

AN ABSTRACT OF THE THESIS OF

Monica Geber _____ for the degree of Master of Science

in Zoology presented on March 11, 1982

Title: Architecture, Size, and Reproduction in plants: A Pollination
Study of *Mertensia ciliata* (James) G. Don.

Abstract approved: _____

Charles E. King

I experimentally test the hypothesis that the potential for selfing increases with plant size in *Mertensia ciliata* (Boraginaceae), a self-compatible, profusely-flowering perennial. This follows from the premises that 1) pollen dispersal by pollinators between flowers is limited, and 2) individual pollinators, in this study bumblebees, will visit more flowers per visit on large than on small plants thereby promoting intra- rather than interplant dispersal of pollen. I show that while *M. ciliata* is self-compatible, outcrossing results in greater reproductive output (seed numbers and seed weight) than selfing. Thus, under the hypothesis above, the reproductive output of flowers should decline with increasing plant size.

I demonstrate, through pollen carryover experiments, that pollen transfer by bumblebees is extensive. Observations of pollinator foraging behavior show that individual bumblebees visit only a few more flowers and stems, and indeed encounter a smaller proportion of a plant's flowers and stems on visits to large than to small plants. Large plants attract more pollinators per minute. I use these results

to predict that flowers on all plants should receive equal amounts of outcrossed and total (self + outcrossed) pollen, and that selfing rates should not differ among plants. This is supported by direct measurements of pollen receipt by flowers, and of the reproductive output of flowers on large and small plants in the field. No differences were found among plants in outcrossed and total pollen receipt, and in seed-set per flower and seed weight.

I examine the pattern of insect visitation in more detail to show that individual bumblebees encounter only a small number and proportion of flowers and stems per visit on all plants, and encounter a smaller proportion on large than on small plants. Individual bees, then, exploit large plants less intensely per visit than small plants. Bees move predominantly between neighboring plants and fly randomly with respect to direction. Many insect visitors are nectar robbers. I propose four factors to explain the short visits of pollinators and the less intense exploitation of large plants by individual bumblebees. These include the complex architecture of the flowering display, the circular geometry and density of stems in plants, the close proximity of plants in the population, and the variance among flowers of plants in nectar reward caused by visits of nectar robbers.

Architecture, Size, and Reproduction in Plants:
A Pollination Study of Mertensia ciliata (James) G. Don

by

Monica Geber

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed 11 March 1982

Commencement 1982

APPROVED:

Professor of Zoology in charge of major

Chairman, Department of Zoology

Dean of Graduate School

Date thesis is presented March 11, 1982

Typed by Suzi Sargent for Monica Geber

ACKNOWLEDGEMENTS

I am grateful to Peter G. Kevan for the use of the Pennsylvania Mountain Research Station, and for his support of my project. I also acknowledge the patience and advice of my committee, Drs. Charles King, Peter Dawson, and Kenneth Chambers. I thank R. Chris Plowright for showing me how to handle bumblebees, for the opportunity of working with him at the University of Toronto, and for many useful discussions on plant architecture. Candi Galen introduced me to Mertensia ciliata, and helped me on many aspects of my research. I have benefited greatly from discussions with William Rice and Nickolas Waser on the design and interpretation of the study. Earlier drafts of the thesis were improved by the criticisms of my committee, Candi Galen, William Rice, Nickolas Waser, and the Zoology Department Ecology Group.

I thank Suzi Sargent for typing the thesis, and John Lucas for making the figures.

The research was supported by a Sigma Xi Grant-in-Aid of research, by the Oregon State University Zoology Department Research funds to the author, and by NSF grant DEB 79-10786 to Peter G. Kevan.

TABLE OF CONTENTS

I.	Introduction	1
II.	Bumblebee Pollination of <u>Mertensia ciliata</u> : The Relationship between Plant Size and Inbreeding	3
	Introduction	3
	Materials	7
	Methods and Results	9
	Breeding system of <u>Mertensia ciliata</u>	9
	Pollen carryover between flowers by bumblebees	11
	Effect of plant size on the receipt of pollen. Pollen deposition in the field	14
	Effect of plant size on the pattern of bumblebee visitation	18
	Effect of plant size on seed-set	21
	Discussion	25
III.	Plant Architecture, Plant Size, and the Cross-Pollination of Profusely-Flowering Plants: The Foraging Behavior of Bumblebees on <u>Mertensia ciliata</u>	31
	Introduction	31
	Materials and Methods	34
	Results	37
	Discussion	40
IV.	Summary and Conclusions	46

V.	Literature cited	49
VI.	Appendix I: Tables and Figures	56
VII.	Appendix II: Tables	86

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1	Outcrossed pollen deposition on emasculated flowers visited in sequence by a bumblebee as a function of the order (i) of a flower in the visit sequence. Circles represent the mean numbers of pollen grains reaching flowers; vertical bars show one standard error around the mean. Data were obtained from fourteen carryover sequences. The line represents the least-squares exponential regression for outcrossed pollen deposition against flower order	64
2	Effect of emascualtion treatments on the receipt of pollen by flowers in the field. The mean number of pollen grains per stigma and one standard error are shown for emasculated (E), clipped (CL), and control (C) plants, of the first and second blocks. Comparisons were made between pairs of means from plants within a given block using Scheffé's method of multiple comparisons (combined $\alpha=0.05$). When the same letter (e.g., a) appears above two means, the means are significantly different from one another	66
3	Effect of plant size on the number of flowers visited per pollinator on a plant. The mean number of flowers visited per bumblebee and one standard error are shown for small (S), medium (M), and large (L) plants of the first and second blocks. Comparisons were made between pairs of means within blocks using Scheffé's method of multiple comparisons (combined $\alpha=0.05$). When the same letter (e.g., a) appears above two means, the means are significantly different from one another	68

- 4 Effect of plant size on the arrival rate of pollinators to plants. The mean number of bumblebees arriving at a plant per minute and one standard error are shown for small (S), medium (M), and large (L) plants of the first and second blocks. Comparisons were made between pairs of means within blocks using Scheffe's method of multiple comparisons (combined $\alpha=0.05$). When the same letter appears above two means, the means are significantly different from one another. 70
- 5 Effect of emasculation treatment and plant size on seed-set per flower. The mean number of seeds per flower and one standard error are shown for emasculated (E), clipped (CL), and control (C) plants of small (S), medium (M), and large (L) sizes in the first and second blocks. Comparisons were made between means of plants of a given size and block and of different emasculation treatment using Scheffé's method of multiple comparisons (combined $\alpha=0.05$). When the same letter (e.g., a) appears above two means, the means are significantly different from one another. 72
- 6 Effect of emasculation treatment and plants size on the weight of seeds. The mean seed weight and one standard error are shown for emasculated (E), clipped (CL), and control (C) plants of small (S), medium (M), and large (L) sizes in the first and second blocks. Comparisons were made between pairs of means from plants of a given size and block and of different emasculation treatments using Scheffé's method of multiple comparisons (combined $\alpha=0.05$). When the same letter (e.g., a) appears above two means, the means are significantly different from one another. 74

Figure

Page

- 7 Effect of plant size on the predicted amount of outcrossed pollen deposited on a flower by a single pollinator (Y_2), and over an hour period by all pollinators visiting the plant (Y_4) (see Discussion for details). 76
- 8 Effect of plant size on the percentage of a plant's flowers visited by a pollinator. The mean percentage of flowers visited per bumblebee on a plant and one standard error are shown for small (S), medium (M), and large (L) plants. Comparisons were made between pairs of means using Scheffé's method of multiple comparisons (combined $\alpha=0.05$). When the same letter (e.g., a) appears above two means, the means are significantly different from one another. 78
- 9 Effect of plant size on the number of stems visited by a pollinator on a plant. The mean number of stems visited per bumblebee on a plant and one standard error are shown for small (S), medium (M), and large (L) plants. Comparisons were made between pairs of means using Scheffé's method of multiple comparisons (combined $\alpha=0.05$). When the same letter (e.g., a) appears above two means, the means are significantly different from one another. 80
- 10 Effect of plant size on the percentage of a plant's stems visited by a pollinator. The mean percentage of stems visited per bumblebee on a plant and one standard error are shown for small (S), medium (M), and large (L) plants. Comparisons were made between pairs of means using Scheffé's method of multiple comparisons (combined $\alpha=0.05$). When the same letter (e.g., a) appears above two means, the means are significantly different from one another. 82
- 11 The distribution of angular changes in direction of bumblebees flying between plants. The distribution does not differ from a uniform distribution when tested with a Kolmogorov-Smirnov one sample test (N = number of flights = 681, $P>0.2$). 84

LIST OF TABLES
Appendix One

<u>Table</u>		<u>Page</u>
1	Design and tests of the breeding experiment on <u>Mertensia ciliata</u> . All five pollination treatments were performed on each of ten plants (sixty flowers/treatment/plant). Plants were considered to be random factors (blocks), and pollination treatments were fixed factors.	56
2	Analysis of seed-set per flower from breeding experiment by 2-way mixed model ANOVA (Plants: random effect; Pollination treatment: fixed effect).	57
3	Mean seed-set, standard errors, and coefficient of variation of means of the five pollination treatments in the breeding experiment. Means are based on ten plants.	58
4	Outcrossed pollen deposition and total (self + outcrossed) pollen deposition on flowers from two emasculated and two non-emasculated pollen carryover runs.	59
5	Summary of ANOVAS on the measures of bumblebee visitation to plants.	60
6	Summary of ANOVAS on pollen receipt by flowers, on seed-set per flower, and on seed weight.	61
7	The pattern of bumblebee pollinator visitation of <u>Mertensia ciliata</u> in 1979 as a function of plant size. Regression equations relate each measure of pollinator visitation (\bar{Y}_i) to plant size (x = no. of stems), except \bar{Y}_2 which is independent of plant size. N = no. of plants from which observations were obtained.	62

TablePage

8	The pattern of insect visitation of <u>Mertensia ciliata</u> in 1979. Regression equations relate each measure of visitation (Y) to plant size (x= no. of stems) and/or distance between a plant and its nearest neighbor (z), (but see remarks a and c). Regression equations were computed separately for a) bumblebee pollinators, b) bumblebee robbers, c) all insect pollinators, and d) all insect robbers. N = no. of plants from which observations were obtained.	63
Appendix Two		
9	Analysis of the number of bumblebees arriving at a plant per minute. The analysis was a 3-way mixed model ANOVA (Block:random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).	86a
10	Analysis of the proportion of a plant's flowers visited per minute. The analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).	87
11	Analysis of the number of flowers visited per bumblebee on a plant. The analysis was a 3-way mixed model ANOVA (Block:random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).	88
12	Analysis of the proportion of a plant's flowers visited per bumblebee. The analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).	89
13	Analysis of the number of stems visited per bumblebee on a plant. The analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).	90

<u>Table</u>		<u>Page</u>
14	Analysis of the proportion of a plant's stems visited per bumblebee. The analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).	91
15	Analysis of the number of pollen grains per stigma. The analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).	92
16	Analysis of seed-set per flower. The analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).	93
17	Analysis of seed weight. The analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).	94

ARCHITECTURE, SIZE, AND REPRODUCTION IN PLANTS:
A POLLINATION STUDY OF MERTENSIA CILIATA (JAMES) G. DON.

CHAPTER I.

INTRODUCTION

Pollinators, in their movements between flowers, transfer pollen from anthers to stigmas and effect matings in plants. Not all pollen reaching a flower stigma through a pollinator will be of equal value to a plant. In self-incompatible species, for example, self pollen on a stigma is ineffective for fertilizations. Even in self-compatible plants, the fecundity of a cross and the viability of offspring from selfing are often lower than from outcrossing. The amount and quality of pollen reaching a flower is a function of the pattern of pollinator visitation to flowers within and between plants, and of the transfer properties of pollen by pollinators. Pollinators, therefore, can strongly affect the reproductive success of a plant. In turn, the plant, through its display of flowers and rewards, can influence the foraging behavior of its pollinators.

In this thesis I examine the pollination by bumblebees of the self-compatible, perennial plant, Mertensia ciliata. The thesis is divided into two parts, corresponding to two manuscripts to be published separately. In the first paper I examine the consequences of pollinator foraging behavior on the transfer of pollen between flowers, and on the level of inbreeding in plants. Plants of this species range in size from a few to over one hundred flowering stems, and bear a few hundred

to several thousand flowers. I show that, while this species is self-compatible, the reproductive output of selfed flowers is lower than that of outcrossed flowers. I ask then: Will the pattern of pollinator visitation to plants lead to greater levels of inbreeding in larger than in smaller plants of this species? This will occur if 1) bumblebees transfer pollen only short distances between flowers; 2) individual pollinators visit more flowers per visit, and thereby deposit less outcrossed pollen per flower, on large than on small plants; and/or 3) large plants cannot compensate for the loss in cross-pollination from a single bumblebee visit by attracting more pollinators. I determine experimentally the transfer properties of pollen between flowers by individual bumblebees, and I examine the relationship between the size of plants and the pattern of pollinator visitation. The receipt of pollen by flowers on large and small plants is also measured directly in the field. Finally, I compare the pattern of seed-set in plants against the predictions of my hypothesis and the results of the other experiments.

In the second part of the thesis, I address the role that the plant plays in shaping the foraging behavior of its pollinators. First, the pattern of insect visitation to plants is described in detail. I then consider the influence of 1) the architecture of the flowering display, 2) the geometry of the plant, 3) the spatial proximity of plants in the population, and 4) variability among flowers in nectar rewards, in promoting pollinator movement between plants.

CHAPTER II.

Bumblebee Pollination of Mertensia ciliata:

The Relationship between Plant Size and Inbreeding

INTRODUCTION

Many long-lived angiosperms produce displays of hundreds to thousands of blossoms in a short flowering season. The consequences of profuse flowering on the reproduction and mating system of a species, and the evolutionary forces shaping display size, have received wide interest (Frankie and Baker, 1974; Frankie, 1976; Frankie, Opler and Bawa, 1976; Gentry, 1974a, b, 1976; Willson and Rathcke, 1974; Willson and Price, 1977; Willson, Miller and Rathcke, 1979; Carpenter, 1976; Stephenson, 1979; Augspurger, 1980; Wyatt, 1980). Factors that have been proposed to favor large floral displays include interspecific competition for pollinators, intraspecific competition for mates, seed predation, and the potential for a large reproductive output (Janzen, 1969, 1977; Platt, Hill, and Clark, 1974; Williams, 1975; Silander, 1978; Willson, 1979; Lloyd, 1979; Lloyd, Webb, and Primack, 1980; Schaffer and Schaffer, 1979; Stephenson, 1979; Zimmerman, 1980; Wyatt, 1980; Augspurger, 1980). It is thought, however, that profuse flowering leads to reduced interplant movement of pollinators because a pollinator can remain constant to the many flowers of a single plant (Stephen, 1958; Free, 1962; Frankie, Opler, and Bawa, 1974; Levin and Kerster, 1974; Augspurger, 1980). Thus, the delivery of foreign pollen to a flower of a plant diminishes, and the dispersal of self pollen within a

plant increases, with display size. Restricted outcrossing lowers seed set in self-incompatible species. In self-compatible plants, the genetic composition, and perhaps fitness of seeds are affected because inbreeding supplants outcrossing. Within a species, where plants vary markedly in size, it is expected that large individuals suffer reduced outcrossing compared with their smaller conspecifics. Thus the negative relationship between seed-set per flower and plant (or inflorescence) size in several profusely-flowering species has been attributed, in part, to a reduction in cross-pollination (Carpenter, 1976; Willson and Rathcke, 1974; Willson and Price, 1977; Schemske, 1980; Wyatt, 1980).

