WILEY

Male and Female Pollination Success in a Deceptive Orchid, a Selection Study
Author(s): Lisa M. O'Connell and Mark O. Johnston
Source: *Ecology*, Vol. 79, No. 4 (Jun., 1998), pp. 1246-1260
Published by: Wiley
Stable URL: http://www.jstor.org/stable/176740
Accessed: 30-05-2016 16:14 UTC

REFERENCES

Linked references are available on JSTOR for this article:
http://www.jstor.org/stable/176740?seq=1&cid=pdf-reference#references_tab_contents
You may need to log in to JSTOR to access the linked references.

MALE AND FEMALE POLLINATION SUCCESS IN A DECEPTIVE ORCHID, A SELECTION STUDY

LISA M. O'CONNELL[1] AND MARK O. JOHNSTON

Department of Biology, Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada

Abstract. We carried out phenotypic selection analyses to assess the relative importance of several floral traits, as well as microhabitat, in determining both male and female pollination success in a nonrewarding orchid (*Cypripedium acaule* Ait.) in two Nova Scotia populations. Fruit production in these one-flowered, cosexual plants was strongly pollen limited within a season: 100% of individuals set fruit following hand-pollination, but only 5% and 13% did so under natural circumstances in the two populations. Male and female reproductive success were highly correlated, with >90% of plants that received a pollinium also dispersing one. Despite very low visitation rates, the chance of a removed pollinium fertilizing another ovary was 36% and 51%, respectively, in the two populations. There was no evidence for selection acting differently, either through the two sexual functions or in the two populations. In contrast, the strength of directional selection on several traits differed among microhabitats within each population. Univariate and multivariate regressions indicated statistically significant directional selection acting on flower-opening date through male, female, and total (combined) pollination success in one population. For female, male, and total success in this population, we also detected correlational selection acting on opening date and flower height. There was also negative correlational selection acting on flower height and labellum length through female success. Microhabitats within the populations had a larger effect on both female and male success than did floral traits. In particular, the presence of ericaceous shrubs and an open canopy appeared to be closely linked with higher pollination rates.

Key words: Bateman's rule; correlational selection; Cypripedium; deceit pollination; flower size; natural selection; Orchidaceae; phenology; plant height; pollen limitation; selection coefficient.

INTRODUCTION

The intuition that natural selection favors those floral dimensions and characteristics that result in precise pollen transfer is supported by at least two general patterns. First, most floral traits display less variation within a species than do vegetative traits (Berg 1960), and second, pollinator types are clustered with floral forms into "syndromes" (Proctor and Yeo 1972, Faegri and van der Pijl 1979). Underlying this view is the concept that floral characters strongly influence the quantity of pollen removed or deposited in a single visit. If this is correct, then it should be possible to detect statistically significant selection acting on floral characters in natural populations. The accumulation of such analyses will reveal what kinds of traits are most important in plants having different pollinators, flower numbers, floral life-spans, and life histories.

The effects of floral traits on pollination success or seed production have been investigated in many species (references in Waser 1983a, Galen 1995, Wilson 1995). The use of regression techniques expresses these ef-

fects as selection coefficients (Lande and Arnold 1983). Such techniques are often useful because the coefficients provide (1) estimates of direct selection as well as indirect selection acting through phenotypically correlated traits; (2) estimates of both linear (directional) and nonlinear (e.g., stabilizing, disruptive, correlational) forms of selection; and (3) estimates that can be expressed in terms of standard deviations and, hence, can be compared among populations, species, or studies. Several selection studies have been conducted and have tended to find that many floral traits experience no detectable selection, whereas other traits experience rather weak selection (Galen and Newport 1987, Campbell 1989, 1991, Schemske and Horvitz 1989, Campbell et al. 1991, Johnston 1991a, Andersson and Widén 1993, Gómez 1993, Herrera 1993, Wilson 1995). Although the discovery of large selection coefficients would support the hypothesis of a strong functional relationship between trait values and fitness, weak selection neither supports nor weakens the hypothesis. In fact, weak selection is expected to occur whenever phenotypic variance is low, even in the presence of a strong functional relationship. Low phenotypic variation, then, will often be expected in characters that are closely related to fitness (Fenster 1991). In such cases, factors other than phenotypic characters, notably microhabitat, will be expected to have a larger influence on

individual fitness (e.g., Johnson and Bond 1992, Herrera 1993). Differences in direction and intensity of viability and fecundity selection have been related to environmental variations such as light level and soil moisture (Stewart and Schoen 1987). In plants with pollen-limited flowers, differences in pollinator assemblage or abundance due to microhabitats can be associated with differences in reproductive success (Dafni 1983, Campbell 1987, Johnson and Bond 1992, Laverty 1992, Alexandersson and Ågren 1996).

Orchids are particularly suitable for measuring both female and male reproductive success at the pollination stage because pollen is dispersed in discrete packets (pollinia), the receipt or removal of which is easily determined. Male reproductive success is ultimately the number of seeds sired, and removed pollen does not necessarily make it on to a stigma of a conspecific flower. However, studies of species with pollinia have shown a positive correlation between pollinia removal and deposition or seeds sired; therefore, pollinia removal can serve as an estimate of male success (Broyles and Wyatt 1990, Nilsson et al. 1992). Species number and diversity of floral morphology in orchids are often attributed to their close association with a specific pollinator (Nilsson 1992, Steiner et al. 1994, but see Dressler 1993). It is estimated that of the 18 000 to 30 000 species of orchids, nearly one-third offer no pollinator reward in the form of nectar or pollen (Gill 1989). Many such nonrewarding (also termed "deceitful" or "deceptive") species probably induce visits by inexperienced pollinators (Nilsson 1980, Ackerman 1981, 1986, Fritz 1990), and other species mimic pollinator mates (Borg-Karlson 1990). Reproductive success in both rewarding and nonrewarding orchids is often limited by pollinators (Firmage and Cole 1988, Johnson and Bond 1992, Burd 1994), and nonrewarding orchids are well-known for their low pollination success rate in nature (e.g., Thien and Marcks 1972, Ackerman 1981, Nilsson 1992, Steiner et al. 1994). The number of visits an individual pollinator makes to flowers of a nonrewarding species is expected to decline with experience. During the learning period, nonrewarding flowers will probably be visited most often when they are near large numbers of rewarding flowers (Dafni 1983, Alexandersson and Ågren 1996). Thus, we expect pollination success in nonrewarding species to be largely determined by spatial and temporal proximity to the more rewarding species. We expect that, if fruit set is limited by pollen rather than resources, then traits involved in pollen acquisition should be under strong selection (Haig and Westoby 1988).

In this study, we investigated pollinator-mediated selection acting through both female pollination success (pollen receipt) and male pollination success (pollen removal) on morphological and phenological traits in two populations of the pink lady's slipper orchid, *Cypripedium acaule* Ait. It is a nonrewarding species bearing a single flower. Flower size would experience pos-

itive directional selection if larger flowers attract more pollinators (Young and Stanton 1990), but it could be under negative directional selection if pollen transfer is more efficient in smaller flowers (Galen and Newport 1987, Armbruster 1990). Flower height is expected to be under positive directional selection if taller inflorescences attract more pollinators. If pollinator abundance is highest early in the season, or bees learn to avoid nonrewarding flowers, flowering date would experience negative directional selection. We first investigated pollination success rates and pollen limitation of fruit set and then asked the following specific questions concerning natural selection. (1) What is the total strength of directional and nonlinear selection acting on each character through male, female, and total pollination success, (a) including the effects of correlated traits, and (b) independently of correlated traits? (2) Does microhabitat affect pollination success? (3) Does selection act differently (a) through male and female function, (b) in different microhabitats within populations, or (c) in different populations?

METHODS

Study system

The pink lady's slipper is a long-lived herbaceous perennial that grows in acidic soil in open pine or mixed woods throughout eastern North America (Brackley 1985). Most plants have two basal leaves with a stalk bearing one flower. The rhizome grows only a few millimeters a year, and genetically separate individuals can easily be distinguished. In our study, <1% of plants produced two or three flower stalks and extra pairs of leaves. The flower has a large, sack-like labellum with a longitudinal slit on its upper surface. There are two anthers, each containing a pollinium. The pollinium is not discrete and waxy, as in most orchids, but is a mass of sticky pollen that is usually removed as a unit when touched (Plowright et al. 1980; L. O'Connell, *personal observation*). In Nova Scotia, *Cypripedium acaule* blooms between late May and early July, and individual flowers remain open for ~3 wk, but senesce ≤5 d after pollination (L. O'Connell, *personal observation*).

The pink lady's slipper is pollinated by large queen bumble bees. *Bombus vagans* has been identified as a definite pollinator and *B. borealis* as a probable pollinator in New Brunswick (Plowright et al. 1980). Despite the flower's bright color, conspicuous "nectar guides," and sweet smell, the pink lady's slipper produces no nectar. Once a bumble bee has entered a flower through the labellum slit, the infolded margins ensure that the sole exit point lies upward, at the labellum base. To exit, the bee first brushes against the stigma and then an anther. The mechanics of pollen transfer in *Cypripedium* are fully described by Darwin ([1877]1984:226–232). No flower visitors were observed during this study. *Cypripedium acaule* is highly fertile and often sets 100% of its fruits after hand-

pollination. A 10-yr study of the pink lady's slipper in Virginia (Gill 1989) found 100% fruit set after hand-pollination, compared to <5% naturally. Davis (1986) obtained 75% fruit set after cross-pollination and 70% fruit set after self-pollination. In another study (Primack and Hall 1990), 100% of cross-pollinated flowers and 90% of self-pollinated flowers set fruit. However, fruit production in natural populations is pollen limited throughout North America (Plowright et al. 1980, Davis 1986, Barrett and Helenurm 1987, Gill 1989, Primack and Hall 1990).

