysis of nectar-tube length in Calathea ovandensis, a plant visited by an array of pollinator types, Schemske and Horvitz (1989) found negative directional selection in one of three reproductive seasons. The most efficient pollinator, a shorttongued bee, was much more common during that year than in the others, and the authors hypothesized that it caused selection for short-tubed plants by visiting them preferentially or being more efficient on them.

Like nectary-stigma distance, plant height

experienced positive directional selection at Glass Creek but not at Wintergreen Lake. Positive directional selection will always occur if pollinators favor taller plants in pollen-limited situations. There is some evidence that hummingbirds. but not bumblebees, favor taller Lobelia: in 6 out of 6 visits to clumps of approximately 10 Glass Creek L. cardinalis plants, hummingbirds visited the plant with the highest flower first (pers. obs.). In half of these cases, the tallest plant did not have the most open flowers. In similar observations on L. si*philitica*, bumblebees never visited the tallest plant first in five observations. Unless all plants in a patch are always visited, those visited first within a patch will receive more visits, and therefore more pollen, than those visited later. Plant height at Glass Creek was the only character found to experience quadratic selection in any population. The positive quadratic selection gradient shows that taller plants reaped accelerating gains in seed production, also consistent with the notion that taller plants receive more visits. These linear and nonlinear effects of height on seed set occurred independently of the positive correlation between height and mean flowers per day. The ratio of plant height to flower number is also greater in Lobelia cardinalis of Glass Creek (4.5) than at Wintergreen Lake (2.9, Table 1). While this pattern could result from habitat differences, it is also consistent with natural selection on plant height occurring independently of flower number at Glass Creek. Selection on plant height may be different in species with indeterminant inforescence development.

TABLE 6. Univariate selection coefficients for six floral and inflorescence characters in <i>Lobelia cardinalis</i> (two populations) and <i>L. siphilitica</i> . Selection differentials express a shift in the mean (s') or variance (C) of characters between the actual and potential parents. Univariate selection gradients (regression coefficients)
express the rate of change of standardized fitness with the character ( $\beta$ ) and with squared deviations from the character mean ( $\gamma$ ) and include the effects of
correlated characters. All coefficients are in units of character standard deviations. Significance levels were determined with a delete-one jackknife procedure on
untransformed data.

			Lobelia	cardinalis				Lobelia siphilitica	
	-	Vintergreen Lake			Glass Creek			Glass Creek	
	s', B'	Ċ	٨.	s', B'	Ċ	X	s', B'	C,	7
Flower number	1.35***	3.3***	0.40	1.30***	2.53***	0.20	1.17***	2.24	-0.16
Mean flowers per day	1.09***	2.6	-0.067	1.1***	1.6***	0.49	1.0***	1.5**	-0.0038
Height	0.97***	1.3**	0.73	1.0***	1.1***	1.2***	0.89***	1.4***	0.65**
Flowering span	0.52*	0.28	0.37	0.61**	0.12	$-0.27^{**}$	0.62**	-0.033	-0.42
Median-flower date	-0.33***	-0.49***	-0.048	-0.43***	-0.31**	0.069	-0.098	-0.048	-0.089
Nectary-stigma distance	0.17	-0.16	-0.15	0.25*	-0.16	-0.14	0.13	-0.16	-0.14
* <i>P</i> < 0.05; ** <i>P</i> < 0.01; *** <i>P</i> < 0.	001.								

In one population of *Impatiens capensis*, for example, Brassard and Schoen (1990) found negative directional selection on plant height measured at the time of flower initiation.