Three factors control the parentage of seeds in animal-pollinated species: 1) the breeding system of the species, 2) the pattern of pollinator visitation to plants, and in particular the tenure of individual pollinators on plants, and 3) the extent of pollen dispersal between flowers and plants. Few researchers in plant-pollination have examined all three factors. In most instances, little is known about pollen dispersal properties. It is often assumed that pollen picked up at one flower is carried to only a few flowers past its source (Feinsinger, 1978; Richards and Ibrahim, 1979; Augspurger, 1980; Schmitt, 1980). That pollen flow is localized in plant populations is suggested by the distribution of pollinator flights between plants. It is typically leptokurtic and pollinators move predominantly between neighboring plants (Levin and Kerster, 1968, 1969a, b, 1974; Frankie, Opler, and Bawa, 1976; Pyke, 1978b; Price and Waser, 1979; Zimmerman, 1979; Schmitt, 1980; Waser and Price, 1982; Waser, in press). The dispersal of marked pollen (Colwell, 1951; Schlising and Turpin, 1971)

or of pollen substitutes (Linhart, 1973; Price and Waser, 1979; Waser and Price, 1982) also indicates short distance transfer of pollen between plants. However, evidence from experiments on actual pollen transfer between flowers by pollinators suggests that the length and variability of pollen carryover may vary with the plant-pollinator system (Levin and Berube, 1972; Thomson and Plowright, 1980; Lertzmann, 1981; Plowright and Hartling, in press; and see Lertzman and Gass, 1982). Unless genetic markers exist by which outcrossed and inbred seeds can be distinguished, a specific understanding of the parameters of carryover clearly is necessary in order to estimate rates of outcrossing of large and small plants of a given species. In the few instances where genetic markers have been used, estimates of pollen flow based on the distribution of pollinator flights underestimate actual gene flow (Schaal, 1980; Levin, in press), or large plants do not experience significantly less outcrossing than small plants (Bateman, 1956).

In this study, I tested the hypothesis that inbreeding may increase with plant size in Mertensia ciliata (Boraginaceae), a profusely-flowering, self-compatible perennial. I actually examined the potential for inbreeding as a function of plant size as I did not have a genetic marker to measure directly the degree of inbreeding. A primary objective of this study was to measure the carryover of pollen between flowers and plants. I addressed the following questions:

- (A) What is the breeding system of M. ciliata? (1) Does self-pollination lower the reproductive output (seed-set, seed

weight) of a flower compared with outcrossing. (2) Does M. ciliata rely on pollinator visitation for reproduction.

- (B) What is the extent of pollen carryover in this species? (1) To how many flowers will a pollinator carry outcrossed pollen. (2) How much outcrossed and total (self + outcrossed) pollen does a flower receive from a single pollinator visit.
- (C) (1) What is the effect of plant size on pollen receipt by flowers in the field? Do flowers on large plants receive less outcrossed pollen than on small plants. (2) Do flowers of large and small plants differ in the amounts of total pollen received?
- (D) What is the pattern of pollinator visitation to plants? (1) Do individual pollinators visit more flowers per visit on large than on small plants? If this is the case, flowers of large plants may receive less outcrossed pollen on average than those of small plants. (2) Could large plants compensate for the loss in cross-pollination from a single pollinator visit by attracting more pollinators.
- (E) Finally, does the reproductive output (seed-set per flower, seed weight) of large and small plants differ? (1) Is it consistent with the hypothesis of greater inbreeding in large plants, and/or (2) with results from inquiries into questions A-D.

MATERIALS

Study organism, site, and pollinators

Mertensia ciliata (James) G. Don (Boraginaceae) is a common herbaceous perennial found in moist habitats of the foothills and higher elevations in the Rocky Mountains and the Sierra Nevadas (Williams, 1937; Pelton, 1961; Hitchcock, Cronquist, Ownbey, and Thompson, 1969). Plants of this species propagate vegetatively by means of a woody caudex and rhizomes to form clones of a few to over one hundred flowering stems. Because rhizomatal connections between portions of a plant may occasionally be severed, plant sizes (number of stems) reported in this paper may underestimate the true extent of clones.

Flowers of M. ciliata change from pink in the bud to blue when they mature at 12-15 mm in length. Anther dehiscence usually begins just after petals open and pollen lasts for one to two days (Pelton, 1961; pers. obs.). Stigmas are receptive to pollen prior to anther dehiscence and remain so for four to five days until corollas wither. Since self-pollen is present in flowers and on plants during the period of stigma receptivity, and M. ciliata is self-compatible (Pelton, 1961; Galen unpubl.), the opportunity for inbreeding therefore exists. A more complete description of flower and inflorescence morphology and development can be found in Pelton (1961).

My study site was located in Park County, Colorado (T9S, R7W, S16) at a dense population of plants along Pennsylvania Creek (elevation 3400-3500 m). Plants extended several meters up the bank from the

water's edge and grew very close to one another. Some isolated individuals were found up to 30 m distant from the main population. Flowering at this site began in early to mid July (1979 and 1980, respectively) and extended through August. Individual plants bloomed for three to five weeks.

M. ciliata was visited by a wide range of insect species at this site (Geber, in prep). However, only three species of long-tongued bumblebees, Bombus flavifrons (Cresson), B. kirbyellus (Curtis), and B. sylvicola (Kirby), regularly pollinated flowers. All other insects robbed nectar through holes pierced in the base of the corolla tubes. They rarely entered flowers for pollen, and hence rarely contacted stigmas. In this paper I report only on the pattern of plant visitation by pollinating bumblebees.

METHODS AND RESULTS

A. Breeding system of *M. ciliata*

Methods

In 1980 I examined the breeding system of *M. ciliata* by comparing the seed-set of flowers subjected to one of five pollination treatments. In Table 1, I present the treatments performed on each of ten plants (sixty flowers/treatment). The difference between seed-set in treatments 3 and 4 measures the relative abilities of outcrossed and self pollen in effecting fertilizations when the two pollens are involved in independent matings. The difference between seed-set in treatments 5 vs. 1 and 2 measures the dependence of *M. ciliata* on pollinator visitation for reproduction.

I excluded insects from flowers in treatments 1 through 4 by placing nylon net bags over stems prior to blooming. Anthers were removed from flowers in treatments 1 and 3 before pollen shedding. I hand-pollinated flowers in treatments 3 and 4 by rubbing a freshly-picked and dehiscing anther across the stigma of a newly-opened flower; I then repeated the procedure on three successive days. I obtained self-pollen from other bagged flowers of the same plant, and outcrossed pollen from a donor plant located between 3 m and 20 m from the recipient plant. I counted the number of seeds per flower (maximum of four) and analyzed the results by 2-way mixed model ANOVA (Plants: random effect; Pollination treatment: fixed effect) (Sokal and Rohlf, 1969). I also compared mean

seed weights of outbred and inbred seeds of the ten plants with a Wilcoxon Signed Ranks test.

Results

In the analysis of seed-set (Table 2), seed-set per flower differed among plants ($P < 0.0005$), among pollination treatments ($P < 0.0001$), and there were significant Plant x Pollination treatment interactions ($P < 0.0001$). There is no obvious interpretation to the interaction term because there was no consistent pattern between the seed-set ranking of a plant in one pollination treatment and its ranking in another treatment. For example, plants that had the highest outcrossed seed-set did not have the lowest (or highest) seed-set from selfing ($r = -0.26$, $P > 0.3$).

In eight out of ten plants, however, outcrossing resulted in greater seed-set per flower than selfing; and the Scheffe comparison of means of the two treatments showed them to be significantly different ($F = 6.37$, d.f. = 1.36, $P < 0.01$). Outcrossed seed-set was about twice that of selfed seed-set (Table 3). Outcrossed seeds also weighed more than inbred seeds (2.14 mg vs. 1.87 mg) (Wilcoxon Signed Ranks test, $T_s = 4$, d.f. = 10, $P < 0.0007$). Thus the results of the hand-pollinations suggest that selfing leads to a loss of reproductive output in both seed number and weight compared with outcrossing. Previous studies of the compatibility system of M. ciliata (Pelton, 1961; Galen, unpubl.) did not find significantly higher seed-set through outcrossing. However, the number of hand-pollinated plants was small, or poor weather

conditions resulted in very low seed-set for all pollination treatments (Galen, pers. comm.).

The dependence of M. ciliata on bumblebee visitation for high seed-set is manifested by a comparison of treatments 5 vs. 1 and 2. Open (insect)-pollinated flowers set ten to fifty times more seed than bagged, unpollinated flowers (Scheffé' comparison of means: $F = 277.89$, d.f. = 1.36, $P < 0.0005$).

B. Pollen carryover between flowers by bumblebees.

Methods

The pollen carryover experiments were designed to measure the amounts of outcrossed and total (self + outcrossed) pollen deposited on successive flowers visited by a bumblebee as a function of the order of flowers in the visit sequence. I also estimated the mean length of outcrossed pollen carryover.

I captured Bombus flavifrons and B. kirbyellus workers on M. ciliata and chilled them for two hours. Chilled bumblebees fed more readily on hand-held flowers inside a 2 m x 2 m x 2 m gazebo where the experiments were performed. All flowers used in the experiments had been screened from prior insect visitation with nylon net bags so that their stigmas were free from pollen. After warming up, a bumblebee was permitted to visit ten non-emasculated donor flowers as sources of 'outcrossed' pollen.

I conducted two types of carryover runs. In the first, a bumblebee proceeded to visit a series of emasculated flowers which contained no self pollen. Counts of pollen grains on stigmas of these flowers provided estimates of outcrossed pollen deposition. These were maximum estimates because self pollen in flowers would normally mix with or conceal the outcrossed pollen carried by bumblebees, and thereby reduce the mean deposition of outcrossed pollen per flower. Mixing and concealment of pollen could also increase the rate of decay in outcrossed pollen deposition on successive flowers, and hence reduce the mean length of pollen carryover (Price and Waser, in press; Waser, in press; Waser and Price, in press; but see Lertzman, 1981; Lertzman and Gass, 1982). In the second type of carryover run, a bumblebee visited a sequence of non-emasculated flowers. Total (self + outcrossed) deposition was estimated from pollen grain counts on stigmas of these flowers.

I conducted fourteen emasculated and ten non-emasculated runs, of ten to thirty-five flowers in length, with different bumblebees and fresh flowers. I removed pollen from a flower by rubbing the stigma three times across a slide coated with a thin film of glycerine jelly (Beattie, 1971; Thomson and Plowright, 1980). Grains adhering to the jelly were counted under a compound scope. The absence of grains from the third rubbing verified that all or most all pollen grains had been removed from a stigma. I regressed pollen grain counts against the order of the flower in the visit sequence for emasculated and non-emasculated runs separately.

Results

The amount and rate of decay in outcrossed pollen deposition on successive flowers was described by the exponential least-squares regression:

$Y_i = 30.5 \exp(-0.095 i)$, Y_i = number of outcrossed grains deposited in the i^{th} flower visited by a bumblebee ($r = -0.68$, $P < 0.001$). The mean dispersal distance of outcrossed pollen, or length of carryover, was 11.6 flowers. Because the rate of decay in deposition was small (9.5%), outcrossed pollen deposition declined very slowly with flower order (Fig. 1). The total amount of pollen deposited on all flowers varied between runs as did the deposition among flowers of a single run (Table 4). Flowers visited late in a run often received more outcrossed pollen than early flowers.

Pollen (self + outcrossed) deposition on stigmas of non-emasculated flowers was also variable (Table 4) but did not decline with flower order ($r = 0.05$, $P > 0.5$). Mean deposition was 140.2 ± 7.2 grains.

As I mentioned earlier, outcrossed pollen deposition and carryover were probably overestimated. Another bias was introduced into estimates of both outcrossed and total (self + outcrossed) deposition because flowers used in the experiments had been screened from prior insect visitation. Screened flowers were usually richer sources of pollen and nectar than flowers in the field. Bumblebees therefore picked up more pollen and spent a longer time at screened than at field flowers. Both factors would tend to increase pollen deposition estimates (Thomson and Plowright, 1980). The bias would be greater for estimates of total

(self + outcrossed) deposition since all flowers of non-emasculated runs contained self pollen.

To overcome the bias introduced by using screened flowers, I also measured pollen receipt by flowers in the field.

C. Effect of plant size on the receipt of pollen. Pollen deposition in the field

Methods

In 1980, I measured the amounts of outcrossed and total (self + outcrossed pollen) on flower stigmas of plants in the field as a function of plant size. Pollen on these stigmas was not the result of a single visit by a bumblebee but rather represented the accumulation of pollen from repeated pollinator visits. Pollen estimates therefore provided a picture of the actual amount of pollen received by a flower over its lifetime, but could not be used to measure carryover. I wanted to determine whether large plants received less outcrossed pollen per flower than small plants, and how total pollen receipt differed among plants.

I selected two groups (blocks) of nine plants from the creek population. Plants within a block grew in the same area and flowered at the same time, although plants of the second block flowered later than the first. Three plants in each block were large (50 stems), three were medium-sized (30 stems), and three were small (10 stems). I emasculated all flowers of one plant (treatment E) in each size class prior to

anther dehiscence. The flowers of these plants, then, had no self pollen, and outcrossed pollen receipt was estimated from pollen grain counts on their stigmas. To speed the emasculation procedure in this experiment, I clipped off the tip of the petal lobes to reach the anthers. This, however, resulted in earlier than usual opening of the shortened flowers which could influence pollen deposition. I therefore clipped all the flowers of a second plant in each size class (treatment CL) but left the anthers intact. The third plant in each size class (treatment C) was untouched and served as a control for the effects of clipping. Thus flowers of clipped and control plants received both self and outcrossed pollen. Total pollen deposition was estimated therefore from pollen counts on their stigmas.

I collected ten 4-day-old flowers at random from each plant every five to six days and counted the number of pollen grains on stigmas. Four collections were obtained from each plant. Results were analyzed by 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatments: fixed effects) (Sokal and Rohlf, 1969).

Because emasculated plants did not offer any pollen to bumblebees, I needed to ascertain whether they were avoided by pollinators. I therefore observed bumblebee visitation to each of the 18 plants for 10 minutes every 2-3 days throughout the flowering of plants. I calculated two measures of visitation for each plant and observation period: 1) the number of bumblebees arriving at a plant per minute and 2) the proportion of a plant's flowers visited per minute. Some plants had no visits during an observation period. For those plants that did attract bumblebees, I also computed 3) the mean number of flowers visited per

bumblebee on a plant, or visit length. Results were again analyzed by 3-way ANOVAs (for balanced design: measures 1 and 2, and unbalanced design: measure 3).

Results

In this section I present the analysis of pollen deposition, and of the effects of emasculation treatment on bumblebee visitation. The effects of plant size on the pattern of bumblebee visitation are discussed later with similar data obtained in 1979 (see Section D).

Effect of emasculation treatment on bumblebee visitation

The results of the ANOVAs for the three measures of visitation are summarized in Table 5. [Individual ANOVAs are presented in Appendix II (Tables 9-11).] Emasculation treatment was important to only one of the three measures of visitation. The 3-way interaction of Block x Size x Emasculation was significant ($P < 0.03$) in the analysis of the mean number of flowers visited per bumblebee on a plant, or visit length (Table 5). Even in this instance, however, only two of the eighteen pairwise comparisons of mean visit length between emasculated, clipped, and control plants of a given block and size were statistically different from zero (Scheffe method of multiple comparisons with combined $\alpha = 0.05$; Neter and Wasserman, 1974). Mean visit length was greater for the large clipped plant of the second block (28.1 ± 14.8 flowers per bumblebee) than on its equal-sized emasculated and control

counterparts (13.3 ± 7.3 and 13.9 ± 8.0 flowers per bumblebee, respectively). The former plant, however, had many more flowers per stem than the latter. Bumblebees did not avoid emasculated plants, and, in general, plants of a given block and size were visited equally. Hence differences among such plants in pollen receipt cannot be attributed to differences in visitation rates resulting from emasculation treatment.

Effect of Plant size and emasculation tretment on pollen receipt by flowers

The analysis of pollen receipt is summarized in Table 6. [The ANOVA of pollen receipt is given in Appendix II (Table 15).] There were significant differences between blocks ($P < 0.03$), among emasculation treatments (Block x Emasculation effect: $P < 0.02$), but not among plant sizes (Block x Size effect: $P > 0.5$; Size effect: $P > 0.25$) in pollen receipt by flowers.