We studied two populations of pink lady's slippers near Halifax, Nova Scotia. Hemlock Ravine Park is an area with mixed deciduous and coniferous trees, with parts of the park containing old-growth hemlocks. Long Lake Provincial Park has areas that are densely covered by ericaceous shrubs and younger trees as a result of recent burns.

Pollen limitation

In Hemlock Ravine, 29 flowers were hand-pollinated (18 self-pollinated and 11 cross-pollinated) to test whether current-year fruit set was limited by pollen receipt. The number of hand-pollinated flowers successfully initiating a fruit was compared to fruit set in open-pollinated flowers in each population.

Traits analyzed

Each population was visited six times, at intervals of five or six days, from late May to early July of 1994. In this study, male pollination success was measured as the number of pollinia removed (0, 1, or 2), whereas female pollination success was measured as the number of pollinia received (0 or 1). All flowers that received pollen subsequently produced a fruit.

Seven morphological or phenological traits on 259 individuals at Hemlock Ravine and 248 individuals at Long Lake were studied. Five of the morphological traits were labellum width and length, flower height, stalk length, and the length of the longest leaf. Labellum width and length were measured at their widest and longest part, respectively, with a digital caliper to the closest 0.1 mm. A sixth morphological trait, labellum frontal area, was estimated as (length × width)/2. The length of the flower stalk and plant height were measured to the nearest 0.5 cm at flower senescence, when the flower reached its greatest height while still being open to pollinators. (The flower stalk continued growing at a rate of ~2 cm/wk after the flower was open.) Flower height was the vertical distance from the ground to the base of the flower. Stalk length was the distance along the stalk from the ground to the base of the flower. These two measures differed only when the stalk was not vertical. Leaf length was included as an estimate of vegetative size. Flower-opening date, the sole phenological trait, was measured as the first day that the petals lifted high enough to allow a pollinator to enter the labellum.
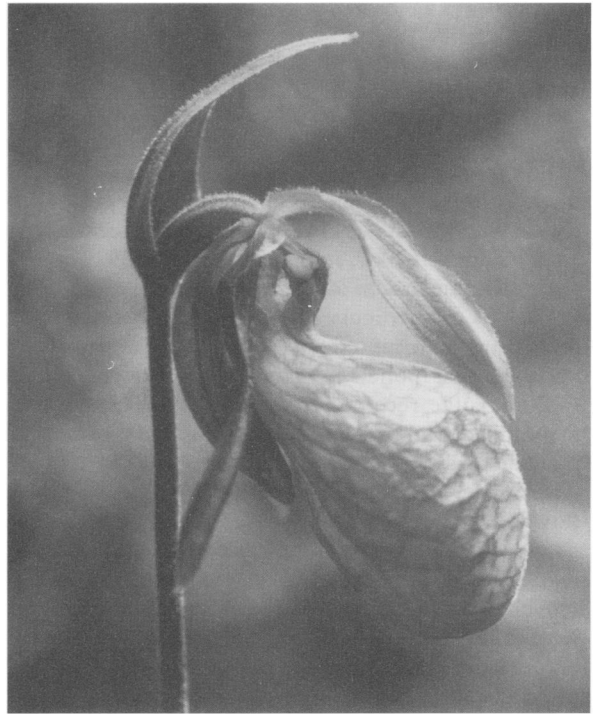

PLATE 1. A pink lady's slipper orchid showing the opening from which bumble bees must exit the flower. An intact pollinium can be seen above the opening.

Microhabitat

Pollinator abundance appeared to vary according to canopy cover as well as identity and density of other species in flower. Within each population, individuals were therefore categorized as belonging to one of four microhabitats characterized by surrounding vegetation and expected to differ in bumble bee abundance: A, areas with dense ericaceous shrubs and no trees; B, areas with dense shrubs and deciduous trees; C, areas with deciduous trees and sparse shrubs; D, areas with deciduous and coniferous trees, but no shrubs. *Cypripedium acaule* flowers in late spring, when deciduous trees have not yet put out their leaves. More sun reaches understory flowers in microhabitats B and C than under coniferous trees in D. Ericaceous shrubs such as blueberry (*Vaccinum* spp.) and huckleberry (*Gaylussacia* spp.) were in flower in late May and early June and attracted a large number of bumble bees (L. O'Connell, *personal observation*).

Statistical analyses

Regression analyses.—Coefficients from a univariate (single-trait) regression estimate the total strength of selection acting on a trait both directly and indirectly through correlated traits. Coefficients from multivariate (multiple-trait) regression estimate the strength of selection acting independently of any potentially correlated traits included in the analysis. When expressed in units of standard deviation, univariate regression co-

efficients are equivalent to selection differentials. We will use "selection differential" to refer to univariate coefficients, and "gradients" to refer to multivariate coefficients (Lande and Arnold 1983). Directional (β') and nonlinear (γ') selection differentials and gradients were estimated using the following regression model:

$$w = \alpha + \sum_{i=1}^{n} \beta_i' z_i' + \frac{1}{2} \sum_{i=1}^{n} \gamma_{ii}' z_i'^2 + \sum_{i=1}^{n} \sum_{j>1}^{n} \gamma_{ij}' z_i' z_j' + \varepsilon$$

(1)

where w is the relative standardized fitness measure, α is a constant, z' is the standardized trait value, and ε is error. Directional differentials and gradients were obtained using the first two terms on the right side of the equation, whereas nonlinear coefficients were obtained using the full model. Directional coefficients were included in the nonlinear analyses to remove any effect of change in mean (due to directional selection) on change in dispersion (Lande and Arnold 1983). Before regression, and separately for pollen receipt and dissemination, each absolute pollination success was divided by the population average to calculate standardized ("relative") fitness, w. To obtain an estimate of the "total" relative pollination success of each plant, we divided the sum of male and female relative success by two (Lloyd and Bawa 1984). Trait values before selection were standardized to zero mean and unit standard deviation. These values were squared and entered into the regression model, yielding $(1/2)\gamma_{ii}'$. All regression coefficients were expressed in units of change in relative fitness per standard deviation, as indicated by the prime symbol, thus facilitating comparison of selection among traits and between populations (Lande and Arnold 1983). As noted, in the case of directional selection, the univariate coefficient, β', represents the regression slope of relative fitness on the standardized character as well as the selection differential, s', which is the shift in the standardized population mean before and after selection. Positive values of γ_{ii}' indicate that the selection surface is concave upwards (disruptive selection), whereas negative values indicate downward concavity (stabilizing selection) (Mitchell-Olds and Shaw 1987, Phillips and Arnold 1989). Values of γ_{ij}' ($i \neq j$) indicate selection favoring positive or negative correlation between the characters i and j. Although logistic regression may be used when fitness has values of 0 or 1 (as for female fitness), least-squares regression is preferred, as it gives essentially identical results and maintains consistency among selection studies (data not shown).

When traits are highly intercorrelated, or important variables are missing from the analysis, selection gradients will not accurately represent selection on a trait (Neter et al. 1983, Mitchell-Olds and Shaw 1987). To avoid problems associated with intercorrelations between morphological traits in multilinear regression, we dropped traits from highly intercorrelated groups (Lande and Arnold 1983). We chose a subset of three characters (opening date, flower height, and labellum length) to be included in the multivariate analyses, for the following reasons: stalk length was highly correlated with flower height; thus, only the latter was included because we would expect pollinators to select for the height of the inflorescence rather than the length of the stalk. Labellum length was chosen, rather than frontal area, because selection differentials for frontal area were not significant in any multivariate analysis. Leaf length was not included in the multivariate analyses because we would expect selection on leaf size acting through pollination success to be indirect.

We wrote programs in BASIC to obtain all multivariate and univariate regression coefficients, as well as the associated 95% CI from 1000 (occasionally 5000) bootstrap iterations (Efron and Tibshirani 1993). For each bootstrap iteration, we randomly sampled observations with replacement from the original observations, and calculated a new regression coefficient. Corresponding two-tailed P values were calculated by determining the smaller of the number of bootstrap coefficients greater or less than zero, multiplying by two, and dividing by the number of bootstrap iterations (Edgington 1987, Crowley 1992). For a few traits in the linear multivariate analyses, P values from standard parametric regression fell on a different side of 0.05 than did those from bootstrapping. We therefore used 5000 iterations for bootstrapping all the linear multivariate analyses.

For each trait, we tested whether female and male regression coefficients differed by randomly assigning observations with replacement to each sex function. The proportion of 1000 outcomes in which the difference between the male and female coefficient was greater or less than the original difference was determined. As before, two-tailed P values were twice the smaller of these two proportions. A similar bootstrap procedure was used to test for differences between populations in univariate coefficients.