Lobelia siphilitica and L. cardinalis.-With all six characters included in the regression model, natural selection was not detected on any trait in L. siphilitica. This lack of selection occurred despite seed production in L. siphilitica being at least as pollen limited as in Glass Creek L. cardinalis. There are at least three possible reasons for this difference between species. First, bumblebees, unlike hummingbirds, actively remove pollen from their bodies. This removal is expected to disperse pollen over a greater portion of the bee's dorsal surface, decreasing the importance of at least one trait, nectary-stigma distance, in the proficiency of pollen receipt. Pollen grooming could also mean that the probability of a visit resulting in fertilization is quite low. Combined with the relative rarity of bumblebee visits, pollen collection by bees could therefore mean that successful fertilizations are not only very infrequent but also highly unpredictable, tending to obscure any relationship between floral traits and fitness. A second potential reason why selection was not detected in Lobelia siphilitica is the presence of male steriles. These female plants produce several times more seeds than the hermaphrodites (Johnston, in prep.). In gynodioecious populations, hermaphrodites are the only pollen producers. Thus, compared to a hermaphroditic population like L. cardinalis, male reproductive success will be relatively more important to the hermaphrodites in gynodioecious populations. Despite pollen limitation of seed set, the characters included in this selection analysis might influence male more than female fitness. Nevertheless, the opportunity for selection through seed production, as measured by the variance in standardized fitness (Arnold and Wade, 1984), is as great in L. siphilitica as in both populations of L. car*dinalis*. The most compelling possible cause for not detecting selection in L. siphilitica is sample size, which sets a lower limit on the selection gradient that can be detected. With only 457 individuals, the intensities of directional selection observed for all six traits would be significant.

TABLE 7	. Estimated	minimum	sample sizes	required	for statistical	significance	$(\alpha = 0.05)$	of multivariat	e se-
lection c	oefficients.					•	. ,		

		Lobelia card	linalis		Lobelia	a siphilitica
	Wintergreen Lake		Glass	Creek	Gla	ss Creek
	β	γ	β	γ	β	γ
Flower number	16	3,410	8	412	73	67.140
Mean flowers per day	66,641	138,130	313	266	241	4.626
Height	378	3,324	30	116	149	486
Flowering span	1,549	1,312	145	4.865	457	708
Median-flower date	121	$46 \times 10^{6}$	1.877	191	156	199
Nectary-stigma distance	1,078	22,673	112	221	188	6.278

Pollen Limitation Pattern with Increased Sample Sizes. - With the sample sizes in this study, the number of significant coefficients in each population did not fit the expectations based on degree of pollen limitation. However, comparisons based on estimates at increased sample sizes are suggestive. If we assume a large but logistically feasible sample size of 500 individuals per population, 8 directional and quadratic coefficients would achieve statistical significance in L. siphilitica, 10 in L. cardinalis at Glass Creek, but only 3 in L. cardinalis at Wintergreen Lake, where seed set is least pollen limited (Table 7).

lection coefficients.

Multivariate Versus Univariate Analyses.-The effect of including several characters in a selection analysis is demonstrated by the contrast between univariate and multivariate coefficients (Tables 5 and 6). Flower number, floral display, plant height and total flowering span were all positively intercorrelated in both species (Tables 2 through 4). Because flower number explains most of seed-set variation, any trait significantly correlated with it will also show directional selection in a univariate analysis. In general, a univariate approach will almost always overestimate or underestimate selection coefficients, depending on the particular pattern of phenotypic correlations. Of the 18 estimated directional coefficients (6 per population), none lost significance in the univariate analysis, 5 remained significant, 3 remained insignificant, 9 gained significance and 1 changed sign. This trait, median-flower date in Wintergreen Lake L. cardinalis, was negatively correlated with both seed production and flower number. Thus, larger plants flowered earlier, which therefore resulted in a negative directional

coefficient in the univariate case. With flower number held constant, however, medianflower date was found to experience positive directional selection. For the quadratic analyses, no traits lost significance in the univariate case, one remained significant, 15 remained insignificant and two gained significance.

While this multivariate selection analysis suggested several functional hypotheses, it remains difficult to assess the importance of the selection coefficients, for two reasons, First, it is not known how selection acts on these characters in other reproductive seasons or through survivorship and male fertility. Second, even the extremely weak selection coefficients may be quite important, because if they are sustained over long time periods, they can result in large phenotypic changes (Lande, 1976a). In particular, we would like to know whether selection is consistent in both magnitude and sign in different reproductive seasons. If not, then the average amount of natural selection could be quite different from that experienced at any particular time. The most illuminating studies in the future will include more than one reproductive season, survival across seasons and both female and male fitness. This information will allow an understanding of how floral traits independently influence male and female reproductive success as well as how selection acts on viability and fecundity.

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