Emasculated plants had consistently smaller pollen doses per stigma than clipped and control plants (Fig. 2). This was to be expected since emasculated plants received only outcrossed pollen, while clipped and control plants had both self and outcrossed pollen. Pollen dose per stigma was invariant with plant size. That is, flowers of large emasculated plants did not receive less outcrossed pollen than flowers of small plants. Furthermore, total pollen (self + outcrossed) doses on clipped and control plants were also constant over all plant sizes. As a result both the amount ($\bar{x} = 61.4 \pm 9.2$ grains) and proportion

(outcrossed dose \div total pollen dose = $1/2 - 2/3$) of pollen outcrossed origin on flowers would be similar for all plants.

Pollen receipt did differ between clipped and control plants of the second block (Fig. 2). It appears that the effect of clipping was to increase, in some cases, pollen deposition. Also, plants of the second block, which were visited more frequently by bumblebees (see Section D) had larger doses per stigma than plants of the first block.

D. Effect of plant size on the pattern of bumblebee visitation

Methods

I studied the pattern of bumblebee visitation to plants to determine 1) whether individual bumblebees visited more flowers per visit and thereby deposited fewer outcrossed grains per flower, on large than on small plants; 2) whether large plants might compensate for the reduced pollen receipt from a single pollinator visit by attracting more bumblebees.

In 1979 I recorded bumblebee visits to each of 80 plants at their peak of flowering during 15-minute observation periods. I watched approximately equal numbers of large (>40 stems), medium (20-40 stems), and small (<20 stems) plants. Sixty plants grew along the creek and were very close to neighboring plants: the mean distance between a plant and its nearest neighbor was 0.10 m. Twenty plants were more isolated and nearest neighbor distances ranged between 1.65 m and 22.70 m. I reduced the size of 12 of the creek-side plants by removing

30-100 stems prior to flowering. I created in this manner six medium and six small plants in order to test experimentally the relationship between plant size and bumblebee visitation. For each plant I calculated 1) the number of bumblebees arriving at the plant per minute, a measure of the rate at which outcrossed pollen is brought to a plant; and 2) the proportion of the plant's flowers visited per minute, a measure of the frequency of flower visitation. Some plants received no visitors during the observation period. For those plants that did attract bumblebees, I also calculated 3) the mean number of flowers visited per bumblebee on a plant, or visit length. These were the same three measures of visitation that I obtained for plants in 1980 (see Section C).

Each measure of visitation from 1979 was analyzed by multiple regression. Independent variables were plant size, square of plant size, nearest neighbor distance, and a dummy variable for size reduction.

Results

The results of the regressions of bumblebee visitation in 1979 are given in Table 7. The analyses of bumblebee visitation in 1980 are summarized in Table 5. [ANOVAs of visitation measures in 1980 are given in Appendix II (Tables 9-11).]

In both years, the mean number of flowers visited per bumblebee on a plant (visit length) increased significantly with plant size (data from 1979, $P < 0.05$; data from 1980, Block x Size effect:

$P < 0.05$, Fig. 3.), but the rate of increase in visit length was very small. Thus, in 1979, for each increment of 10 stems on a plant, bumblebees visited an average of only 1.45 additional flowers (Table 7). The number of bumblebees arriving at a plant per minute also increased with plant size in 1979 ($P < 0.001$, Table 7) and in the second block of plants in 1980 (Block \times Size effect: $P < 0.003$, Fig. 4). The proportion of a plant's flowers visited per minute was independent of plant size in both years (data from 1979: $P > 0.5$, Table 7; data from 1980, Size effect: $P > 0.3$, $\bar{x}\%$ flowers visited per minute = $1.5 \pm 0.2\%$).

The indicator variable for size reduction never entered the regression equations (Table 7), and thus reduced plants in 1979 were visited as if they had been originally smaller in size. Square of plant size and nearest neighbor distance were unimportant variables in the regressions (Table 7). In 1980, pollinator services differed between the two blocks of plants: the number of bees arriving at a plant per minute (Block effect: $P < 0.05$), and the proportion of a plant's flowers visited per minute (Block effect: $P < 0.01$) were greater in the second than in the first block. The greater visitation of second block plants could explain the larger pollen doses on stigmas of their flowers (Section C).

In summary, the pattern of pollinator visitation was consistent between years. While bumblebee visit length increased with plant size, it was not substantially greater on larger plants. Furthermore, large plants attracted more bumblebees, and hence more outcrossed pollen, per minute than small plants; large plants therefore might compensate for

reduced outcrossed pollen deposition per flower from single pollinator visits by attracting more pollinators (see Discussion). Bumblebee densities on plants responded perfectly to plant size in that the proportion of a plant's flowers visited per minute, and hence the frequency of flower visitation, were similar for all plant sizes. This could explain the equal deposition of pollen on flowers of large and small plants in 1980 (Section C).

E. Effect of Plant Size on Seed-set.

Methods

In 1979 I measured the mean number of seeds per flower on 95 creek-side plants and on 12 isolated plants (range of sizes: 1-160 stems). I reduced the size of 19 creek-side plants prior to flowering to test experimentally the relationship between plant size and seed-set per flower. Because size reduction of plants could alter resource availability for seed-set, and resource limitation (if any) could vary with plant size, I tested for resource limitation to seed-set in the following manner: I removed half of the flowers from 23 of the creek-side plants after pollination but before seed development occurred; nine of the creek-side plants were both reduced in size and had flowers removed. I analyzed seed-set results by multiple regression. Independent variables were plant size, number of flowers per stem, nearest neighbor distance, and three indicator variables, one for stem removal,

one for flower removal, and one for the combined effects of stem and flower removal.

In 1980, I counted the number of seeds in 100 flowers of each of the large, medium, and small treatment E, CL and C plants (18 plants) from the experiment in Section C. I also measured the weights of 20 seeds from each plant. Recall that emasculated plants (treatment E) received only outcrossed pollen, and thus seeds from these plants were outcrossed. Clipped and control plants (treatment CL and C), on the other hand, could set both inbred and outbred seeds since they received both self and outcrossed pollen. Data on seed-set per flower and seed weight were analyzed by 3-way mixed ANOVAs (see Section C).

Results

In this section, I simply present the results of seed-set per flower and seed weight. I later discuss the consistency of the results with predictions derived from 1) the hypothesis of increased inbreeding with increasing plant size and 2) the alternate hypothesis of no greater inbreeding in larger plants.

Mean seed-set per flower in 1979 was independent of plant size, the number of flowers per stem, nearest neighbor distance, and the three indicator variables for size reduction and flower removal (\bar{x} seed-set per flower = 1.92 ± 0.02 , $R = -0.198$, $P > 0.2$). Thus seed-set did not decline in larger plants; and there was no evidence for resource limitation to seed-set.

The analyses of seed-set per flower and seed weight from 1980 are summarized in Table 6 [ANOVAs of seed-set and seed weight are given in Appendix II (Tables 16 and 17)]. In the analysis of seed-set, the 3-way interaction of Block x Size x Emasculation treatment was highly significant ($P < 0.001$). In pairwise comparisons between plants of a given block and size, control plants set better seed than their emasculated and clipped counterparts in one and three comparisons respectively, and had fewer seeds per flower in only one contrast (Scheffe method of multiple comparisons with combined $\alpha = 0.05$, Fig. 5). The generally lower seed-set of emasculated and clipped plants may have resulted from damage done to their stigmas and styles during the emasculation and clipping procedure (pers. obs.). Emasculated (outcrossed) plants did not set better seed than clipped (selfed + outcrossed) plants (Fig. 5). There were significant effects of Block x Size ($P < 0.001$) and Block ($P < 0.01$) in the analysis of seed-set but their meaning is difficult to interpret given the very large 3-way interaction (see also Fig. 5). Seed-set of clipped and control plants did not decline with increasing plant size; and there was no pattern to the relative magnitudes of seed-set in emasculated and clipped plants as a function of plant size (Fig. 5).

In the analysis of seed weight, the 3-way interaction of Block x Size x Emasculation was again significant ($P < 0.01$); and mean seed weights of plants followed no consistent pattern (Fig. 6). Outcrossed seeds of emasculated plants did not weigh more than seeds of clipped and control plants of the same block and size (Fig. 6). The effect of Block x Size was also significant ($P < 0.001$) but its meaning is again

unclear. Mean seed weight did not appear to decrease with increasing plant size (Fig. 6). Plants of the first block had heavier seeds than those of the first (Block effect: $P < 0.0001$, Fig. 6).

In summary, seed-set per flower in 1979 and 1980, and seed weight in 1980, did not decline in larger plants. There were also no consistent differences in seed-set per flower and seed weight between clipped (selfed + outcrossed) and emasculated (outcrossed) plants in 1980. Finally, there was no evidence for resource limitation to seed-set in 1979.

DISCUSSION

The hypothesis that inbreeding increases in large plants of Mertensia ciliata is based on the supposition that large plants receive less outcrossed pollen per flower than small plants. This premise, however, is not supported by the results of my experiments measuring pollen deposition on plants in the field: pollen doses on stigmas of emasculated flowers did not differ among large and small plants (Section C).

The amount of outcrossed pollen on a flower is a function of 1) the carryover distance of pollen, 2) the number of flowers visited by a pollinator on a plant (visit length) and 3) the number of pollinators arriving at a plant (i.e. the arrival rate of outcrossed pollen). Pollen carryover between flowers in M. ciliata appears to be extensive, the average pollen grain being transferred to the eleventh or twelfth flower past its source (Section B). Bumblebees on M. ciliata do visit a few more flowers (Section D), and will therefore deposit slightly less outcrossed pollen on average per flower on large than on small plants. However, large plants also attract bumblebees at a faster rate (Section D). One can ask, therefore, whether the greater arrival rate of pollinators to larger plants is sufficient to make up for the smaller receipt of pollen from a single pollinator visit. In other words, are the combined effects of the pattern of pollinator visitation and pollen carryover consistent with the finding of equal pollen deposition on emasculated flowers of large and small plants?

Let:

$Y_1 = f_1(x)$ = mean number of bumblebees arriving per minute at a plant of size x .

$Y_2 = f_2(x)$ = mean amount of outcrossed pollen deposited on a flower by a single bumblebee during its visit to a plant of size x

$Y_3 = f_3(x)$ = mean visit length of a pollinator on a plant of size x .

$Y_4 = f_4(x)$ = mean amount of outcrossed pollen deposited on a flower by all pollinators visiting a plant of size x per minute.

Using the carryover function of outcrossed pollen derived in Section B, I can estimate Y_2 and Y_4 as follows:

$$Y_2 = \frac{\int_0^{Y_3} 30.5 \exp(-0.095 y) dy}{Y_3}$$

and

$$Y_4 = Y_1 Y_2$$

By substituting for Y_1 and Y_3 the regressions of bumblebee arrival rate and visit length on plant size from 1979 (see Table 7), I can then compute values of Y_2 and Y_4 for large and small plants. The results of these calculations are graphed in Fig. 7. Values of Y_4 were multiplied by 60 in order to plot them on the same graph, and thus represent the average amount of outcrossed pollen brought to a flower per hour by all visitors to a plant. This estimation procedure therefore predicts, and my measurements of pollen on flowers in the field confirm, that emasculated flowers of large and small plants get equal amounts of outcrossed pollen. Large plants compensate for slightly reduced outcrossed pollen deposition from single pollinator visits (Y_2) by

attracting more visitors. Thus the combination of extensive pollen dispersal and the pattern of bumblebee visitation should result in similar rates of selfing in large and small plants. Actually, the amount of outcrossed pollen received by a flower is not the only parameter affecting the potential for selfing. Selfing is also likely to depend on the proportion of pollen of outcrossed origin (i.e., outcrossed (self+ outcrossed)) on a flower. However, all non-emasculated flowers in the carryover experiments (Section B), and on large and small plants in the field (Section C), received equal total (self + outcrossed) amounts of pollen. Hence the proportion of pollen of outcrossed origin in a flower should also be the same for large and small plants.

The assumption, in many pollination studies, of low pollen carryover (Feinsinger, 1978; Richards and Ibrahim, 1979; Augspurger, 1980; Schmitt, 1980) is based on the early work of Levin and Berube (1972) which pointed to a rapid and efficient transfer of pollen between flowers by Colias butterflies on Phlox (but see Lertzman and Gass, 1982). In more recent studies, including the present study, with bumblebees (Thomson and Plowright, 1980; Waser and Price, 1982; Plowright and Hartling, in press) and hummingbirds (Perkins, 1977; Lertzman, 1981; Waser and Price, 1982; Price and Waser, in press; Waser, in press) on a variety of plant species, pollen dispersal was more extensive. It is imperative, then, in pollination studies dealing with outcrossing rates in plants, that pollen carryover be examined.

A note of caution about my results on carryover is necessary at this point. Recall that outcrossed pollen deposition and carryover in this

study (and in many other studies) was always measured on emasculated flowers which did not contain self pollen. If self pollen in flowers normally mixes with or partially conceals the outcrossed load carried by a bumblebee mean outcrossed pollen deposition per stigma would most likely decline. Mixing or layering of pollen may also affect the rate of outcrossed pollen deposition, and hence the mean dispersal distance or carryover, of outcrossed pollen. Lertzman (1981) and Lertzman and Gass (1982) have modelled the effects of pollen mixing and layering on the distance over which pollen from one source flower is carried. Their results suggest that under certain conditions both the mean and variance of carryover distance are greater with mixing or layering than when there is no interaction among pollens from different flowers. In particular, when pollens are layered over one another on the body of a pollinator, long-buried pollen may resurface only after many subsequent flowers have been visited. Detection of this sort of carryover would be difficult, however, since it requires studies of very long visitation sequences (but see Lertzman, 1981). Price and Waser (in press) in a recent study found that the presence of pollen-bearing anthers in flowers reduced the carryover of dye particles (pollen mimics) between flowers compared with carryover on emasculated flowers. As they point out, the discrepancy between estimates of carryover in different plant-pollinator systems (see citations in Introduction) may be due in part to the different methodologies used (emasculated vs. non-emasculated flowers) to obtain these estimates, and more careful studies of carryover are needed.

If the presence of self pollen in flowers of M. ciliata shortens carryover considerably, both the mean amount and proportion of pollen of outcrossed origin on a flower would decline with increasing plant size. (I would only have been able to detect this had I been able to distinguish outcrossed from self pollen.) Under these circumstances, the potential for selfing would be greater in larger plants. I can, however, turn to the results of seed-set per flower and seed weight (Section E) to see whether they are consistent with the hypothesis of 1) increased selfing in larger plants, or, alternatively 2) of no difference in selfing rates among plants.

Given that selfing reduces the number and weight of seeds produced by a flower (Section A), that flowers of large and small are visited with equal frequency (Section D), and receive the same total amounts of pollen (Section C), and that there is no evidence of resource limitation to seed-set (Section E), the first hypothesis predicts that seed-set per flower and seed weight should decline with increasing plant size; the second hypothesis predicts no such decline. The latter is what I found: seed-set and seed weight did not decrease in larger plants (Section E). Hence the reproductive output of flowers on large and small plants do not support my original hypothesis of more inbreeding in larger plants of M. ciliata.

Under both of the above hypotheses, seed-set per flower and seed weight of clipped plants (selfed + outcrossed) should have been smaller than for emasculated plants (outcrossed) in 1980. However, I found no consistent differences among plants in these two measures of reproductive output. There are two possible mechanisms that may explain

the lack of differences: 1) Flowers of clipped plants, even with the added presence of self pollen, are substantially outcrossed, or 2) all plants mate primarily with close neighbors who are also close relatives.

There is mounting evidence that plants are capable of distinguishing among pollens of different origins and degrees of genetic relatedness (Bateman, 1956; see Mulcahy, 1975 for review, 1979; Waser in Price, 1982, Levin, in press), and as a result all pollens do not contribute to viable seed production in proportion to their abundances on stigmas. This remains to be demonstrated for M. ciliata, but plants of this species may be capable of limiting selfing as long as they receive sufficient amounts of outcrossed pollen.

Plants at my study site will exchange pollen primarily with neighbor plants because pollinators fly predominantly between a plant and its nearest neighbors (Geber, in prep). From the results of carryover and pollinator visitation patterns, it is expected that pollen will be dispersed two to five plants removed from its source. If pollen dispersal were the only component of gene flow in M. ciliata, genetic neighborhood sizes (sensu Wright, 1950) would be small, and neighbors would be closely related. However, M. ciliata frequently grows along watercourses and thus seed dispersal by water and melting snows may be extensive. It is difficult to evaluate how closely related neighboring plants are at my study site without genetic data. While I cannot safely attribute the pattern of reproductive output in M. ciliata to one or the other of the two mechanisms, both processes, however, predict equal selfing rates among large and small plants.