Chi-square and Monte Carlo simulations.—To test whether female and male pollination success differed among microhabitats, a χ^2 or G test was not suitable, because some table cells contained expected frequencies less than five (Sokal and Rohlf 1981). Therefore, a Monte Carlo simulation was conducted in BASIC by generating data sets having the same number of observations per microhabitat as in the original data. For each microhabitat, the number of observations in each fitness level was then determined by random draws, with probability determined by the fraction of all observations in each fitness level in the original data. The resulting χ^2 value was then compared with that from the original data (Hall and Wilson 1991), and P values were calculated after 20 000 iterations as for regression coefficients.

Tukey test.—Mean values for opening date, flower height, and labellum length were compared among microhabitats using a Tukey multiple-comparison test

TABLE 1. Distribution of male and female pollination success in the orchid *Cypripedium acaule* in two Nova Scotia populations, expressed as percentage of the total observations (number of individuals in parentheses). Plants may receive 0 or 1 pollinium and may disperse 0, 1, or 2 pollinia.

Male fitness	Female fitness		
	0	1	Total
Hemlock Ravine			
0	86.5% (224)	0.4% (1)	86.9% (225)
1	7.0% (18)	4.2% (11)	11.2% (29)
2	1.1% (3)	0.8% (2)	1.9% (5)
Total	94.6% (245)	5.4% (14)	100% (259)
Long Lake			
0	77.4% (192)	1.2% (3)	78.6% (195)
1	8.5% (21)	8.9% (22)	17.3% (43)
2	1.2% (3)	2.8% (7)	4.0% (10)
Total	87.1% (216)	12.9% (32)	100% (248)

(SYSTAT 1992. These results were identical to those from bootstrapping).

ANOVA.—To test whether microhabitat explained a significant portion of the variation in fitness through male, female, and total pollination success, we conducted an ANOVA. Variables included in the model were microhabitat, flower opening date, flower height, and labellum length (SYSTAT 1992).

ANCOVA.—To determine whether the direction or strength of selection differed among microhabitats, we conducted a separate analysis for each of the seven original traits. To test whether slopes differed among microhabitats, we calculated the F ratio for the interaction between microhabitat and the trait by using the procedure in Zar (1974:300). We calculated the F ratio for 1000 randomly sampled data sets with replacement from the original data. Here, P values were the pro-

portion of bootstrap results in which F exceeded that from the original data set.

Bonferroni corrections.—Because of the large number of correlational and regression analyses on the same data, we performed sequential Bonferroni corrections to reduce the probability of obtaining significant results by chance alone (Rice 1989). For correlations, regression analyses, and ANCOVA, separate corrections were done for each population. For regression analyses, we also performed separate sequential Bonferroni corrections for each sex (male, female, and total).

RESULTS

Male and female pollination success

All 29 hand-pollinated flowers (selfed and crossed) initiated and matured fruits. Among open-pollinated flowers, the percentage receiving a pollinium, all of which produced a fruit, was 5.4% at Hemlock Ravine and 12.9% at Long Lake (Table 1). At Hemlock Ravine, 11.2% of the flowers had one pollinium removed and 1.9% had two removed. At Long Lake, 17.3% had one removed and 4.0% had two removed. Male and female reproductive success were highly correlated in both populations (Pearson r: Hemlock Ravine $r = 0.54$, $P < 0.00001$; Long Lake $r = 0.65$, $P < 0.00001$; Table 2). Of flowers that received pollen, 92% and 91% had at least one pollinium removed at Hemlock Ravine and Long Lake, respectively (Table 1). By dividing the number of pollinia received (the number of flowers with female success) by the number of total pollinia dispersed, we estimated the chance of a pollinium being deposited on a stigma once it has been removed from a flower as 36% at Hemlock Ravine and 51% at Long Lake.

TABLE 2. Pearson product-moment correlation coefficients (r) for morphological and phenological traits, and male and female pollination success in two populations of *Cypripedium acaule*.

Trait	Female success	Male success	Opening date	Stalk length	Flower height	Labellum width	Labellum length	Labellum frontal area
Hemlock Ravine								
Male success	**0.54******							
Opening date	**−0.19****	−0.17**						
Stalk length	0.16**	0.12	−0.01					
Flower height	0.16**	0.14*	−0.13*	**0.77******				
Labellum width	0.058	0.040	−0.087	**0.48******	**0.39******			
Labellum length	−0.073	−0.052	−0.12	**0.53******	**0.42******	**0.75******		
Labellum frontal	0.050	0.029	−0.13*	**0.54******	**0.44******	**0.98******	**0.92******	
Leaf length	0.022	−0.015	0.069	**0.72******	**0.51******	**0.40******	**0.47******	**0.46******
Long Lake								
Male success	**0.65******							
Opening date	−0.15*	−0.12						
Stalk length	0.077	0.089	0.055					
Flower height	0.098	0.12	0.017	**0.89******				
Labellum width	0.020	0.11	0.00026	**0.47******	**0.40******			
Labellum length	0.020	0.15*	0.0045	**0.57******	**0.50******	**0.76******		
Labellum frontal	0.048	0.15*	−0.017	**0.54******	**0.46******	**0.95******	**0.92******	
Leaf length	0.036	0.05	0.050	**0.68******	**0.56******	**0.39******	**0.48******	**0.45******

$* P \leq 0.05$; $** P \leq 0.01$; $*** P \leq 0.001$; $**** P \leq 0.0001$. Statistically significant coefficients after sequential Bonferroni correction are boldface.

TABLE 3. Means and standard deviations of morphological and phenological traits before selection in two populations of *Cypripedium acaule* (all morphometric measures are in cm and frontal area in cm²). *P* values are from *t* tests for difference in means between populations for each trait.

| | Hemlock Ravine | | Long Lake | | |
Trait	*n*	Mean ± 1 SD	*n*	Mean ± 1 SD	*P*
Opening date	259	8 June 1994 ± 4.9 d	248	6 June 1994 ± 3.8 d	<0.0001
Stalk length	258	31.83 ± 6.47	248	33.90 ± 7.9	0.0014
Flower height	258	29.71 ± 8.03	248	32.88 ± 8.02	0.00011
Labellum width	254	2.60 ± 0.260	245	2.71 ± 0.294	<0.0001
Labellum length	259	5.29 ± 0.432	248	5.45 ± 0.499	0.00013
Frontal area	254	6.93 ± 1.16	245	7.45 ± 1.39	<0.0001
Leaf length	241	17.87 ± 3.21	239	17.60 ± 3.68	0.40

Traits means and correlation between traits

In both populations, all of the morphological traits (stalk length, flower height, labellum width, labellum length, labellum frontal area, and leaf length) were intercorrelated (Table 2). Female fitness at Hemlock Ravine was negatively correlated with flower-opening date (Table 2). The values for morphological traits were all greater in Long Lake than in Hemlock Ravine, with the exception of leaf length, which did not differ between the populations (Table 3). Plants at Long Lake also flowered earlier, on average.

Univariate selection analyses

The r^2 values from the univariate linear regression were <0.04 in every case. The selection differentials for flower-opening date were negative and statistically significant for female, male, and total pollination success at Hemlock Ravine (Table 4). At Long Lake, the selection differentials for flower-opening date were also negative for male, female, and total pollination success, although they were not statistically significant. Selection differentials were positive for flower height (or stalk length) at Hemlock Ravine for male, female, and total reproductive success. No directional selection differential was statistically significant in Long Lake. No trait had a significant nonlinear coefficient (γ_{ii} or γ_{ij}) in the univariate analyses.

There were no significant differences between the slopes of male and female univariate regressions (all 14 *P* values >0.2).

Multivariate selection analyses

In all multivariate analyses, the R^2 values were always small (from 0.03 to 0.08). As in the univariate regressions, coefficients for flower-opening date were negative in Hemlock Ravine for female, male, and total pollination success (Table 5). The regression coefficient for flower height was significant and positive for both male and total reproductive success in Hemlock Ravine. The analyses showed no significant regression coefficients for any trait in Long Lake. Correlational selection coefficients (γ'_{ij}) for the interaction between opening date and flower height were negative and significant for female, male, and total success in Hemlock

Ravine. Correlational selection for flower height and labellum length was significant and negative for female success. There was no significant quadratic or correlational selection detected in Long Lake.

Microhabitat

Reproductive success.—At Hemlock Ravine, Monte Carlo simulations revealed a significant difference in female pollination success among microhabitats ($P = 0.009$), but not in male success ($P = 0.13$; Fig. 1a, b). At Long Lake, both male ($P = 0.0004$) and female ($P = 0.002$) pollination success differed among microhabitats (Fig. 1c, d). Figure 1 suggests that both male and female success was highest in microhabitats A and B, which have ericaceous shrubs and an open canopy. Flowers in microhabitat D, under coniferous trees, had both low female and male reproductive success.

A general linear model (ANOVA), including microhabitat as a class variable and opening date, flower height, and labellum length as covariates, showed that microhabitat explained a significant proportion of the variance in pollination success in all cases except male reproductive success at Hemlock Ravine (results not shown). R^2 values remained low in all cases (0.076 to 0.15). In analyses in which microhabitat was statistically significant, the significance levels of the traits included in the analyses generally remained similar to those of the multivariate analyses. However, inclusion of microhabitat rendered labellum length significant for female success at Hemlock Ravine and flower height significant for female success at Long Lake.

Trait values.—Mean opening date differed among microhabitats only in the Hemlock Ravine population (Table 6). In contrast, flower height and labellum length differed among microhabitats only at Long Lake. Here, plants tended to be shorter in A, the microhabitat with the greatest pollination success.

Differences in selection among microhabitats and between populations

Selection measured through male success was indistinguishable among microhabitats in both populations (Table 7). Such relationships between traits and success did differ, however, for several traits acting

TABLE 4. Univariate selection acting through male, female, and total fitness on seven traits in the two populations of *Cypripedium acaule*. Directional (s', β') and nonlinear (γ') selection differentials represent direct effects of traits on pollination success as well as indirect effects of correlated traits.

Trait	n	s', β'	95% CI	γ'	95% CI
Hemlock Ravine, male					
Opening date	259	**−0.47*****	−0.77, −0.19	0.16	−0.23, 0.54
Stalk length	258	0.32*	0.05, 0.62	−0.20	−0.57, 0.14
Flower height	258	**0.38***	0.09, 0.68	−0.04	−0.44, 0.34
Labellum width	254	0.12	−0.31, 0.51	0.11	−0.24, 0.64
Labellum length	259	−0.14	−0.49, 0.20	0.14	−0.14, 0.82
Frontal area	254	0.08	−0.32, 0.43	0.02	−0.32, 0.53
Leaf length	241	−0.04	−0.34, 0.24	−0.23	−0.58, 0.15
Hemlock Ravine, female					
Opening date	259	**−0.81*****	−1.46, −0.35	0.58*	0.001, 1.21
Stalk length	258	**0.68***	0.18, 1.20	0.03	−0.52, 0.72
Flower height	258	0.68*	0.10, 1.25	0.38	−0.15, 1.15
Labellum width	254	0.27	−0.28, 0.83	−0.12	−0.70, 0.49
Labellum length	259	−0.31	−1.02, 0.30	0.46	−0.19, 1.89
Frontal area	254	0.24	−0.29, 0.80	−0.34	−0.73, 0.44
Leaf length	241	0.09	−0.35, 1.73	−0.16	−0.83, 0.66
Hemlock Ravine, total					
Opening date	259	**−0.64*****	−1.03, −0.29	0.37	−0.08, 0.84
Stalk length	258	**0.50***	0.15, 0.88	−0.09	−0.47, 0.40
Flower height	258	0.53**	0.16, 0.92	0.17	−0.23, 0.70
Labellum width	254	0.19	−0.15, 0.59	−0.01	−0.41, 0.49
Labellum length	259	−0.22	−0.71, 0.21	0.30	−0.19, 1.35
Frontal area	254	0.16	−0.22, 0.05	−0.06	−0.49, 0.45
Leaf length	241	0.02	−0.34, 0.36	−0.20	−0.66, 0.33
Long Lake, male					
Opening date	248	−0.24	−0.50, 0.01	0.32	−0.08, 0.87
Stalk length	248	0.18	−0.07, 0.45	0.01	−0.34, 0.33
Flower height	248	0.24*	0.01, 0.48	0.02	−0.33, 0.32
Labellum width	245	0.24*	0.01, 0.49	−0.23	−0.64, 0.17
Labellum length	248	0.30*	0.08, 0.55	0.06	−0.30, 0.46
Frontal area	245	0.32*	0.06, 0.57	−0.24	−0.55, 0.14
Leaf length	239	0.11	−0.21, 0.42	0.21	−0.25, 0.67
Long Lake, female					
Opening date	248	−0.38*	−0.76, −0.38	0.21	−0.20, 0.73
Stalk length	248	0.20	−0.12, 0.54	0.05	−0.34, 0.53
Flower height	248	0.25	−0.10, 0.61	0.05	−0.38, 0.43
Labellum width	245	0.06	−0.25, 0.36	−0.17	−0.75, 0.43
Labellum length	248	0.05	−0.29, 0.42	0.11	−0.34, 0.65
Frontal area	245	0.13	−0.17, 0.48	−0.21	−0.63, 0.37
Leaf length	239	0.10	−0.29, 0.48	0.40	−0.22, 0.88
Long Lake, total					
Opening date	248	−0.31*	−0.60, −0.03	0.26	−0.09, 0.80
Stalk length	248	0.19	−0.08, 0.44	0.03	−0.30, 0.40
Flower height	248	0.24	−0.03, 0.52	0.04	−0.31, 0.40
Labellum width	245	0.15	−0.10, 0.42	−0.20	−0.69, 0.27
Labellum length	248	0.18	−0.10, 0.46	0.03	−0.27, 0.57
Frontal area	245	0.22	−0.02, 0.50	−0.23	−0.55, 0.21
Leaf length	239	0.10	−0.20, 0.41	0.30	−0.24, 0.70

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. Selection differentials that remained significant after sequential Bonferroni correction are boldface.

through both female and total success at Hemlock Ravine (stalk length, flower height, leaf length).

There were no significant differences between the two populations in the selection differentials measured through male, female, and total pollination success (all 21 P values >0.05).

DISCUSSION

Similarity of male and female selection

Examining seven traits in two populations, we found no evidence for a difference between male and female selection coefficients or gradients. A unit (standard deviation) change in a trait value, therefore, changed relative male and female success to the same degree. In both populations, the male and female 95% CI overlapped for all seven directional differentials, all seven nonlinear differentials, all three directional gradients, and all six nonlinear gradients (Tables 4 and 5). This conclusion is supported by explicit tests for differences between male and female directional differentials, which represent the total effects of direct and indirect (through correlated traits) directional selection.

Why does selection act similarly through male and female functions in the pink lady's slipper? The variance in relative fitness (I; Crow 1958) places an upper bound on selection coefficients and can be viewed as the "opportunity for selection" (Arnold and Wade 1984). It is often suggested that I is higher through male function than through female, and that male selection might therefore be stronger. In a California population of honey bee-pollinated wild radish, *Raphanus sativus,* the variance in absolute female fertility exceeded that of estimated male fertility (Devlin and Ellstrand 1990). Likewise, in both populations of orchids studied here, variances in relative fitness were higher through female function than through male function. Female and male values were ~6.9 and 4.2, respectively, at Long Lake, and ~16.5 and 7.0, respectively, at Hemlock Ravine. Thus, there was no less opportunity for selection through pollen receipt than through pollen dispersal. Furthermore, female selection coefficients and gradients, although not significantly different, tended to be greater than male selection coefficients in absolute value. In the case of *Cypripedium acaule,* it seems inappropriate to assume that selection acts on floral characteristics primarily through male function, as is often suggested (e.g., Queller 1983, Bell 1985; see Murcia 1990, Wilson et al. 1994).

A more likely explanation for similarity of male and female selection coefficients is as follows. In the pink lady's slipper and many other single-flowered species with two pollen packets, relative pollination success is constrained to only two levels in the female case (zero and high), and only three in the male case (zero, medium, and high). At the pollination stage, a single pollinator visit will almost certainly result in the removal of one pollinium. Therefore, two visits saturate the male fitness gain curve, and one visit can potentially saturate the female curve. Some plants experience relatively high numbers of pollinator visits, primarily as a result of microhabitat. Thus, both the male and female fitness-gain curves saturate readily in those microhabitats with a high chance, or frequency, of pollinator visits, such as microhabitat A in these Nova Scotia populations. Under such circumstances, male and female success will be positively correlated, as was found. The selection coefficients are similar because most plants receiving any pollinator visits receive a sufficient number to obtain relatively high female and male pollination success.

Selection differences among microhabitats and between populations

Differences in reproductive success among microhabitats can be associated with differences in the strength of selection on a phenotypic trait. In this study, we found that at least two of four microhabitats differed significantly in selection coefficients for several traits. In contrast to these differences among microhabitats within populations, we found no differences between the two populations in the strength of selection (see also Schemske 1984, Galen 1989, Brassard and Schoen 1990, Campbell et al. 1991, Johnston 1991a, Wilson 1995).

Because the pink lady's slipper is a long-lived perennial, selection in one year may not represent selection in another. Disturbances and community succession will alter microhabitat type over time. Stuckey (1967) observed that the pink lady's slipper occurred in larger numbers after brush fires, and that peak blooming was reached 10–15 yr later. Temporally variable selection is well-known in the case of germination date (Kalisz 1986, Miller 1987), and, although we are not aware of statistical comparisons of selection coefficients in different seasons for reproductive traits, some studies have found statistically significant selection coefficients in some years, but not others (Campbell 1989, Scheiner 1989, Schemske and Horvitz 1989, Widén 1991).

In this study, *Cypripedium* plants near ericaceous shrubs, particularly blueberry, appeared to be far more successful than *Cypripedium* plants in other areas. In Long Lake, pink lady's slippers growing in microhabitats A and B had pollination success rates higher than the average for the population. As bumble bees visit the nectar-producing blueberry flowers, they may be tempted to explore the large pink lady's slipper flower. When bumble bees are abundant among blueberry bushes, these exploratory visits may be frequent, leading to a high pollination success rate. Rather than competing with the huckleberry and blueberry flowers, the pink lady's slipper benefits from their proximity. Davis (1986) also observed, in Massachusetts, that there was an increase in the number of pollinator visits to *C. acaule* in the presence of ericaceous shrubs. Pollination of the deceitful orchid *Orchis caspia* also depends on the proximity of nectariferous species (Dafni 1983). The main pollinators of *O. caspia* are several species of bees with an apparently low ability to learn to avoid the deceptive flowers. Laverty (1992) similarly found an increase in fruit set in the nectarless mayapple (*Podophyllum peltatum*) when it was in proximity of nectar-producing lousewort (*Pedicularis canadensis*).