CHAPTER III.

Plant Architecture, Plant Size, and the Cross-pollination of
Profusely-Flowering Plants:The Foraging Behavior of Bumblebees on Mertensia ciliataINTRODUCTION

Cross-pollination is essential for seed set in self-incompatible plants; it is also beneficial to self-compatible plants where the number or viability of seeds from outcrossing are greater than from selfing (Price and Waser, 1979, 1982; Levin, in press). In many species, animals are responsible for the transfer of pollen between plants. However, pollen dispersal by animals is localized, and pollen picked up at one flower (or plant) is rapidly deposited at subsequent flowers (Colwell, 1951; Schlising and Turpin, 1971; Levin and Berube, 1972; Price and Waser, 1979, in press; Thomson and Plowright, 1980). If a pollinator visits many flowers on a plant, successive flowers receive diminishing amounts of foreign pollen, and progressively larger doses of self pollen. The likelihood, then, that a given flower sets seed by outcrossing, or donates pollen to another plant, declines with increased length of a pollinator's visit. Short visits coupled with frequent returns to plants by pollinators are most conducive to cross-pollination. How might this be achieved in profusely-flowering plants with hundreds to thousands of blossoms?

Researchers in foraging theory predict that the length of time an animal spends in a patch (plant) depends on the amount of rewards or on

the capture rate of rewards in the current and previously visited patches, and on the cost of traveling between patches (Krebs, 1973; Charnov, 1976; Pyke, Pulliam and Charnov, 1977; Ollason, 1980). In studies on natural and artificial plants and on arrays of artificial flowers, the number of flowers visited by bees and hummingbirds is positively correlated with the size and number of rewards obtained (Pyke, 1978a; Gass and Montgomerie, 1979; Heinrich, 1979; Morse, 1980; Waddington, 1980). Conversely, the frequency of interplant moves and the distance flown between flowers and plants increases with declining floral rewards, and with the number of visits to empty flowers (Gill and Wolf, 1975; Pyke, 1978b; Hartling and Plowright, 1979a, b; Heinrich, 1979). The frequency of encounters with unrewarding flowers will depend not only on the reward status of a plant but also on the pollinator's ability to avoid previously visited flowers. Pollinators may find it difficult to keep track of their foraging path on profusely-flowering plants with large and complex floral displays, and may thus return to flowers quickly (see Gass and Montgomerie, 1979; and below). The architecture, i.e. the spatial arrangement of blossoms, of a plant may then be important in promoting pollinator movement. The presentation of variable flower rewards by a plant could also enhance pollinator movement, since some flowers will appear unrewarding compared to others. Bees and wasps have been shown to avoid flowers with variable rewards in favor of those with constant nectar return (Real, 1981). Reward levels can vary between flowers due to innate differences in nectar and pollen production. Variation will also arise automatically because of recent visitation of some flowers by a pollinator (Pleasants

and Zimmerman, 1979). Finally, as plant density increases, and the cost of travel between plants declines, pollinators visit fewer flowers per plant (Bateman, 1956; Levin and Kerster, 1974; Hartling and Plowright, 1979a).

There are few reports in the literature on the length of pollinator visits to profusely-flowering plants. In three studies of shrubs and herbaceous perennials, pollinators probed only a small number (5-10) and percentage (3-10%) of available flowers per visit on plants (Silander and Primack, 1978; Zimmerman, 1979; Augspurger, 1980). Pollinator fidelity to very large and often widely-dispersed tropical trees may be considerably greater (Frankie, Opler and Bawa, 1974). Indeed, large tropical plants often support territorial flower visitors which do not contribute directly to cross-pollination (Wolf, 1970; Linhart, 1973; Frankie and Baker, 1974; Frankie, 1976; Carpenter, 1976; Gould, 1978; Stiles, 1979; Schemske, 1980).

In this paper I wish to focus attention primarily on the cross-pollination of plants the size of bushes or small trees. I report first on the pattern of insect visitation to the large herbaceous perennial, Mertensia ciliata (James) G. Don (Boraginaceae). Plants of this species produce a cluster of a few to over one hundred flowering stems each year. At peak bloom, individual plants bear a few hundred to more than five thousand flowers. Insect visitation is examined in relation to plant size. I then discuss the roles of plant architecture, variable floral rewards (properties of individual plants), and plant density (property of a population of plants) in promoting cross-pollination.

MATERIALS AND METHODS

A. Study Site and Insect Visitors

Insect observations were conducted in a dense population of Mertensia ciliata along Pennsylvania Creek (elevation 3400-3500m) in Park Co., Colorado (T9S, R7W, S16). M. ciliata is visited by a wide variety of insect species at my study site: four species of bumblebees (Bombus, Apidae), two species of solitary bees (Megachilidae, Colletidae), and ten species of flies (Muscidae, Sarcophagidae, Tachinidae, Anthomyiidae). However, only three species of bumblebees, Bombus flavifrons (Cresson), B. kirbyellus (Curtis), and B. sylvicola (Kirby), have sufficiently long proboscides to reach the nectaries of flowers, and regularly pollinate flowers. The short-tongued bumblebee, B. mixtus (Cresson), pierces holes at the base of the corolla tube and robs nectar. The solitary bees and flies make use of the holes, and are primarily nectar thieves, although they occasionally enter flowers for pollen.

B. Insect Visitation

In 1979, I recorded insect visitation to each of 80 plants at their peak of flowering during 15-minute observation periods. I watched approximately equal numbers of large (>40 stems), medium (20-40 stems), and small (<20 stems) plants (range: 3-113 stems). Sixty plants grew along the creek and very close to neighboring plants; the mean distance

between a creek-side plant and its nearest neighbor was 0.10 m. Twenty plants were more isolated and nearest neighbor distances ranged between 1.65 m and 22.70 m. I reduced the size of 12 large creek-side plants by removing between 30 and 100 stems prior to flowering. I created in this manner six medium and six small plants in order to test experimentally the relationship between plant size and insect visitation.

The proportion of a plant's flowers visited per minute was calculated for each plant by dividing the total number of flower visits per minute by the number of available flowers. Some plants did not receive visitors during the observation period. For those plants that attracted insects, the mean number of flowers and the proportion of a plant's flowers visited by each visitor were also obtained. Measures of insect visitation were calculated for a) bumblebee pollinators, b) bumblebee robbers, c) all insect pollinators, and d) all insect robbers. Multiple regression was used to relate each measure of visitation to plant size, distance between a plant and its nearest neighbor, and a dummy variable for size reduction.

In 1980, bumblebee pollinator visitation was recorded on 18 plants (6 large, 6 medium, and 6 small) during 10-minute observation periods every 2 to 3 days throughout the flowering of plants. The same measures of visitation were obtained as in 1979. In addition, the mean number of stems and the proportion of a plant's stems visited per bumblebee on a plant were calculated. The 18 plants were used in a 3-way factorial experiment measuring the deposition of outcrossed and self pollen on flowers of different-sized plants (Geber, in prep). Factors were blocks (two blocks of nine plants), size (three size classes), and emasculation

treatment (three treatments, anthers were removed from all flowers of some plants but not from the flowers of other plants). Measures of bumblebee visitation were analyzed by 3-way mixed model ANOVA (block: random effect; plant size and emasculation treatment: fixed effects). Only the significant main and interaction effects of size on bumblebee visitation are presented in detail in this paper. Visitation differed in some analyses between blocks (plants of the second block flowered later and were visited more frequently than plants of the first block). Emasculation treatment had an effect in only one analysis (Block x Size x Emasculation effect was significant in the analysis of the mean number of flowers visited per bumblebee), but in most comparisons plants of a given block and size were not visited differently by bumblebees (Geber, in prep) and therefore can be considered as replicates.

In 1979, I mapped the foraging routes of 49 bumblebees between plants. Moves between plants were classified as between nearest neighbor plants or non-nearest neighbor plants. The change in direction (angle of departure from a plant minus angle of arrival) between successive moves was calculated; turns to the left were assigned a positive sign, and turns to the right a negative sign (Levin, Kerster, and Niedzlek, 1971). In 1980, flights by bumblebees from the 18 plants were again classified as nearest neighbor or non-nearest neighbor moves. In addition, where stems from adjacent plants abutted on one of the 18 plants, the percentage of moves made to abutting stems was obtained.

RESULTS

The results of pollinator and robber visitation of plants in 1979 are presented in Table 8. Regression equations of visitation measures are given separately for 1) bumblebee pollinators, b) bumblebee robbers, c) all insect pollinators, and d) all insect robbers. The effects of plant size on bumblebee pollinator visitation in 1980 are given in the text below and are illustrated in Figures 3, 8, 9 and 10 (see also Table 5 and Appendix II [Tables 10-14]).

The indicator variable for size reduction never entered the regression equations of visitation (Table 8) and hence reduced plants in 1979 were visited as if they were originally smaller in size. Nearest neighbor distance was important in only one measure of visitation: the mean number of flowers probed per bumblebee robber, or visit length, increased with increasing distance between plants ($P < 0.003$, Table 8). Visit length of bumblebee pollinators did not increase significantly with isolation of plants. However, the sample size of visit lengths to isolated plants was small because many of these plants were not visited during the 15-minute observation period. In both years, bumblebees visited significantly more flowers per visit on large than on small plants (data of 1979; $P < 0.05$, Table 8; data of 1980, effect of Block x Size: $P < 0.05$, Fig. 3). However, the rate of increase in visit length was very small. Thus, in 1979, for each increment of ten stems on a plant, bumblebee pollinators visited an average of only 1.45 additional flowers (Table 8). In 1979, when data

from all insect pollinators or robbers were combined, there was no relationship between visit length and plant size (Table 8).

Because visit length increased very slowly with plant size, the proportion of a plant's flowers visited per visitor was negatively correlated with plant size (data of 1979, $P < 0.05$ for all classes of visitors, Table 8; data of 1980, effect of Size: $P < 0.06$, Fig. 8). If the proportion of flowers visited per visitor on a plant is taken as a measure of the intensity of exploitation of the plant by the visitor, it is seen that individual insects exploited large plants less intensely than small plants. This was not due to differing reward statuses of large and small plants. Large plants attracted more insects per minute than small plants (Geber, in prep) so that overall, the proportion of a plant's flowers visited per minute by all visitors, and hence the proportion of empty flowers, was constant over all plant sizes (data of 1979; $P > 0.5$ for all classes of visitors, Table 8; data of 1980, effect of Size: $P > 0.3$, $\bar{x}\%$ of flowers visited per minute = 0.015 ± 0.002).

The pattern of bumblebee pollinator visitation to stems of plants mirrored that to flowers. Bumblebee pollinators in 1980 visited significantly more stems per plant (effect of Size: $P < 0.05$, Fig. 9) but encountered a smaller proportion of stems per plant effect of Block \times Size : $P < 0.004$, Fig. 10) on large than on small plants. Thus flowers and stems of large plants were exploited less fully by individual bumblebees than those of small plants. Also, bumblebees visited only a small number and percentage of flowers and stems on plants of all sizes.

M. ciliata was extensively robbed at my study site (98% of the flowers on plants were pierced). Visitation by robbers accounted for approximately half of the flower visits ($55.9 \pm 3.3\%$ of visits for all insects, $44.1 \pm 5.6\%$ for bumblebees, 1979).

Eighty percent of the moves between plants by bumblebees were between nearest neighbors ($77.1 \pm 7.1\%$ in 1979, $80.3 \pm 2.4\%$ in 1980). In 1980, the percentage of moves that were to abutting stems of adjacent plants was positively correlated with the number of abutting stems ($r = 0.60$, $P < 0.01$), but not with plant size ($r = -0.14$, $P > 0.5$). Of the 18 plants from which data were obtained, large plants did not have more abutting stems than small plants. In general, however, the number of abutting stems on a plant increased with plant size ($r = 0.63$, $P < 0.001$, $N = 62$ plants).

The distribution of angular changes in direction is shown in Fig. 11. The distribution does not differ from a uniform distribution when tested with a Kolgomorov-Smirnov one sample test ($N = 681$, $P > 0.2$). Thus bumblebees did not maintain a constant direction of flight, but rather foraged randomly with respect to direction. These results are in agreement with those of Zimmerman (1979) for B. flavifrons foraging on the large perennial Polemonium foliossissimum, but do not agree with the directional flight pattern of bumblebees on the smaller flowering spikes of Delphinium nelsonii and Aconitum columbianum plants (Pyke, 1978b).

DISCUSSION

There are several factors which may contribute to the short visits of insects on M. ciliata plants and to the less intense exploitation of large plants by individual visitors at my study site. Some of them may also be important in other large flowering species.

A. Plant architecture and flower revisitation

The ability of individual pollinators to avoid revisiting flowers is likely to depend on the architecture, i.e. the spatial arrangement of flowers, of the plant. For example, the simple and well-defined structure of a Delphinium nelsonii flowering spike allows a pollinator to systematically visit a large proportion of the plant's flowers (at least 30 to 40%, Pyke 1978c) without revisits. Thus pollinators on vertical spikes tend to begin foraging at the bottom flowers of the spike and proceed upwards to a neighbor flower along the stem (Macior, 1969; Kevan, 1972; Pyke, 1978c, 1979; Heinrich, 1979; Waddington and Heinrich, 1979). The strongly directional flight pattern of pollinators both within and between flowering spikes (Pyke, 1978b, c, 1979) minimizes the probability of returning to previously searched areas (Pyke 1978b, Pleasants and Zimmerman, 1979). The architecture of M. ciliata and other large plants is more complex as it may consist of a hierarchy of flower arrangements: flowers in inflorescences, inflorescences on stems, and stems in plants. The architecture is also less easily defined because variability in the size and orientation of

inflorescences and stems leads to an irregular display of flowers. The complexity and/or irregularity of the floral arrangement could make it difficult for a pollinator to keep track of previously visited flowers since there is no easily defined entry point (bottom flower) to, or systematic pathway through the plant. The random flight of bumblebees on M. ciliata, and on the large perennial Polemonium foliossissimum (Zimmerman, 1979) suggests that bees do not keep track of where they have been. Bumblebees on P. foliossissimum, as on M. ciliata, visit only a small percentage (3-4%) of the available flowers. Zimmerman (1979) has argued, from optimal foraging considerations, that random flight is expected on P. foliossissimum because the probability of returning to a specific flower of a patch or plant is small. Just because a pollinator visits only a small percentage of a plant's flowers does not mean that the frequency of flower revisitation is negligible. Furthermore, random flight may result from the inability of pollinators to remember the precise location of flowers, rather than from the ability to forage optimally. I suggest that as the complexity and size of the floral arrangement increases, the percentage of flowers visited before revisits occur declines. In a laboratory experiment on artificial "complex" plants, Bombus impatiens workers were found to return quickly to previously visited flowers (Plowright and Geber, unpubl.). Each of 12 bumblebees foraged from a small (44 flowers), medium (104 flowers), and large (256 flowers) spherical "plants" on which artificial flowers were arranged in approximate geodesic arrays. The mean number and percentage of flowers probed before the first revisit was small on all three plants (small plant: 7.5 ± 0.97 flowers,

17.0 \pm 2.2% of available flowers; medium plant: 13.3 \pm 1.64 flowers, 12.8 \pm 1.6% of flowers; large plant: 14.2 \pm 2.47 flowers, 5.5 \pm 0.96% of flowers). The mean number of flowers visited before the first revisit increased logarithmically with plant size. Hence the mean percentage of flowers visited before the first revisit was inversely related to plant size. The same patterns held for subsequent revisits.

If pollinators do begin to revisit flowers soon after arriving at a plant, they may depart from the plant quickly. The complex architecture of M. ciliata and other large perennial shrubs, then, could enhance cross-pollination. Furthermore, the results of the laboratory experiment suggest that flower revisitation begins on large plants after a smaller proportion of the flowers have been probed than on small plants; and thus individual pollinators should exploit large plants less intensely than small plants. This was true for M. ciliata and for Oenothera fruticosa (Silander and Primack, 1978). If a plant is very large (e.g. a tropical tree), early flower revisitation may not be sufficient to cause a pollinator to depart from the plant: the pollinator may not fly far enough after encountering empty flowers to leave the plant entirely.