ANOVAs that included microhabitat as well as opening date, height, and labellum length showed that microhabitat significantly explains more variance in pollination success than the floral traits for female fitness in both populations and for male fitness in Long Lake. We therefore agree with Herrera (1993) that environmental conditions may determine fitness to a greater degree than do plant characters themselves.

Pollen limitation and selection

Natural fruit-set in the pink lady's slipper was 5% in Hemlock Ravine and 13% in Long Lake. These low percentages indicate high levels of pollen limitation within a season, as every experimentally and naturally pollinated flower produced a fruit. Pollen limitation

TABLE 5. Multivariate selection on three traits in *Cypripedium acaule* acting through male, female, and total fitness. Linear (β') and nonlinear (γ') selection gradients are the direct effect of traits on relative fitness.

Trait	Male			
	β'	95% CI	γ'	95% CI
Hemlock Ravine (N = 258)				
Opening date	−0.45**	−0.73, −0.18	0.13	−0.23, 0.46
Flower height	0.49***	0.16, 0.86	−0.05	−0.52, 0.44
Labellum length	−0.40*	−0.81, −0.003	0.37	−0.11, 1.02
Date × height			−0.40***	−0.71, −0.13
Date × labellum			0.36	−0.08, 0.75
Height × labellum			−0.29	−0.79, 0.10
Long Lake (N = 248)				
Opening date	−0.25	−0.51, 0.02	−0.11	−0.52, 0.33
Flower height	0.11	−0.16, 0.38	0.26	−0.11, 0.33
Labellum length	0.25	−0.003, 0.53	−0.03	−0.37, 0.35
Date × height			−0.19	−0.48, 0.14
Date × labellum			0.19	−0.21, 0.37
Height × labellum			0.05	−0.20, 0.47

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. Statistically significant coefficients after Bonferroni correction are boldface.

appears to be characteristic of *Cypripedium acaule*, because its presence, or that of low fruit-set, has been documented in many parts of eastern North America (Plowright et al. 1980, Davis 1986, Barrett and Helenurm 1987, Gill 1989, Primack and Hall 1990). A similar range of natural fruit-set occurs in other nectarless orchid species (e.g., Ackerman and Montalvo 1990). Single-season studies of pollen limitation do not demonstrate that lifetime seed production is limited by pollinators, because seed production may have costs expressed in reduced survival or future fertility (Janzen et al. 1980, Calvo and Horvitz 1990). In Massachusetts populations of *C. acaule*, Primack and Hall (1990) found that continual fruit production significantly reduces both leaf area and the likelihood of flowering in the next season. Similar effects have been documented in other orchids (Montalvo and Ackerman 1987, Ackerman 1989, Snow and Whigham 1989, Zimmerman and Aide 1989, Ackerman and Montalvo 1990, but see Calvo 1990).

It has been hypothesized that selection acting through female function should increase as the degree of pollen limitation increases (Johnston 1991*a, b*). Some support for this idea was found in *Lobelia cardinalis*, where the more pollen-limited of two populations showed a higher number of statistically significant standardized selection coefficients, as estimated for hypothesized larger sample sizes. (Of course, because selection acts on relative, not absolute, fitness, this prediction requires some degree of nonlinearity between trait value and female success.) In the present study, all experimentally (and naturally) pollinated plants subsequently produced a fruit. Because the natural levels of fruit-set were ~13% in Long Lake and ~5% in Hemlock Ravine, one can conclude that pollen limitation of fruit production was greater in Hemlock Ravine. We found no significant differences between the two populations in (univariate) selection coefficients; such potential differences were apparently over-

whelmed by microhabitat effects within the populations. Nevertheless, in accordance with the prediction, absolute values of these differentials were larger in Hemlock Ravine than in Long Lake for six of seven coefficients and for all nine multivariate gradients.

Once inside a labellum, a bee is probably very efficient at removing a pollen sac. Of those flowers receiving a pollinium in the present study, 92% (Hemlock Ravine) and 91% (Long Lake) also had at least one removed. Thus, the number of pollen packets removed is probably determined by the number of bumble bees lured into the flowers. A pink lady's slipper pollinium detaches as a large, sticky unit and can adhere to the pollinator until it visits another lady's slipper. The pollinium is viscous and probably remains viable for hours or days after it has been removed from the flower. Pollen removed from two other species of orchids, *Cypripedium reginae* and *Calopogon tuberosus*, and applied to stigmas 8 d later can still initiate fruit production and fertilize a large number of ovules (H. Proctor, *personal communication*). For the pink lady's slipper, the proportion of removed pollinia successfully transferred to another flower appears to be high for a deceptive species (36% in Hemlock Ravine and 51% in Long Lake). This suggests that pollen remains attached to pollinators long enough for successful deposition on a stigma, and that it remains viable for this period, as all flowers receiving pollen subsequently set fruit. The chance of pollen reaching a conspecific stigma can be higher in orchids (20% for *Cymbidiella flabellata* [Orchidaceae; Nilsson et al. 1986]) and milkweeds (25% for *Asclepias syriaca* [Asclepiadaceae; Pleasants 1991]) than in species without pollen packages (3% for *Polemonium viscosum* [Polemoniaceae; Galen and Stanton 1989] and 0.6% for *Erythronium grandiflorum* [Liliaceae; Harder and Thomson 1989]).

Selection on floral traits

Flower-opening date.—The relationship between flowering time and total seed production has been in-

TABLE 5. Extended.

	Female				Total			
β'	95% CI	γ'	95% CI		β'	95% CI	γ'	95% CI
−0.79***	−1.31, −0.31	0.54*	0.01,	1.07	**−0.62*****	−1.01, −0.27	0.34	−0.07, 0.71
0.91*	0.23, 1.64	0.53	−0.18,	1.56	**0.70****	0.22, 1.19	0.24	−0.34, 0.84
−0.78	−1.59, 0.02	1.18*	0.20,	2.64	−0.59*	−1.17, −0.03	0.77*	0.07, 1.78
		−0.79***	−0.51, −0.26				**−0.60*****	−1.04, −0.22
		0.79*	0.06,	1.44			0.57*	0.07, 1.05
		−1.02**	−2.11, −0.25				−0.66*	−1.42, −0.13
−0.39*	−0.77, −0.03	0.09	−0.49,	0.71	−0.32*	−0.61, −0.04	0.23	−0.14, 0.72
0.31	−0.06, 0.68	0.20	−0.24,	0.77	0.21	−0.08, 0.52	0.01	−0.50, 0.45
−0.10	−0.47, 0.29	0.10	−0.34,	0.69	0.07	−0.22, 0.37	0.03	−0.37, 0.55
		−0.15	−0.58,	0.31			−0.02	−0.49, 0.20
		0.02	−0.48,	0.43			0.16	−0.20, 0.51
		0.26	−0.19,	0.70			0.11	−0.28, 0.45

vestigated in several species (e.g., *Fouquieria splendens,* Waser 1979; *Polemonium foliosissimum,* Zimmerman and Gross 1984). In both Hemlock Ravine and Long Lake, earlier flowering plants had the greatest total (male and female) pollination success. There are at least two reasons for this. First, deceptive flowers may have a higher success earlier in the season because pollinators eventually learn to avoid them (Nilsson 1980, Dafni 1984, Fritz 1990). Second, pollinator abundance and, therefore, pollination success, may be correlated with flowering in another species. In these Nova Scotia orchid populations, bumble bee abundance ap-
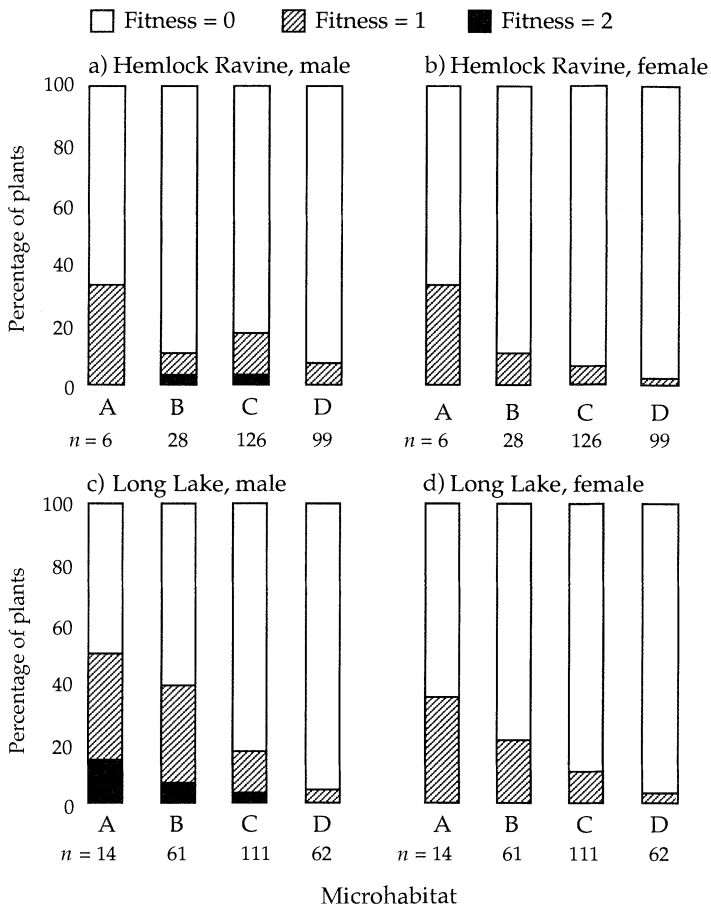

FIG. 1. Percentage of *Cypripedium acaule* plants in each fitness class (0, 1, or 2) in each of the four microhabitats: A no trees, shrubs; B deciduous trees, shrubs; C deciduous trees, no shrubs; and D coniferous trees, no shrubs. *P* values from 20 000 Monte Carlo simulations for differences in reproductive success in different microhabitats are: (a) male fitness in Hemlock Ravine, *P* = 0.13; (b) female fitness in Hemlock Ravine, *P* = 0.009; (c) male fitness in Long Lake, *P* = 0.0004; (d) female fitness in Long Lake, *P* = 0.002.

TABLE 6. Means of three phenotypic traits in two populations of *Cypripedium acaule* in four microhabitats: A, no trees, shrubs; B, deciduous trees, shrubs; C, deciduous trees, no shrubs; and D, coniferous trees, no shrubs. Opening date is the day in June on which flowers opened.

Population	Trait	*P* value	Microhabitat			
			A	B	C	D
Hemlock Ravine	opening date	<0.0001	1.3[a]	9.7[b]	6.5[c]	10.2[b]
	flower height	0.53	32.7[a]	30.8[a]	29.9[a]	29.0[a]
	labellum length	0.16	53.4[a]	52.3[a]	53.4[a]	52.2[a]
Long Lake	opening date	0.16	4.6[a]	5.8[a]	5.3[a]	6.7[a]
	flower height	0.02	27.4[a]	33.0[a,b]	34.0[b]	31.9[a,b]
	labellum length	0.01	52.0[a,b]	54.6[a,b]	55.3[a]	53.2[b]

Note: Means within a row with different superscripts are significantly different from each other at $\alpha = 0.05$ (Tukey multiple-comparison test).

peared to decrease when the pink lady's slippers were at their peak blooming period. Blueberry (*Vaccinum myrtilloides*) and huckleberry (*Gaylussacia baccata*) bloomed during late May and the first two weeks of June, and attracted a large number of bumble bees (L. O'Connell, *personal observation*). This is the period when almost all pink lady's slippers were successfully pollinated (data not shown). Later in June, deciduous trees had flushed their leaves, bumble bee abundance was low, and few pink lady's slippers were pollinated.

The effect of the presence and/or phenology of one species on the pollinator service to another has been documented in both nonrewarding and rewarding plants (Schemske et al. 1978, Thomson 1978, Rathcke 1983, 1988, Waser 1983b, Laverty 1992). On a Baltic island, the highest pollination rate of the nonrewarding *Orchis spitzelii* occurred prior to the population peak in number of open flowers, during the flowering phase of *Arctostaphylos uva-ursi,* the main food plant for bumble bee pollinators (Fritz 1990). At one site where *A. uva-ursi* was absent, fruit-set was lower. Pollination rates of another deceptive orchid, *Calypso bulbosa,*

also corresponded positively with bumble bee abundance (Ackerman 1981). In multiflowered plants, co-ordination of flowering time with pollinator activity does not necessarily increase seed production, even in pollen-limited situations. For example, in a Michigan population of nectariferous *Lobelia cardinalis,* larger plants flowered earlier, as measured by the date that the median flower opened (Johnston 1991a). Thus, ear-lier flowering individuals produced more seeds per plant. Seed number per flower, in contrast, increased at later median-flower dates, as the much more abun-dant hummingbird food plant, *Impatiens capensis,* reached peak flowering. These opposing processes led to a negative selection differential, but a positive se-lection gradient.

If there is selection for earlier opening flowers, why do pink lady's slippers not evolve to open earlier? This species is one of the first flowers to open in late spring. A late frost can destroy buds, flowers, and early-form-ing capsules (Luer 1975). It is also possible that another strong selective pressure not included in the analyses counteracts the effect of selection on early flowering

TABLE 7. Analyses of covariance in univariate selection coefficients among the four microhabitats within two populations of *Cypripedium acaule*. A different analysis was performed for each variable. *F* ratios and *P* values are for the interaction between trait and microhabitat.

Trait	Male		Female		Total	
	F	*P*	*F*	*P*	*F*	*P*
Hemlock Ravine						
Opening date	0.69	0.56	1.15	0.33	1.22	0.304
Stalk length	1.68	0.17	**5.94**	**0.00063**	**5.07**	**0.002**
Flower height	2.22	0.086	**8.05**	**0.00004**	**6.86**	**0.0002**
Labellum width	0.47	0.70	1.02	0.038	0.97	0.41
Labellum length	0.36	0.078	0.81	0.49	0.73	0.53
Frontal area	0.39	0.76	0.53	0.66	0.60	0.61
Leaf length	1.87	0.14	**6.15**	**0.00048**	**5.37**	**0.0014**
Long Lake						
Opening date	1.39	0.29	3.53	0.057	2.53	0.11
Stalk length	1.07	0.34	1.57	0.24	1.61	0.21
Flower height	2.59	0.04	1.50	0.25	2.35	0.087
Labellum width	1.20	0.24	0.30	0.76	0.67	0.50
Labellum length	1.16	0.35	0.34	0.82	0.32	0.81
Frontal area	1.49	0.19	0.44	0.69	0.88	0.43
Leaf length	2.25	0.18	2.44	0.16	2.93	0.085

Note: For the *F* ratios, numerator degrees of freedom are 3, and denominator degrees of freedom are $n - 8$; see Table 4 for sample sizes (n). Values that are statistically significant after sequential Bonferroni correction are boldface.

date. In a multiyear study of *Senecio integrifolius,* Widén (1991) found consistent and strong selection against late flowering, but weak and inconsistent selection against early flowering. Stabilizing selection thus occurred in some years, but not in others. Attempts to measure selection on flowering date in other species have found stabilizing (Augspurger 1981, Schmitt 1983) or inconsistent directional selection that changes from year to year (Primack 1980; see also Gross and Werner 1983, Zimmerman and Gross 1984). Selection at the flowering stage is not necessarily indicative of selection at the stage of seed production or establishment of offspring (Galen 1995). In hummingbird-pollinated *Ipomopsis aggregata,* earlier flowering was associated with greater flower number and seed number, both as expected, but the amount of experimental dye (and presumably pollen) received per flower was higher instead among later flowering plants (Campbell 1991).

Flower height.—Male, female, and total pollination success increased with plant (flower) height at Hemlock Ravine, but not at Long Lake. Microhabitat factors such as soil and sunlight might be expected to have an influence on plant height, creating an apparent causal relationship. There is no evidence for this, however, as plants in the more successful microhabitats at Hemlock Ravine were no taller or shorter than those in other areas. Therefore, the positive association between height and success did not appear to have resulted from microhabitat conditions separately affecting both pollinator abundance and height. At Long Lake, individuals in the best microhabitat, A, had shorter stalks. This negative association between a trait and a fitness-enhancing microhabitat apparently caused two selection directional differentials and all three directional gradients to be indistinguishable from zero (see Rausher 1992). At Hemlock Ravine, height, opening date, and pollination success were correlated. Mulivariate analysis showed that height and opening date each had direct effects on success and remained statistically significant when the other trait was held constant. Correlational selection for early opening and greater height was also detected.

It is unknown whether plant height varies from one year to another. Primack et al. (1994) found that there was a decrease in leaf area in pink lady's slippers that had produced a fruit in the previous year, but they did not indicate whether there was a decrease in plant height or flower size. In this study, leaf size was more highly correlated with stalk length than with floral characters. A shorter plant during one season may be the result of successful fruit production in the previous year.

In its effects on reproductive success, plant height appears to be less well studied than many other traits. Nevertheless, it is known that pollinators often forage at a particular height, causing assortative pollination. In *Lythrum salicaria,* the correlation between heights of successively visited plants exceeds 0.8 (Levin and

Kerster 1973). The sphecid pollinators of the sexually deceptive orchid *Chilogolottis trilabra* prefer flowers within a narrow height range (Peakall and Handel 1993). Because most natural plants are below this height, there may be positive directional selection for height. In many cases, pollinators might consistently favor taller plants because they have more open flowers. For some species, however, it is known that taller plants achieve disproportionately greater seed-set than shorter plants, independently of effects of total flower number or number of open flowers per day (Johnston 1991*a*).

Flower size: labellum length.—"Flower size" is a general term that can include corolla flare, tube depth, and other aspects of petal area. Corolla flare may often act as a signal to pollinators, causing a positive relationship with visitation frequency (Stanton and Preston 1988, Eckhart 1991, Galen 1995, but see Mitchell 1994). Size may often be indirectly selected because pollinators use it as a cue for a correlated trait, such as nectar reward (Cresswell and Galen 1991). It is not yet known whether flowers that offer no reward benefit especially by being large and obvious. Schemske and Ågren (1995) found that bees preferentially visited larger experimental (artificial) flowers modeled after the partially deceit-pollinated tropical herb *Begonia involucrata* (see also Schemske et al. 1995).