B. Plant architecture and proximity of neighbor plants.

When plants grow close together, a pollinator may be drawn away from the edge of one plant by neighboring plants. My studies on bumblebee movement suggest that this occurs at my study site: bumblebees moved

predominantly between nearest neighbor plants; and the percentage of moves to abutting stems of adjacent plants was positively correlated with the number of abutting stems. Abutting stems are often as close in the line of flight of a bumblebee as stems of the plant on which the bee is foraging. For this reason, bumblebees move readily between adjacent plants. Conversely, bumblebee robbers remained longer on plants that were isolated. The high density of plants at my study site, therefore, enhances cross-pollination. Plant density, however, is an attribute of a population of plants and cannot be seen as an adaptation of individual plants.

There are two properties of M. ciliata plants that may favor cross-pollination as plant size increases. The first of these is the circular geometry of plants. In a random walk through a plant, a pollinator will encounter stems in proportion to the plant's radius:

$$\text{No. of stems visited} = c_1 r, \quad (1)$$

where c_1 is a constant, and r is the radius of the plant. Assuming that the density of stems (stems per unit area) is the same for large and small plants (see below), the total number of stems in a plant (plant size) is proportional to the area of the plant:

$$\text{plant size} = c_2 \pi r^2, \quad c_2 \text{ a constant} \quad (2)$$

Dividing equation (1) by (2):

$$\% \text{ stems visited on a plant} = \frac{c_3}{r\pi}, \text{ where } c_3 = \frac{c_1}{\pi c_2} \quad (3)$$

Since the radius increases with plant size, a bumblebee would tend to arrive at the edge of a large plant after visiting a smaller proportion of stems (and flowers) than of a small plant. Equation (3) predicts

that the product of the proportion of stems visited per bumblebee times the radius of the plant does not vary with plant size. However, when I use the results of bumblebee visitation of 1980 and measures of the radii of plants, I find that this product declines with increasing plant size ($r = -0.66$, $P < 0.05$, $N = 18$ plants). The second property of M. ciliata plants may account for this negative correlation. The density of stems within a plant is not constant (as assumed in equation (2)) but rather increases significantly with plant size ($r = 0.63$, $P < 0.001$, $N = 62$ plants). In other words, stems grow closer together in large than in small plants. Just as a pollinator misses stems of one plant by moving to those of a closely adjacent plant, it can also bypass stems within a plant by flying to only one of many possible neighbor stems. The number of stems bypassed in this manner will increase with stem density. Indeed, if two plants differ only in the density of stems, but not in size, a pollinator would have to employ a more circuitous route on the denser plant in order to visit the same percentage of stems on both plants.

In summary, high plant density, the circular geometry and the density of stems of M. ciliata plants all favor cross-pollination. The two attributes of individual plants (geometry and stem density) may also contribute to the less intense exploitation of large plants by individual visitors.

C. Nectar robbing and variable flower rewards.

M. ciliata is extensively robbed at my study site and at other locations (Pelton, 1961). M. arizonica, another profusely-flowering member of the genus, is also robbed (Matthews and Conrad, 1968). Robbery has been reported for many large plants (Cazier and Lindsley, 1974; Frankie and Baker, 1974; Barrows, 1976; Augspurger, 1980; Wyatt, 1980). Robbery may be direct as in the hole piercing of B. mixtus on M. ciliata; or it may be indirect when flowers are visited normally but the morphology and/or behavior of the flower visitors are such that pollination is not effected. Territorial animals on large self-incompatible plants (see citations in Introduction) and many unspecialized flower visitors reported from large plants (Frankie, 1973, 1976; Frankie and Baker, 1974; Carpenter, 1976; Augspurger, 1980) may act primarily as thieves. Heinrich and Raven (1972) suggested that robbery, by reducing the average flower reward, may enhance cross-pollination, because pollinators would be forced to visit more flowers. Robbery also increases the number of empty flowers and the variance among flowers in reward (Frankie and Baker, 1974). Both of these factors can promote more rapid pollinator movement (see citations in Introduction), and hence cross-pollination.

CHAPTER IV.
SUMMARY AND CONCLUSIONS

In the first manuscript of this thesis, I propose and test the hypothesis that inbreeding increases with plant size in Mertensia ciliata, a profusely-flowering, self-compatible perennial.

I first show that selfing in this species lowers the number and weight of seeds produced by a flower compared with outcrossing; and thus, increased selfing in larger plants would result in a loss to the reproductive output of individual flowers. I measure experimentally the transfer of outcrossed and total (self + outcrossed) pollen between flowers by individual bumblebees, and find that pollen carryover in this species is extensive. Observations of pollinator foraging behavior on plants demonstrate that 1) individual bumblebees visit only a few more flowers per visit to large than to small plants; and 2) large plants attract more bumblebees per minute than small plants. I use this information to predict the amount of outcrossed pollen to reach a flower of different-sized plants from a single pollinator visit and over the lifetime of a flower. I conclude that large plants will receive slightly less outcrossed pollen per flower from single visits than small plants, but that they can compensate for this loss by attracting more bumblebees. In the end, flowers of all plants should receive equal amounts of outcrossed pollen. This prediction is supported by measurements of pollen on flowers in the field. As a result, rates of selfing should not differ among plants. I show that the reproductive output of flowers is similar on all plants; a result that is

inconsistent with the original hypothesis, but that supports the conclusion of equal rates of selfing in large and small plants.

In the second chapter, a more detailed examination of insect visitation to plants is presented. I show that 1) individual insects visit only a small number and proportion of flowers on a plant; 2) individual bumblebee visit only a few more flowers and stems, and thus encounter a smaller percentage of flowers and stems on large than on small plants. Single bumblebees, therefore, exploit large plants less intensely than small plants, and this is not due to differing reward statuses of plants; 3) bumblebees in flights between plants move predominantly between adjacent plants; 4) they do not maintain a constant direction of flight, but rather forage randomly with respect to direction; 5) many visits to plants are by nectar robbing insects.

I discuss the role of four factors in promoting pollinator movement between plants, and cross-pollination, and in explaining the less intense exploitation of large plants. 1) The complex architecture of the flowering display in M. ciliata may make it difficult for a pollinator to keep track of its path on plants. The random flight of bees suggest that this is so. A pollinator may therefore return to a previously-visited and empty flower soon after arriving at a plant. If a visit to empty flowers prompts the pollinator to leave the plant, it will visit only a small number and proportion of flowers. Revisits should begin to occur after a smaller proportion of flowers are visited on large than on small plants. Laboratory evidence for this hypothesis is presented. 2) The close proximity of plants in the population enhances pollinator movement. 3) The circular geometry of plants and

the greater density of stems in large plants mean that a pollinator will traverse a large plant after visiting a smaller percentage of stems and flowers than on a small plant. 4) Nectar robbing, by increasing the variance among flowers in reward, may promote rapid pollinator movement between plants.

Literature Cited

- Auspurger, C. K. 1980. Mass-flowering of a tropical shrub (Hybanthus prunifolius): influence on pollinator attraction and movement. *Evolution* 34: 475-488.
- Barrows, E. M. 1976. Nectar robbing and pollination of Lantana camara (Verbenaceae). *Biotropica* 8: 132-135.
- Bateman, A. J. 1956. Cryptic self-incompatibility in the wallflower: Cheiranthus cheiri L. *Heredity* 10: 257-261.
- Beattie, A. J. 1971. A technique for the study of insect-borne pollen. *Pan Pacif. Ent.* 47:82.
- Carpenter, F. L. 1976. Plant-pollinator interactions in Hawaii: pollination energetics of Metrosideros collina (Myrtaceae). *Ecology* 57:1125-1144.
- Cazier, M. A., and E. G. Linsley. 1974. Foraging behavior of some bees and wasps at Kallstroemia grandiflora flowers in southern Arizona and New Mexico. *Amer. Mus. Nov.* 2546: 1-20.
- Charnov, E. L. 1976. Optimal foraging, the marginal value theorem. *Theor. Pop. Biol.* 9: 129-135.
- Colwell, R. 1951. The use of radioactive isotopes in determining spore dispersal patterns. *Amer. J. Bot.* 38: 511-523.
- Feinsinger, P. 1978. Ecological interactions between plants and hummingbirds in a successional tropical community. *Ecol. Monog.* 48: 269-287.
- Frankie, G. W. 1973. Tropical forest phenology and pollinator-plant coevolution, p. 192-209. In L. E. Gilbert and P. H. Raven (eds.), *Coevolution of Animals and Plants*. University of Texas Press, Austin and London.
- Frankie, G. W. 1976. Pollination of widely dispersed trees by animals in Central America, with emphasis on bee pollination systems, p. 151-159. In J. Burley and B. T. Stiles (eds.), *Variation, Breeding, and Conservation of Tropical Forest Trees*. Academic Press, London.
- Frankie, G. W., and H. G. Baker. 1974. The importance of pollinator behavior in the reproductive biology of tropical trees. *An. Inst. Biol. Univ. Nat. Auton. Mexico* 45, Ser. Botanica 1: 1-10.
- Frankie, G. W., P. A. Opler, and K. S. Bawa. 1976. Foraging behavior of solitary bees: implications for outcrossing of a neotropical forest tree species. *J. Ecology* 64: 1049-1057.

- Free, J. B. 1962. The effect of distance from pollinizer varieties on the fruit set of trees in plum and apple orchards. *J. Hort. Sci.* 37: 262-271.
- Gass, C. L., and R. D. Montgomerie. 1979. Hummingbird foraging behavior: decision making and energy regulation, p. 92-111. In A. C. Kamil and T. D. Sargent (eds.), *Mechanisms of Foraging Behavior*. Garland Press.
- Gentry, A. H. 1974a. Flowering phenology and diversity in tropical Bignoniaceae. *Biotropica* 6: 64-68.
- Gentry, A. H. 1974b. Coevolutionary patterns in Central American Bignoniaceae. *Annals of Missouri Bot. Gar.* 61: 728-759.
- Gentry, A. H. 1976. Bignoniaceae of Southern Central America: distribution and ecological specificity. *Biotropica* 10: 184-193.
- Gill, F. B., and L. L. Wolf. 1975. Economics of feeding territoriality in the golden-winged sunbird. *Ecology* 56: 333-345.
- Gould, E. 1978. Foraging behavior of Malaysian nectar-feeding bats. *Biotropica* 10: 184-193.
- Hartling, L. K., and R. C. Plowright. 1979a. Foraging by bumblebees on patches of artificial flowers: a laboratory study. *Can. J. Zool.* 57: 1866-1870.
- Hartling, L. K., and R. C. Plowright. 1979b. An investigation of inter- and intra-inflorescence visitation rates by bumblebees on red clover with special reference to seed-set. *Proc. IV. Int. Symp. on Pollination*. Maryland Agricultural Experiment Station Special Miscellaneous Publ. No. 1: 457-460.
- Heinrich, B. 1979. Resource heterogeneity and patterns of movement in foraging bumblebees. *Oecologia* 40: 235-245.
- Heinrich, B., and P. H. Raven. 1972. Energetics and pollination ecology. *Science* 176: 597-602.
- Hitchcock, C. L., A. Cronquist, M. Ownbey, and J. W. Thompson. 1969. Vascular Plants of the Pacific Northwest. Univ. of Washington Press. Seattle and London.
- Janzen, D. H. 1969. Seed eaters versus seed size, number, toxicity, and dispersal. *Evolution* 21: 1-27.
- Janzen, D. H. 1977. A note on optimal mate selection by plants. *Amer. Natur.* 111: 365-371.

- Kevan, P. G. 1972. Insect-flower associations in the high arctic with special reference to nectar. *J. Ecol.* 60: 831-847.
- Krebs, J. R. 1973. Behavioral aspects of predation, p. 73-111. In P. G. Bateson and P. H. Klopfer (eds.). *Perspectives in Ethology*. New York, Plenum Press.
- Lertzman, K. P. 1981. Pollen transfer kinetics: processes and consequences. Unpublished MS Thesis. Univ. of British Columbia, Vancouver.
- Lertzman, K. P., and C. L. Gass. 1982. Alternative models of pollen transfer. In C. E. Jones and R. J. Little (eds.), *Handbook of Experimental Pollination Ecology*. Van Nostrand Reinhold, New York, in press.
- Levin, D. A. in press. Plant parentage: another view of the breeding structure of plant populations. In C. E. King and P. S. Dawson (eds.), *Population Biology: Retrospect and Prospect*. Columbia Univ. Press.
- Levin, D. A., and D. E. Berube. 1972. Phlox and Colias: the efficiency of a pollination system. *Evolution* 26: 242-250.
- Levin, D. A., and H. W. Kerster. 1968. Local gene dispersal in Phlox. *Evolution* 22: 130-139.
- Levin, D. A., and H. W. Kerster. 1969a. Density-dependent gene dispersal in Liatris. *Amer. Natur.* 103: 61-74.
- Levin, D. A., and H. W. Kerster. 1969b. The dependence of bee-mediated pollen and gene dispersal upon plant density. *Evolution* 23: 560-571.
- Levin, D. A., and H. W. Kerster. 1974. Gene flow in plants. *Evolutionary Biology* Vol. 7: 138-220.
- Levin, D. A., H. W. Kerster, and M. Niedzlek. 1971. Pollinator flight directionality and its effects on pollen flow. *Evolution* 25: 113-118.
- Linhart, Y. B. 1973. Ecological and behavioral determinants of pollen dispersal in hummingbird-pollinated Heliconia. *Amer. Natur.* 107: 511-523.
- Lloyd, D. G. 1980. Sexual strategies in plants. I. An hypothesis of serial adjustment of maternal investment during one reproductive session. *New Phytol.* 86: 69-79.

- Lloyd, D. G., C. J. Webb, and R. B. Primack. 1980. Sexual strategies in plants. II. Data on the temporal regulation of maternal investment. *New Phytol.* 86: 81-92.
- Macior, L. W. 1969. Pollination adaptation in Pedicularis lanceolata. *Amer. J. Bot.* 56: 853-859.
- Matthews, V. B., and P. W. Conrad. 1968. An ecological life history of the tall bluebell (Mertensia arizonica var. leonardi) in Utah. *Ecology* 49: 1113-1123.
- Morse, D. H. 1980. The effect of nectar abundance on foraging patterns of bumblebees. *Ecol. Ent.* 5:53-59.
- Mulchahy, D. L. (ed.). 1975. Gamete Competition in Plants and Animals. North-Holland Publ., Co., Amsterdam and Oxford.
- Mulchahy, D. L. 1979. The rise of angiosperms: a genecological factor. *Science* 206: 20-23.
- Neter, J., and W. W. Wasserman. 1974. Applied Linear Statistical Models. Richard D. Irwin, Inc.
- Ollason, J. G. 1980. Learning to forage - optimally? *Theor. Pop. Biol.* 18: 44-56.
- Pelton, J. 1981. An investigation of the ecology of Mertensia ciliata in Colorado. *Ecology* 42: 38-52.
- Perkins, M. D. C. 1977. Dynamics of hummingbird-mediated pollen flow in a subalpine meadow. Unpubl. MS Univ. of British Columbia, Vancouver.
- Platt, W. J., G. R. Hill, and S. Clark. 1974. Seed production in a prairie legume (Astragalus canadensis L.). *Oecologia* 17: 55-63.
- Pleasants, J. M., and M. Zimmerman. 1979. Patchiness in the dispersion of nectar resources: evidence for hot and cold spots. *Oecologia* 41: 283-288.
- Plowright, R. C., and L. K. Hartling. in press. Red clover pollination by bumblebees: a study of the dynamics of a plant-pollinator relationship. *J. Ecology*.
- Price, M. W., and N. M. Waser. 1979. Pollen dispersal and optimal outcrossing in Delphinium nelsoni. *Nature* 277: 294-296.
- Price, M. V., and N. M. Waser. in press. Experimental studies of pollen carryover: hummingbirds and Ipomopsis aggregata. *Oecologia*.