Multivariate analysis for Hemlock Ravine showed negative directional coefficients for male, female, and total reproductive success when the effects of opening date and height were held constant (although they were not statistically significant after Bonferroni correction). In this population, no relationship with any of the success measures was apparent in the univariate analysis. Taller plants were both more successful and tended to have a longer labellum. Thus, the negative direct effect of labellum length was masked in the univariate analysis by its positive correlation with height, which was associated with greater success. The negative correlational selection gradient for female success showed that the most successful plants combined greater height with a smaller labellum. The negative relation between labellum length and reproductive success did not result from any tendency for smaller flowers to occur in the best microhabitats. Instead, it is possible that a larger labellum does not constrain a pollinator to brush against a pollinium or stigma with sufficient force to ensure pollen transfer.

Conclusions

In this study, we found selection for earlier opening flowers, taller plants, and smaller flowers. In contrast to the commonly hypothesized antagonism between pollen receipt and dispersal, selection acted similarly through male and female pollination success. These effects of phenology and morphology, however, were overwhelmed by environmental variation. Incorporation of environmental effects on fitness is not necessary

for the quantification of selection, but results in a fuller understanding of the causes of fitness variation and permits a test of the hypothesis that morphological selection is weak because of environmental variation.

LITERATURE CITED

Ackerman, J. D. 1981. Pollination biology of Calypso bulbosa var. occidentalis (Orchidaceae): a food-deception system. Madroño 28:101–110.
———. 1986. Mechanisms and evolution of food-deceptive pollination systems in orchids. Lindleyana 1:108–113.
———. 1989. Limitations to sexual reproduction in Encyclia krugii (Orchidaceae). Systematic Botany 14:101–109.
Ackerman, J. D., and A. M. Montalvo. 1990. Short- and long-term limitations to fruit production in a tropical orchid. Ecology 71:263–272.
Alexandersson, R., and J. Ågren. 1996. Population size, pollinator visitation and fruit production in the deceptive orchid Calypso bulbosa. Oecologia 107:533–540.
Andersson, S., and B. Widén. 1993. Pollinator-mediated selection on floral traits in a synthetic population of Senecio integrifolius (Asteraceae). Oikos 66:72–79.
Armbruster, W. S. 1990. Estimating and testing the shapes of adaptive surfaces: the morphology and pollination of Dalechampia blossoms. American Naturalist 135:14–31.
Arnold, S. J., and M. J. Wade. 1984. On the measurement of natural and sexual selection: theory. Evolution 38:709–719.
Augspurger, C. 1981. Reproductive synchrony of a tropical shrub: experimental studies on effects of pollinators and seed predators on Hybanthus prunifolius (Violaceae). Ecology 62:775–788.
Barrett, S. C. H., and K. Helenurm. 1987. The reproductive biology of boreal forest herbs. I. Breeding systems and pollination. Canadian Journal of Botany 65:2036–2046.
Bell, G. 1985. On the function of flowers. Proceedings of the Royal Society of London: Series B, 224:223–265.
Berg, R. L. 1960. The ecological significance of correlation pleiades. Evolution 14:171–180.
Borg-Karlson, A.-K. 1990. Chemical and ethological studies of pollination in the genus Ophrys (Orchidaceae). Phytochemistry 29:1359–1387.
Brackley, F. E. 1985. The orchids of New Hampshire. Rhodora 87:1–117.
Brassard, J. T., and D. J. Schoen. 1990. Analysis of phenotypic selection among locations in Impatiens pallida and Impatiens capensis. Canadian Journal of Botany 68:1098–1105.
Broyles, S. B., and R. Wyatt. 1990. Paternity analysis in a natural population of Asclepias exaltata: multiple paternity, functional gender, and the "pollen-donation hypothesis." Evolution 44:1454–1468.
Burd, M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. Botanical Review 60:83–139.
Calvo, R. N. 1990. Four-year growth and reproduction of Cyclopogon cranichoides (Orchidaceae) in south Florida. American Journal of Botany 77:736–741.
Calvo, R. N., and C. C. Horvitz. 1990. Pollinator limitation,

cost of reproduction, and fitness in plants: a transition-matrix demographic approach. American Naturalist 136:499–516.
Campbell, D. R. 1987. Interpopulational variation in fruit production: the role of pollination limitation in the Olympic mountains. American Journal of Botany 74:269–273.
———. 1989. Measurements of selection in a hermaphroditic plant: variation in male and female pollination success. Evolution 43:318–334.
———. 1991. Effects of floral traits on sequential components of fitness in Ipomopsis aggregata. American Naturalist 137:713–737.
Campbell, D. R., N. M. Waser, M. V. Price, E. A. Lynch, and R. J. Mitchell. 1991. Components of phenotypic selection: pollen export and flower corolla width in Ipomopsis aggregata. Evolution 45:1458–1467.
Cresswell, J. E., and C. Galen. 1991. Frequency-dependent selection and adaptive surfaces for floral character combinations: the pollination of Polemonium viscosum. American Naturalist 138:1342–1353.
Crow, J. F. 1958. Some possibilities for measuring selection intensities in man. Human Biology 30:1–13.
Crowley, P. H. 1992. Resampling methods for computation-intensive data analysis in ecology and evolution. Annual Review of Ecology and Systematics 23:405–447.
Dafni, A. 1983. Pollination of Orchis caspia, a nectarless plant which deceives the pollinators of nectariferous species from other plant families. Journal of Ecology 71:476–474.
———. 1984. Mimicry and deception in pollination. Annual Review of Ecology and Systematics 15:259–278.
Darwin, C. [1877] 1984. The various contrivances by which orchids are fertilised by insects. University of Chicago Press, Chicago, Illinois, USA.
Davis, R. W. 1986. The pollination biology of Cypripedium acaule (Orchidaceae). Rhodora 88:445–450.
Devlin, B., and N. C. Ellstrand. 1990. Male and female fertility variation in wild radish, a hermaphrodite. American Naturalist 136:87–107.
Dressler, R. L. 1993. Phylogeny and classification of the orchid family. Dioscorides Press, Portland, Oregon, USA.
Eckhart, V. M. 1991. The effects of floral display on pollinator visitation vary among populations of Phacelia linearis (Hydrophyllaceae). Evolutionary Ecology 5:370–384.
Edgington, E. S. 1987. Randomization tests. Second edition. Marcel Dekker, New York, New York, USA.
Efron, B., and R. J. Tibshirani. 1993. An introduction to the bootstrap. Chapman and Hall, New York, New York, USA.
Faegri, K., and L. van der Pijl. 1979. The principles of pollination ecology. Third edition. Pergamon Press, Oxford, UK.
Fenster, C. B. 1991. Selection on floral morphology by hummingbirds. Biotropica 23:98–101.
Firmage, D. H., and F. R. Cole. 1988. Reproductive success and inflorescence size of Calopogon tuberosus (Orchidaceae). American Journal of Botany 75:1371–1377.
Fritz, A. 1990. Deceit pollination of Orchis spitzelii (Orchidaceae) on the island of Gotland in the Baltic: a suboptimal system. Nordic Journal of Botany 9:577–587.
Galen, C. 1989. Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine sky pilot, Polemonium viscosum. Evolution 43:882–890.
———. 1995. The evolution of floral form: insights from an alpine wildflower, Polemonium viscosum (Polemoniaceae). Pages 273–291 in D. G. Lloyd and S. C. H. Barrett, editors. Floral biology: studies on floral evolution in animal-pollinated plants. Chapman and Hall, New York, New York, USA.
Galen, C., and M. E. A. Newport. 1987. Bumble bee behavior

and selection on flower size in the sky pilot, *Polemonium viscosum*. Oecologia **74**:20–23.

Galen, C., and M. L. Stanton. 1989. Bumble bee pollination and floral morphology: factors influencing pollen dispersal in the alpine sky pilot, *Polemonium viscosum* (Polemoniaceae). American Journal of Botany **76**:419–426.

Gill, D. E. 1989. Fruiting failure, pollinator inefficiency, and speciation in orchids. Pages 458–481 *in* D. Otte and J. A. Endler, editors. Speciation and its consequences. Sinauer Associates, Sunderland, Massachusetts, USA.

Gómez, J. M. 1993. Phenotypic selection on flowering synchrony in a high mountain plant, *Hormathophylla spinosa* (Cruciferae). Journal of Ecology **81**:605–613.

Gross, R. S., and P. A. Werner. 1983. Relationships among flowering phenology, insect visitors, and seed-set of four co-occurring species of goldenrod (*Solidago*: Compositae). Ecological Monographs **53**:95–117.

Haig, D., and M. Westoby. 1988. On limits to seed production. American Naturalist **131**:757–759.

Hall, P., and S. R. Wilson. 1991. Two guidelines for bootstrap hypothesis testing. Biometrics **47**:757–762.

Harder, L. D., and J. D. Thomson. 1989. Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. American Naturalist **133**:323–344.

Herrera, C. M. 1993. Selection on floral morphology and environmental determinants of fecundity in a hawkmoth-pollinated violet. Ecological Monographs **63**:251–275.