- Pyke, G. H. 1978a. Optimal foraging in hummingbirds: testing the marginal value theorem. *Amer. Zool.* 18: 739-752.
- Pyke, G. H. 1978b. Optimal foraging: movement patterns of bumblebees between inflorescences. *Theor. Pop. Biol.* 13: 72-98.
- Pyke, G. H. 1978c. Optimal foraging in bumblebees and coevolution with their plants. *Oecologia* 36: 281-293.
- Pyke, G. H. 1979. Optimal foraging in bumblebees: rule of movement between flowers within inflorescences. *Anim. Behav.* 27: 1167-1181.
- Pyke, G. H., H. R. Pulliam, E. L. Charnov. 1977. Optimal foraging: a selective review of theory and tests. *Quart. Rev. Biol.* 52: 137-154.
- Real, L. A. 1981. Uncertainty and pollinator-plant interactions: the foraging behavior of bees and wasps on artificial flowers. *Ecology* 62: 20-26.
- Richards, A. J., and H. Ibrahim. 1978. Estimation of neighborhood size in two populations of Primula veris. In A. J. Richards (ed.), the Pollination of Flowers by Insects. Linnean Society Symposium Series 6: 165-174.
- Schaal, B. A. 1980. Measurement of gene flow in Lupinus texensis. *Nature* 284: 450-451.
- Schaffer, W. M., and M. V. Schaffer. 1979. The adaptive significance of variations in reproductive habit in Agavaceae II: pollinator foraging behavior and selection for increased reproductive expenditure. *Ecology* 60: 1051-1069.
- Schemske, D. W. 1980. Floral ecology and hummingbird pollination of Combretum farinosum in Costa Rica. *Biotropica* 12: 169-181.
- Schlising, R. A., and R. A. Turpin. 1971. Hummingbird dispersal of Delphinium cardinale pollen treated with radioactive iodine. *Amer. J. Bot.* 58: 401-406.
- Schmitt, J. 1980. Pollinator foraging behavior and gene dispersal in Senecio (Compositae). *Evolution* 34: 934-943.
- Silander, J. A. 1978. Density-dependent control of reproductive success in Cassia biflora. *Biotropica* 10: 292-296.
- Silander, J. A., and R. B. Primack. 1978. Pollination intensity and seed set in the evening primrose (Oenothera fruticosa). *Amer. Midl. Natur.* 100: 213-216.

- Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co. San Francisco.
- Stephenson, A. G. 1979. An evolutionary examination of the floral display of Catalpa speciosa (Bignoniaceae). *Evolution* 33: 1200-1209.
- Stiles, F. G. 1979. Ecology, flowering phenology, and hummingbird pollination of some Costa Rican Heliconia species. *Ecology* 56: 285-301.
- Thomson, J. D., and R. C. Plowright. 1980. Pollen carryover, nectar rewards, and pollinator foraging behavior with special reference to Diervilla lonicera. *Oecologia* 46: 68-74.
- Waddington, K. D. 1980. Flight patterns of foraging bees relative to density of artificial flowers and distribution of nectar. *Oecologia* 44: 199-204.
- Waddington, K. D., and B. Heinrich. 1979. The foraging movements of bumblebees on vertical 'inflorescences': an experimental analysis. *J. Comp. Physiol.* 134: 113-117.
- Waser, N. M. in press. A comparison of distances flown by different visitors to flowers of the same species. *Oecologia*.
- Waser, N. M., and M. V. Price. 1982. Optimal and actual outcrossing in plants, and the nature of the plant-pollinator interaction. In C. E. Jones and R. J. Little (eds.), Handbook of experimental pollination ecology. Van Nostrand Reinhold, New York, in press.
- Waser, N. M., and M. V. Price. in press. A comparison of pollen and fluorescent dye carryover by natural pollinators of Ipmopsis aggregata (Polemoniaceae).
- Willson, M. F. 1979. Sexual selection in plants. *Amer. Natur.* 113: 777-790.
- Willson, M. F., and J. B. Rathcke. Adaptive design of the floral display in Asclepias syriaca L. *Amer. Midl. Natur.* 92: 47-57.
- Willson, M. F., L. J. Miller, and B. J. Rathcke. 1979. Floral display in Phlox and Geranium: adaptive aspects. *Evolution* 33: 52-63.
- Wolf, L. L. 1970. The impact of seasonal flowering on the biology of some tropical hummingbirds. *Condor* 72: 1-14.
- Wyatt, R. 1980a. The reproductive biology of Asclepias tuberosa: I. Flower number, arrangement, and fruit set. *New Phytol.* 85: 119-131.

- Wyatt, R. 1980b. The impact of nectar-robbing ants on the pollination system Asclepias curassavica. Bull. Torr. Bot. Club 107: 24-28.
- Wright, S. 1950. The genetical structure of populations. Nature 166: 247-249.
- Zimmerman, M. 1979. Optimal foraging: A case for random movement. Oecologia 43: 261-267.

APPENDIX I

Table 1. Design and tests of the breeding experiment on *Mertensia ciliata*. All five pollination treatments were performed on each of ten plants (sixty flowers/treatment/plant). Plants were considered to be random factors (blocks), and pollination treatments were fixed factors.

Pollination Treatment	Insect Exclusion	Anther Removal	Pollination	Test for seed set due to:
1	Yes	Yes	None	Apomixis ^a
2	Yes	No	None	Autogamy ^a
3	Yes	Yes	Hand-outcrossed	Outcrossing ^b
4	Yes	No	Hand-selfed	Selfing ^b
5	No	No	Open-pollination	Pollination by insects ^a

a. A comparison of Treatment 5 vs. Treatments 1 and 2 measures the dependence of *M. ciliata* on insect pollination for seed set.

b. A comparison of Treatments 3 and 4 measures the relative abilities of outcrossed and self pollen in fertilizing ovules when the two pollens are involved in independent matings.

Table 2. Analysis of seed set per flower from breeding experiment by 2-way mixed model ANOVA (plants: random effect; pollination treatment: fixed effect).

Source	d.f.	MS	F	P <
Plant	9	2.89	3.32	0.0005
Pollination Treatment	4	373.51	68.79 ⁺	0.0001
Plant x Pollination Treatment	36	5.44	6.25	0.0001
Error	2950	0.87		

⁺ The MS (Plant x Pollination Treatment) was used to test the effects of pollination treatment (Sokal and Rohlf, 1969).

Table 3. Mean seed set, standard errors, and coefficient of variation of means of the five pollination treatments in the breeding experiment. Means are based on ten plants.

Pollination Treatment	$\bar{x} \pm \text{s.e.}$	C.V.
1. Apomixis	0.040 ± 0.020	157.2
2. Autogamy	0.164 ± 0.044	85.3
3. Outcrossing	0.723 ± 0.142	62.1
4. Selfing	0.393 ± 0.072	57.9
5. Open	2.045 ± 0.080	12.4

Table 4. Outcrossed pollen deposition and total (self + outcrossed) pollen deposition on flowers from two emasculated and two non-emasculated pollen carryover runs.

Outcrossed pollen deposition on emasculated flowers	Flower order																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
no. of pollen grains	1. 101	15	6	41	*	15	0	6	30	7	*	43	5	8	16	7	4	1	1	3	1				
	2. 14	4	13	3	3	7	2	3	3	1	2	2	1	0	5	1	8	4		0	3	0	0	0	1
Total (self + outcrossed) pollen deposition on non-emasculated flowers																									
no. of pollen grains	1. 157	172	128	181	180	230	86	102	138	82	263	299	41	34	49	109	137	227							
	2. 246	245	*	124	22	94	60	96	69	20	30	60	188	58	189	251	32	159	94						

* Pollen counts are not available because flowers were lost or stigmas had been damaged during the emasculation procedure.

TABLE 5. Summary of ANOVAS on the measures of bumblebee visitation to plants.

Source ^a	no. bees arriving/ minute	% flowers visited/ minute	no. flowers visited/ bee	% flowers visited/ bee	no. stems visited/ bee	% stems visited/ bee
B	*** ^b	**	NS	NS	*	***
S	NS	NS	NS	NS ^c	*	NS
E	NS	NS	NS	NS	NS	NS
BxS	*	NS	*	NS	NS	**
BxE	NS	NS	NS	NS	NS	NS
SxE	NS	NS	NS	NS	NS	NS
BxSxE	NS	NS	*	NS	NS	NS

a. B = Block, S = Size, E = Emasculation treatment

b. NS: not significant; * : $P < 0.05$; ** : $P < 0.01$; *** : $P < 0.001$

c. Effect of Size: $P < 0.06$

TABLE 6. Summary of ANOVAS on pollen receipt by flowers, on seed-set per flower, and on seed weight.

Source ^a	no. pollen grains/ stigma	no. seeds/ flower	seed weight
B	*	**	***
S	NS	NS	NS
E	NS	NS	NS
BxS	NS	*	**
BxE	**	***	NS
SxE	NS	NS	NS
BxSxE	NS	**	**

a. B = Block, S = Size, E = Emasculation treatment

b. NS: not significant; * : $P < 0.05$; ** : $P < 0.01$;
*** : $P < 0.001$

Table 7. The pattern of bumblebee pollinator visitation of *Mertensia ciliata* in 1979 as a function of plant size. Regression equations relate each measure of pollinator visitation (\hat{Y}_1) to plant size (x = no. of stems), except for \bar{Y}_2 which is independent of plant size. N = no. of plants from which observations were obtained.

No. of bumblebees arriving at a plant/minute ($N = 80$)	Percentage of a plant's flowers visited/minute ($N = 80$) ⁺	No. of flowers visited/bumblebee on a plant ($N = 44$)
$\hat{Y}_1 = 0.025 + 0.002x$	$^+ \bar{Y}_2 = 0.001 \pm 0.0003$	$\hat{Y}_3 = 4.15 + 0.145x$
$r^2 = 0.18$		$r^2 = 0.11$
$P < 0.0001$	$P > 0.5$	$P < 0.05$

⁺ Y_2 is independent of plant size.

Table 8. The pattern of insect visitation of *Mertensia ciliata* in 1979. Regression equations relate each measure of visitation (\bar{Y}_i) to plant size (x = no. of stems) and/or distance between a plant and its nearest neighbor (z), (but see remarks a and c). Regression equations were computed separately for a) bumblebee pollinators, b) bumblebee robbers, c) all insect pollinators, and d) all insect robbers. N = no. of plants from which observations were obtained.

Class of Insect Visitor	Proportion of a plant's ^a Flowers visited / minute	Mean no. Flowers visited / visitor	Mean proportion of a plant's flowers visited / visitor
Bumblebee Pollinators	$\bar{Y}_1 = 0.0014 \pm 0.0003$ $p > 0.5$ ($N = 80$)	$\hat{Y}_2 = 4.15 + 0.145x$ $r^2 = 0.11, p < 0.05$ ($N = 44$)	$\hat{Y}_3 = 0.108 - 0.001x$ $r^2 = 0.14, p < 0.01$ ($N = 44$)
Bumblebee Robbers	$\bar{Y}_1 = 0.0015 \pm 0.0003$ $p > 0.5$ ($N = 80$)	$\hat{Y}_2 = 3.28 + 0.0117x^b + 4.53z$ $r^2 = 0.22, p < 0.05,$ $p < 0.003$ ($N = 33$)	$\hat{Y}_3 = 0.115 - 0.001x$ $r^2 = 0.12, p < 0.05$ ($N = 33$)
All Insect Pollinators	$\bar{Y}_1 = 0.0021 \pm 0.0003$ $p > 0.5$ ($N = 80$)	$\bar{Y}_2 = 2.87 \pm 0.79^c$ $p > 0.2$ ($N = 73$)	$\hat{Y}_3 = 0.055 - 0.006x$ $r^2 = 0.10, p < 0.007$ ($N = 73$)
All Insect Robbers	$\bar{Y}_1 = 0.0024 \pm 0.0003$ $p > 0.5$ ($N = 80$)	$\bar{Y}_2 = 2.29 \pm 0.36^c$ $p > 0.2$ ($N = 73$)	$\hat{Y}_3 = 0.43 - 0.004x$ $r^2 = 0.09, p < 0.008$ ($N = 73$)

- a. The proportion of a plant's flowers visited per minute; \bar{Y}_1 , is independent of plant size for all classes of visitors
b. The first p value refers to plant size (x), the second to nearest neighbor distance (z)
c. The mean no. of flowers visited / visitor is independent of plant size when observations of all insect pollinators or robbers are combined.

Figure 1. Outcrossed pollen deposition on emasculated flowers visited in sequence by a bumblebee as a function of the order (i) of a flower in the visit sequence. Circles represent the mean numbers of pollen grains reaching flowers; vertical bars show one standard error around the mean. Data were obtained from fourteen carryover sequences. The line represents the least-squares exponential regression for outcrossed pollen deposition against flower order.

Figure 1

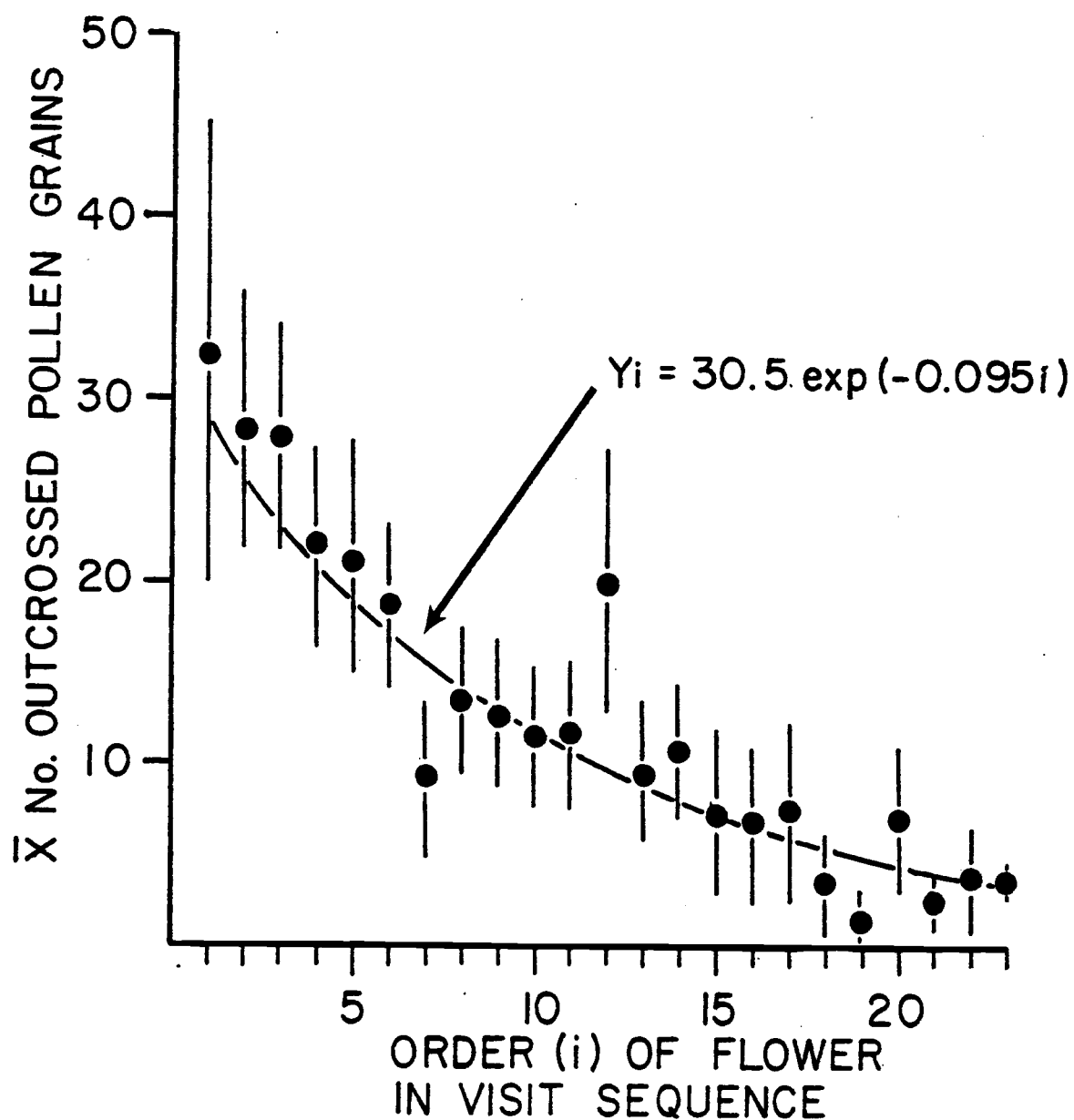
OUTCROSSED POLLEN DEPOSITION AND
CARRYOVER ON SUCCESSIVE FLOWERS

Figure 2. Effect of emasculation treatments on the receipt of pollen by flowers in the field. The mean number of pollen grains per stigma and one standard error are shown for emasculated (E), clipped (CL), and control (C) plants of the first and second blocks. Comparisons were made between pairs of means from plants within a given block using Scheffé's method of multiple comparisons (combined $\alpha=0.05$). When the same letter appears above two means (e.g., a), the means are significantly different from one another.