Janzen, D. H., P. DeVries, D. E. Gladstone, M. L. Higgins, and T. M. Lewinsohn. 1980. Self- and cross-pollination of *Encyclia cordigera* (Orchidaceae) in Santa Rosa National Park, Guanacaste, Costa Rica. Biotropica **12**:72–74.

Johnson, S. D., and W. J. Bond. 1992. Habitat dependent pollination success in a Cape orchid. Oecologia **91**:455–456.

Johnston, M. O. 1991a. Natural selection on floral traits in two species of *Lobelia* with different pollinators. Evolution **45**:1468–1479.

———. 1991b. Pollen limitation of female reproduction in *Lobelia cardinalis* and *L. siphilitica*. Ecology **72**:1500–1503.

Kalisz, S. 1986. Variable selection on the timing of germination in *Collinsia verna* (Scrophulariaceae). Evolution **40**:479–491.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution **37**:1210–1226.

Laverty, T. M. 1992. Plant interactions for pollinator visits: a test of the magnet species effect. Oecologia **89**:502–508.

Levin, D. A., and H. W. Kerster. 1973. Assortative pollination for stature in *Lythrum salicaria*. Evolution **27**:144–152.

Lloyd, D. G., and K. S. Bawa. 1984. Modification of the gender of seed plants in varying conditions. Evolutionary Biology **17**:255–338.

Luer, C. A. 1975. The native orchids of the United States and Canada excluding Florida. New York Botanical Garden, New York, New York, USA.

Miller, T. E. 1987. Effects of emergence time on survival and growth in an early old-field plant community. Oecologia **72**:272–278.

Mitchell, R. J. 1994. Effects of floral traits, pollinator visitation, and plant size on *Ipomopsis aggregata* fruit production. American Naturalist **143**:870–889.

Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. Evolution **41**:1149–1161.

Montalvo, A. M., and J. D. Ackerman. 1987. Limitations to fruit production in *Ionopsis utricularioides*. Biotropica **19**:14–31.

Murcia, C. 1990. Effect of floral morphology and temper-ature on pollen receipt and removal in *Ipomoea trichocarpa*. Ecology **71**:1098–1109.

Neter, J., W. Wasserman, and M. H. Kutner. 1983. Applied linear regression models. Irwin, Homewood, Illinois, USA.

Nilsson, L. A. 1980. The pollination ecology of *Dactylorhiza sambucina* (Orchidaceae). Botaniska Notiser **133**:367–385.

———. 1992. Orchid pollination biology. Trends in Ecology and Evolution **7**:255–259.

Nilsson, L. A., L. Jonsson, L. Rason, and E. Randrianjohany. 1986. The pollination of *Cymbidiella flabellata* (Orchidaceae) in Madagascar: a system operated by sphecid wasps. Nordic Journal of Botany **6**:411–422.

Nilsson, L., E. Rabakonandrianina, and B. Pettersson. 1992. Exact tracking of pollen transfer and mating in plants. Nature **360**:666–668.

Peakall, R., and S. N. Handel. 1993. Pollinators discriminate among floral heights of a sexually deceptive orchid: implications for selection. Evolution **47**:1681–1687.

Phillips, P. C., and S. J. Arnold. 1989. Visualizing multivariate selection. Evolution **43**:1209–1222.

Pleasants, J. M. 1991. Evidence for short-distance dispersal of pollinia in *Asclepias syriaca* L. Functional Ecology **5**:75–82.

Plowright, R. C., J. D. Thomson, and G. R. Thaler. 1980. Pollen removal in *Cypripedium acaule* (Orchidaceae) in relation to aerial fenitrothion spraying in New Brunswick. Canadian Entomologist **112**:765–769.

Primack, R. B. 1980. Variation in the phenology of natural populations of montane shrubs in New Zealand. Journal of Ecology **68**:849–862.

Primack, R. B., and P. Hall. 1990. Costs of reproduction in the pink lady's slipper orchid: a four-year experimental study. American Naturalist **136**:638–656.

Primack, R. B., S. L. Miao, and K. R. Becker. 1994. Costs of reproduction in the pink lady's slipper orchid (*Cypripedium acaule*): defoliation, increased fruit production, and fire. American Journal of Botany **81**:1083–1090.

Proctor, M., and P. Yeo. 1972. The pollination of flowers. Taplinger, New York, New York, USA.

Queller, D. C. 1983. Sexual selection in a hermaphroditic plant. Nature **305**:706–707.

Rathcke, B. J. 1983. Competition and facilitation among plants for pollinators. Pages 309–329 *in* L. Real, editor. Pollination biology. Academic Press, New York, New York, USA.

———. 1988. Interactions for pollination among co-flowering shrubs. Ecology **69**:446–457.

Rausher, M. D. 1992. The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. Evolution **46**:616–626.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution **43**:223–225.

Scheiner, A. M. 1989. Variable selection along a successional gradient. Evolution **43**:548–562.

Schemske, D. W. 1984. Population structure and local selection in *Impatiens pallida* (Balsaminaceae), a selfing annual. Evolution **38**:817–832.

Schemske, D. W., and J. Ågren. 1995. Deceit pollination and selection on female flower size in *Begonia involucrata*: an experimental approach. Evolution **49**:207–214.

Schemske, D. W., J. Ågren, and J. Le Corff. 1995. Deceit pollination in the monoecious, neotropical herb *Begonia oaxacana* (Begoniaceae). Pages 292–318 *in* D. G. Lloyd and S. C. H. Barrett, editors. Floral biology: studies on floral evolution in animal-pollinated plants. Chapman and Hall, New York, New York, USA.

Schemske, D. W., and C. C. Horvitz. 1989. Temporal variation in selection on a floral character. Evolution **43**:461–465.

Schemske, D. W., M. F. Willson, M. N. Melampy, L. J. Miller,

L. Verner, K. M. Schemske, and L. B. Best. 1978. Flowering ecology of some spring woodland herbs. Ecology **59**: 351–366.

Schmitt, J. 1983. Individual flowering phenology, plant size, and reproductive success in *Linanthus androsaceus,* a California annual. Oecologia **59**:135–140.

Snow, A. A., and D. F. Whigham. 1989. Costs of flower and fruit production in *Tipularia discolor* (Orchidaceae). Ecology **70**:1286–1293.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. Second edition. W. H. Freeman, New York, New York, USA.

Stanton, M. L., and R. E. Preston. 1988. Ecological consequences and phenotypic correlates of petal-size variation in wild radish, *Raphanus sativus* (Brassicaceae). American Journal of Botany **75**:528–539.

Steiner, K. E., V. B. Whitehead, and S. D. Johnson. 1994. Floral and pollinator divergence in two sexually deceptive South African orchids. American Journal of Botany **81**: 185–194.

Stewart, S. C., and D. J. Schoen. 1987. Pattern of phenotypic viability and fecundity selection in a natural population of *Impatiens pallida.* Evolution **41**:1290–1301.

Stuckey, I. H. 1967. Environmental factors and the growth of native orchids. American Journal of Botany **54**:232–241.

SYSTAT. 1992. Statistics, Version 5.2 edition. SYSTAT, Evanston, Illinois, USA.

Thien, L. B., and B. G. Marcks. 1972. The floral biology of *Arethusa bulbosa, Calopogon tuberosus,* and *Pogonia ophioglossoides* (Orchidaceae). Canadian Journal of Botany **50**:2319–2325.

Thomson, J. D. 1978. Effects of stand composition on insect visitation in two-species mixtures of *Hieracium.* American Midland Naturalist **100**:431–440.

Waser, N. M. 1979. Pollinator availability as a determinant of flowering time in ocotillo (*Fouquieria spendens*). Oecologia **39**:107–121.

———. 1983a. The adaptive nature of floral traits: ideas and evidence. Pages 241–285 *in* L. Real, editor. Pollination biology. Academic Press, New York, New York, USA.

———. 1983b. Competition for pollination and floral character differences among sympatric plant species: a review of evidence. Pages 277–293 *in* C. E. Jones and R. J. Little, editors. Handbook of experimental pollination biology. Van Nostrand-Reinhold, New York, New York, USA.

Widén, B. 1991. Phenotypic selection on flowering phenology in *Senecio integrifolius,* a perennial herb. Oikos **61**: 205–215.

Wilson, P. 1995. Selection for pollination success and the mechanical fit of *Impatiens* flowers around bumblebee bodies. Biological Journal of the Linnean Society **55**:355–383.

Wilson, P., J. D. Thomson, M. L. Stanton, and L. P. Rigney. 1994. Beyond floral Batemania: gender biases in selection for pollination success. American Naturalist **143**:283–296.

Young, H. J., and M. L. Stanton. 1990. Influences of floral variation on pollen removal and seed production in wild radish. Ecology **71**:536–547.

Zar, J. H. 1974. Biostatistical analysis. Second edition. Prentice-Hall, Englewood Cliffs, New Jersey, USA.

Zimmerman, J. K., and T. M. Aide. 1989. Patterns of fruit production in a neotropical orchid: pollinator vs. resource limitation. American Journal of Botany **76**:67–73.

Zimmerman, M., and R. S. Gross. 1984. The relationship between flowering phenology and seed set in an herbaceous perennial plant, *Polemonium foliosissimum* Gray. American Midland Naturalist **111**:185–191.