Figure 2

EFFECT OF EMASCULATION TREATMENTS ON POLLEN COUNTS PER STIGMA

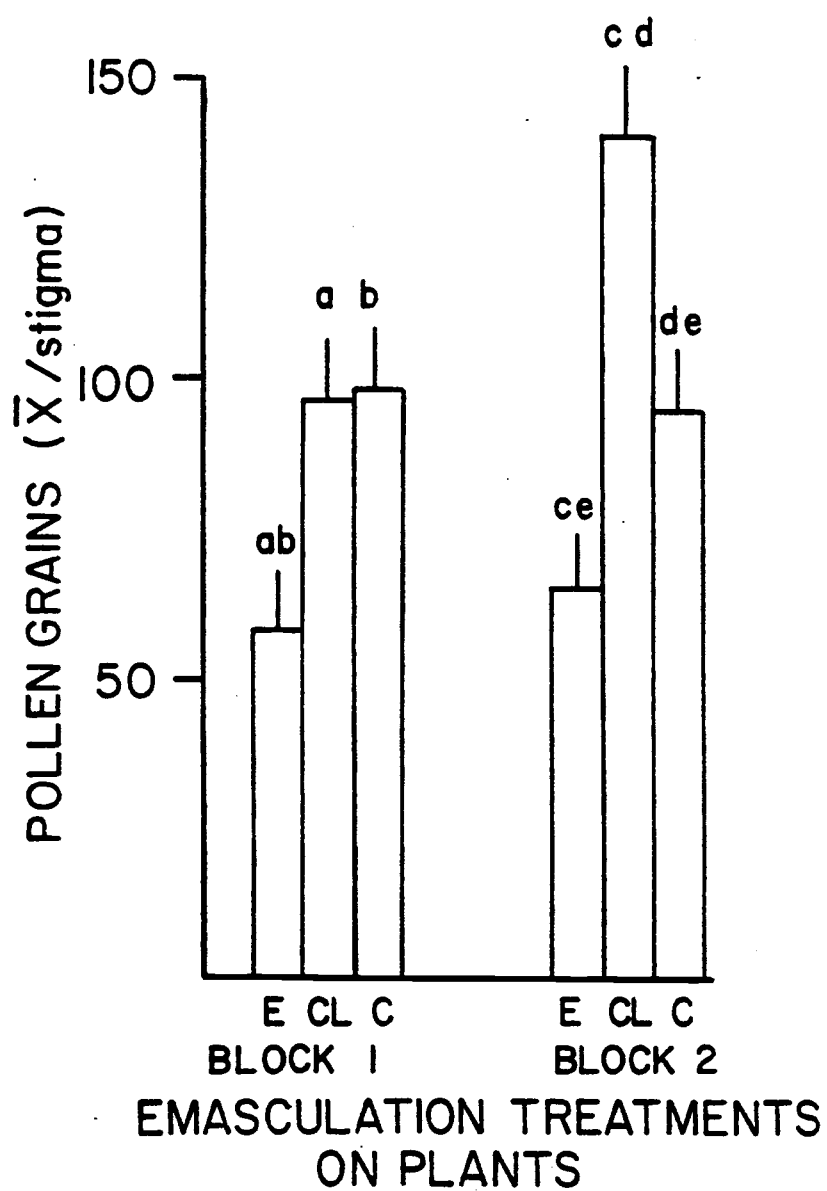


Figure 3. Effect of plant size on the number of flowers visited per pollinator on a plant. The mean number of flowers visited per bumblebee and one standard error are shown for small (S), medium (M), and large (L) plants of the first and second blocks. Comparisons were made between pairs of means within blocks using Scheffé's method of multiple comparisons (combined $\alpha=0.05$). When the same letter (e.g., a) appears above two means, the means are significantly different from one another.

Figure 3

EFFECT OF PLANT SIZE ON VISIT LENGTH OF BUMBLEBEES ON PLANTS

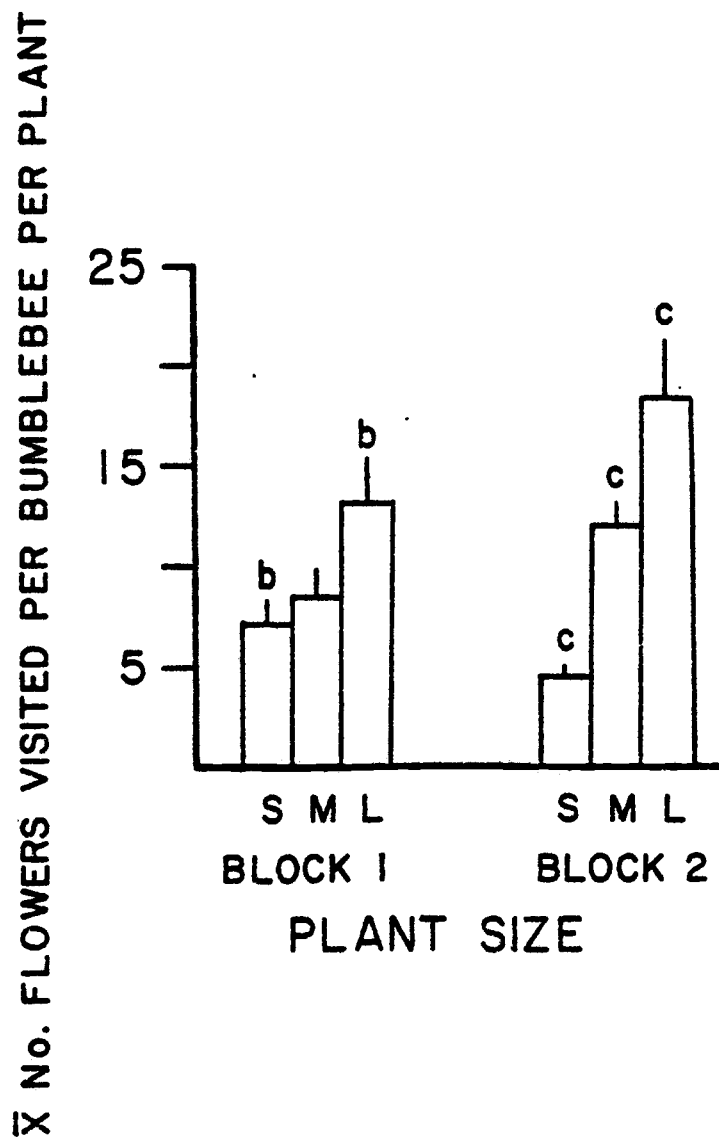


Figure 4. Effect of plant size on the arrival rate of pollinators to plants. The mean number of bumblebees arriving at a plant per minute and one standard error are shown for small (S), medium (M), and large (L) plants of the first and second blocks. Comparisons were made between pairs of means within blocks using Scheffé's method of multiple comparisons (combined $\alpha=0.05$). When the same letter appears above two means, the means are significantly different from one another.

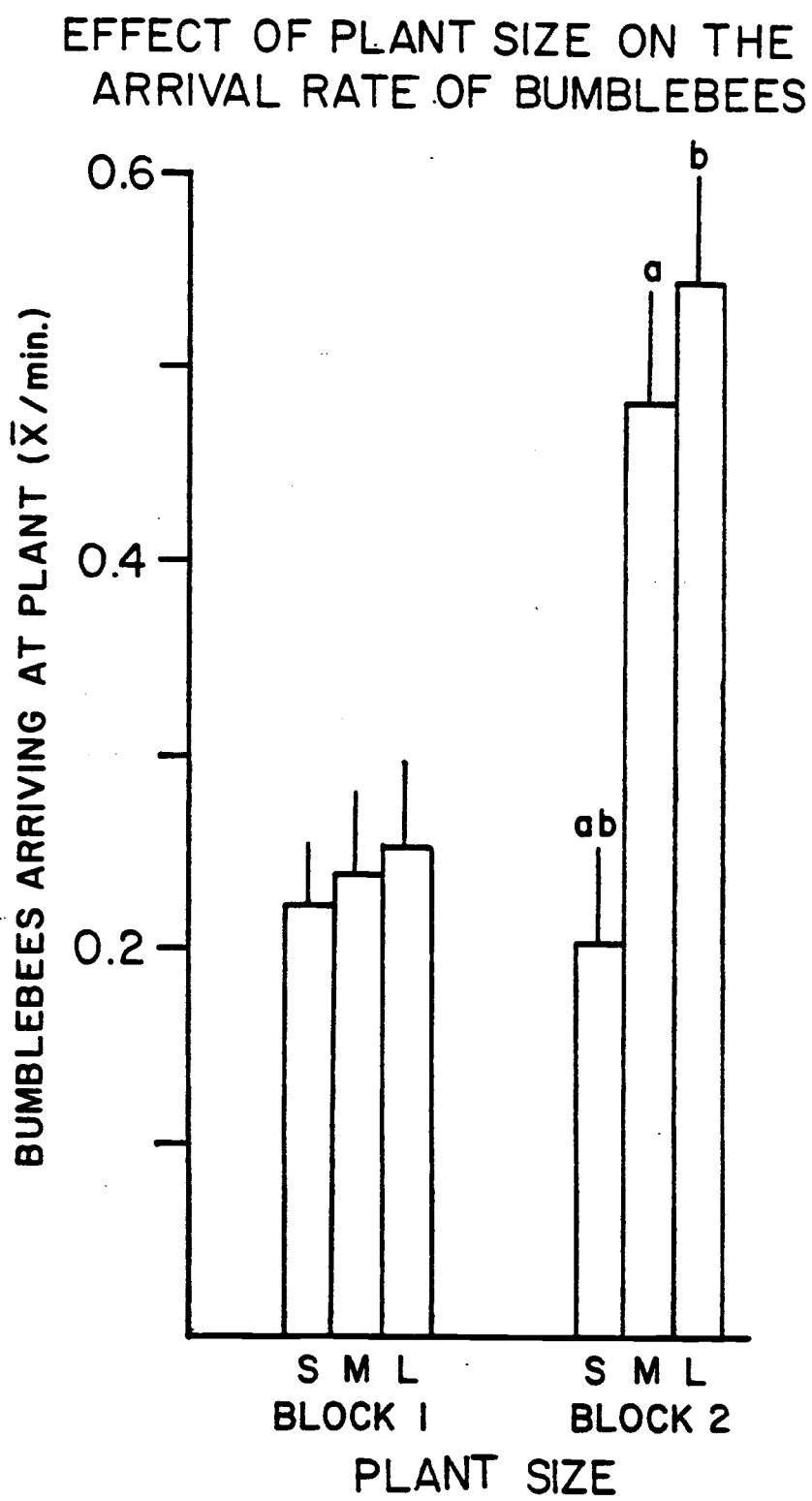


Figure 4

Figure 5. Effect of emasculation treatment and plant size on seed-set per flower. The mean number of seeds per flower and one standard error are shown for emasculated (E), clipped (CL), and control (C) plants of small (S), medium (M), and large (L) sizes in the first and second blocks. Comparisons were made between means of plants of a given size and block and of different emasculation treatment using Scheffé's method of multiple comparisons (combined $\alpha=0.05$). When the same letter (e.g., a) appears above two means the means are significantly different from one another.

Figure 5

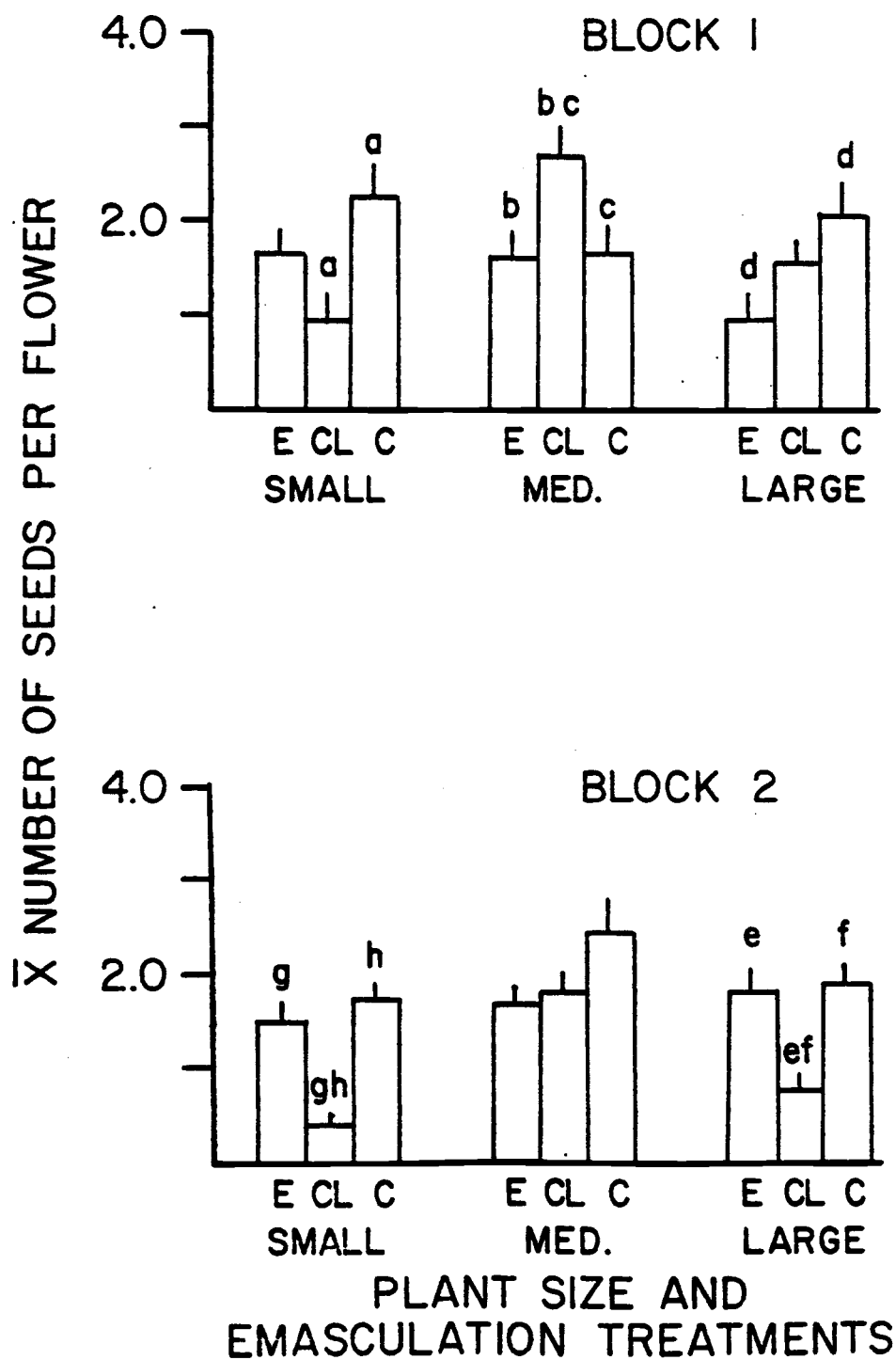
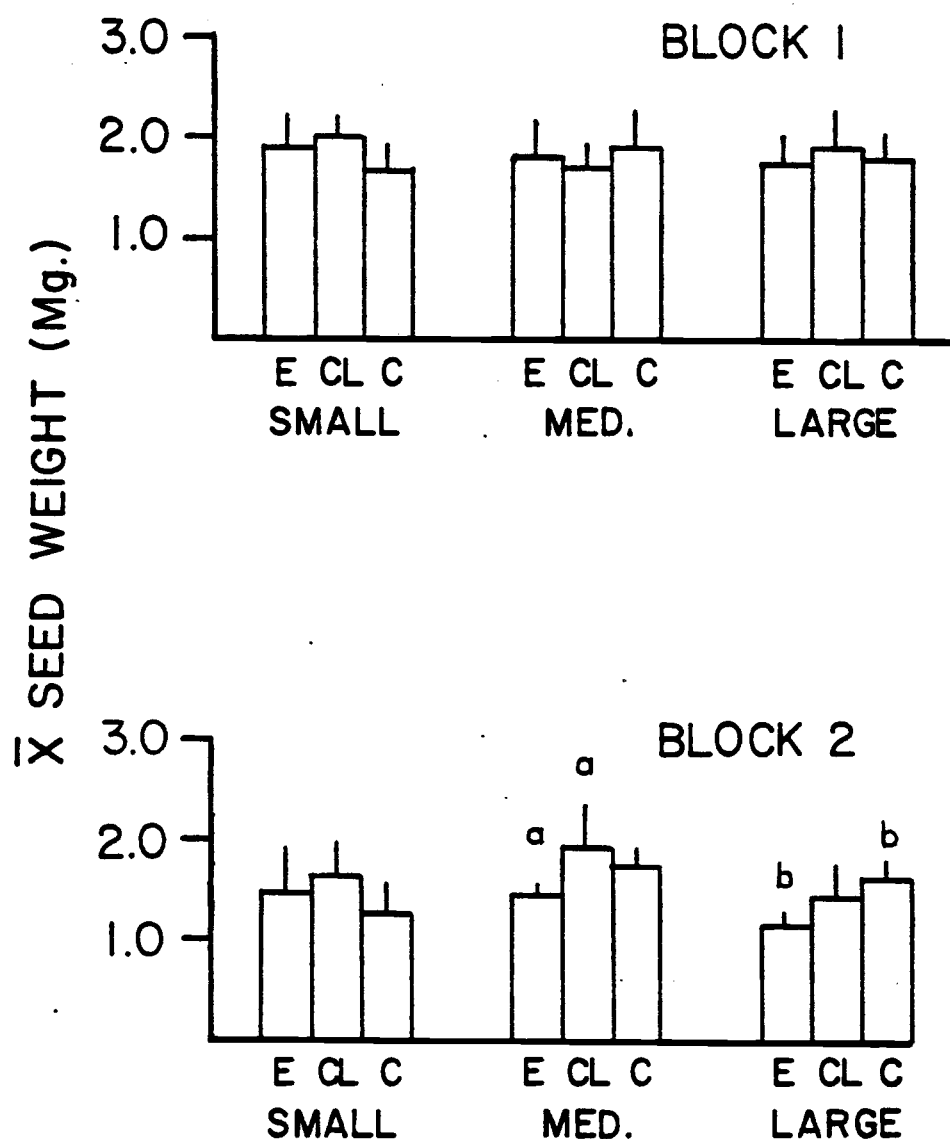
EFFECT OF PLANT SIZE AND
EMASCULATION TREATMENT ON SEED SET

Figure 6. Effect of emasculation treatment and plant size on the weight of seeds. The mean seed weight and one standard error are shown for emasculated (E), clipped (CL), and control (C) plants of small (S), medium (M), and large (L) sizes in the first and second blocks. Comparisons were made between pairs of means from plants of a given size and block and of different emasculation treatments using Scheffé's method of multiple comparisons (combined $\alpha=0.05$). When the same letter (e.g., a) appears above two means, the means are significantly different from one another.

EFFECT OF PLANT SIZE AND EMASCULATION TREATMENT ON SEED WEIGHT



PLANT SIZE AND EMASCULATION TREATMENT

Figure 6

Figure 7. Effect of plant size on the predicted amount of outcrossed pollen deposited on a flower by a single pollinator (y_2), and over an hour period by all pollinators visiting the plant (y_4) (see Discussion for details).

Figure 7

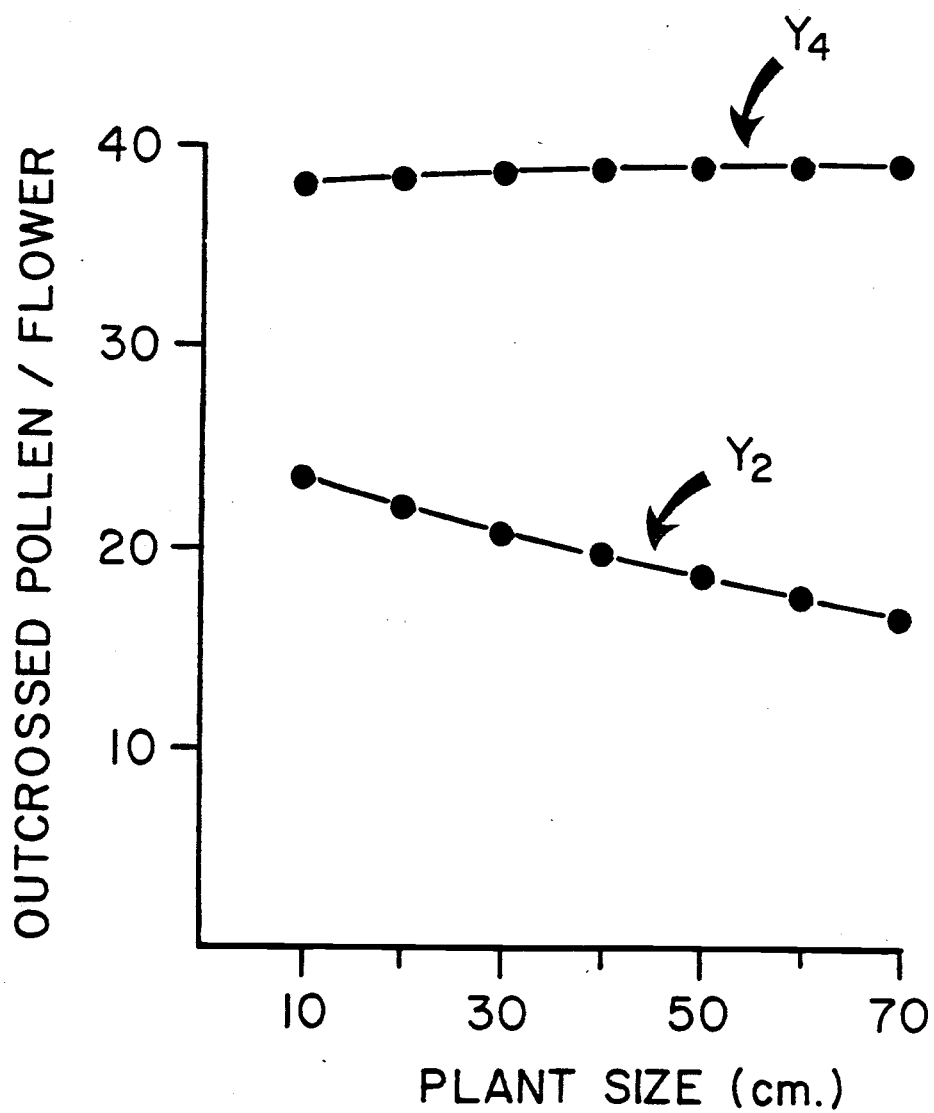


Figure 8. Effect of plant size on the percentage of a plant's flowers visited by a pollinator. The mean percentage of flowers visited per bumblebee on a plant and one standard error are shown for small (S), medium (M), and large (L) plants. Comparisons were made between pairs of means using Scheffé's method of multiple comparisons (combined $\alpha=0.10$). When the same letter (e.g., a) appears above two means are significantly different from one another.

Figure 8

EFFECT OF PLANT SIZE ON THE
PERCENTAGE OF A PLANT'S FLOWERS
VISITED PER BUMBLEBEE

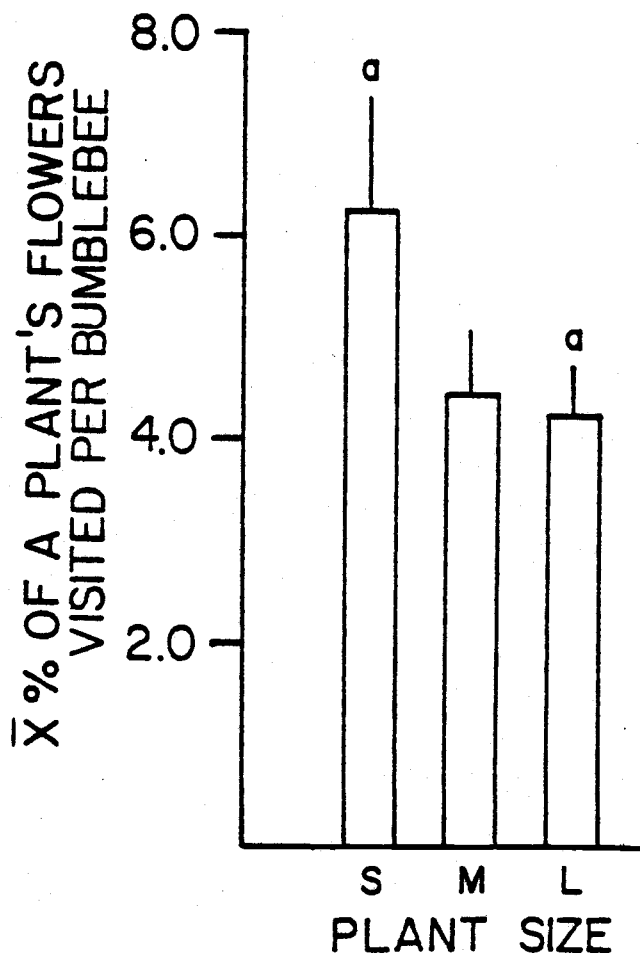


Figure 9. Effect of plant size on the number of stems visited by a pollinator on a plant. The mean number of stems visited per bumblebee on a plant and one standard error are shown for small (S), medium (M), and large (L) plants. Comparisons were made between pairs of means using Scheffé's method of multiple comparisons (combined $\alpha=0.05$). When the same letter (e.g., a) appears above two means the means are significantly different from one another.

Figure 9

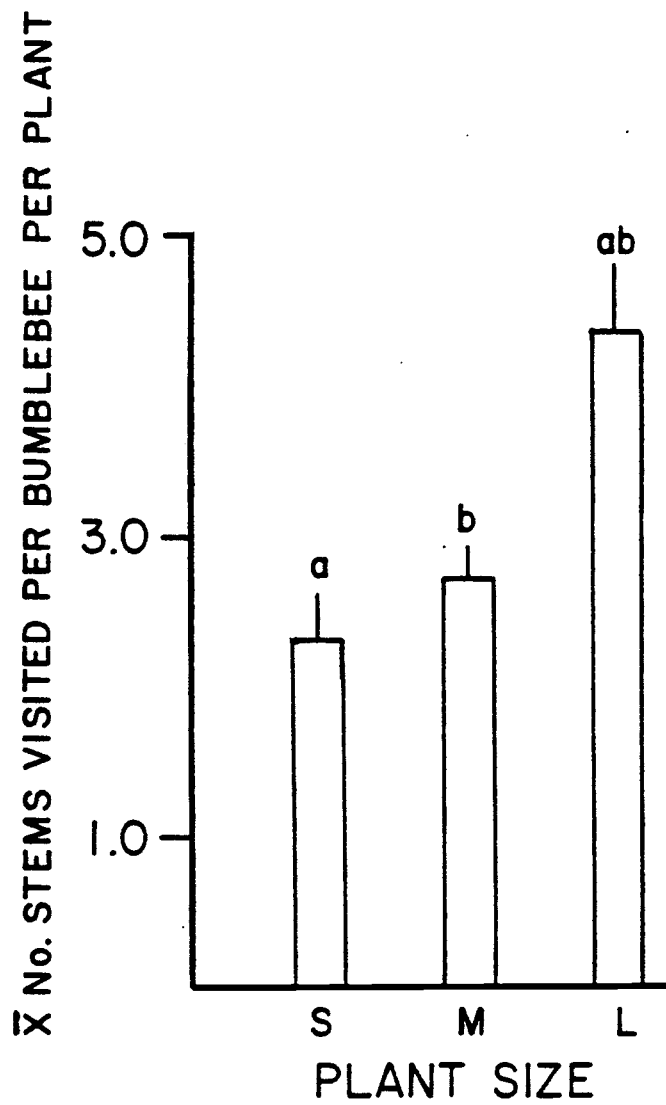


Figure 10. Effect of plant size on the percentage of a plant's stems visited by a pollinator. The mean percentage of stems visited per bumblebee on a plant and one standard error are shown for small (S), medium (M), and large (L) plants for the first and second blocks. Comparisons were made between pairs of means within blocks using Scheffé's method of multiple comparisons (combined $\alpha = 0.05$). When the same letter (e.g., a) appears above two means, the means are significantly different from one another.

Figure 10

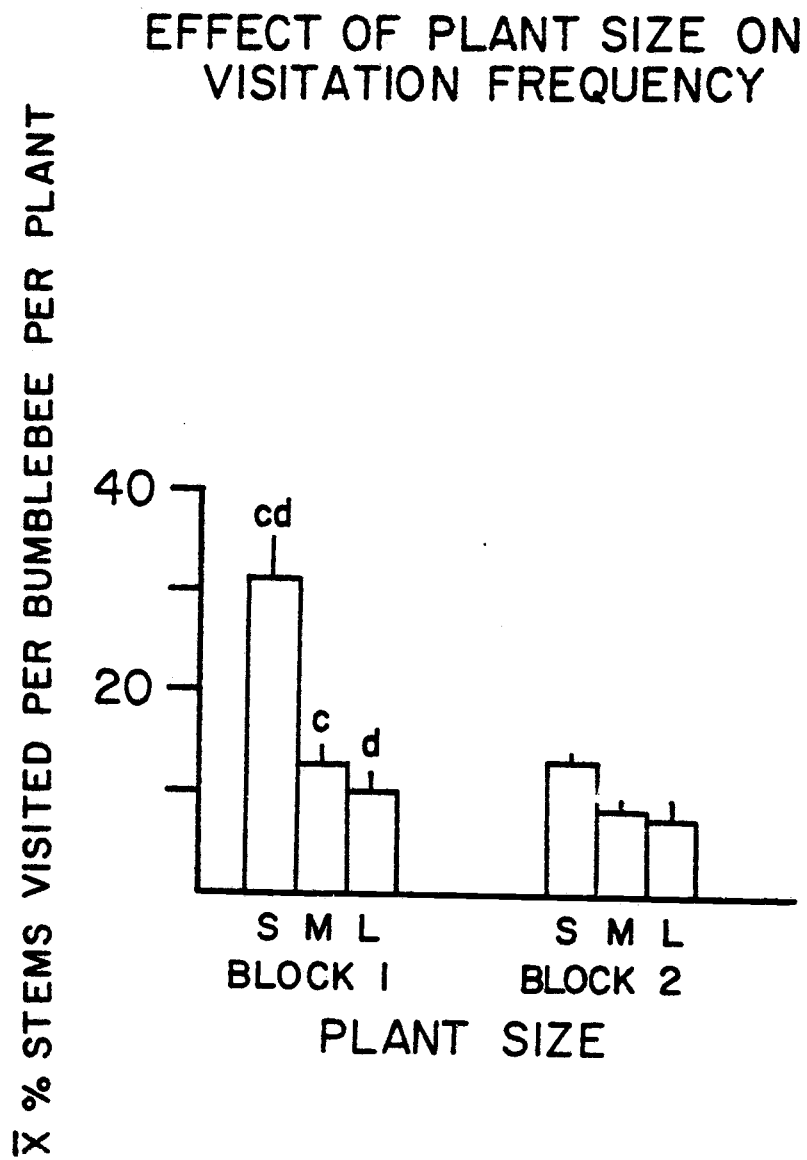
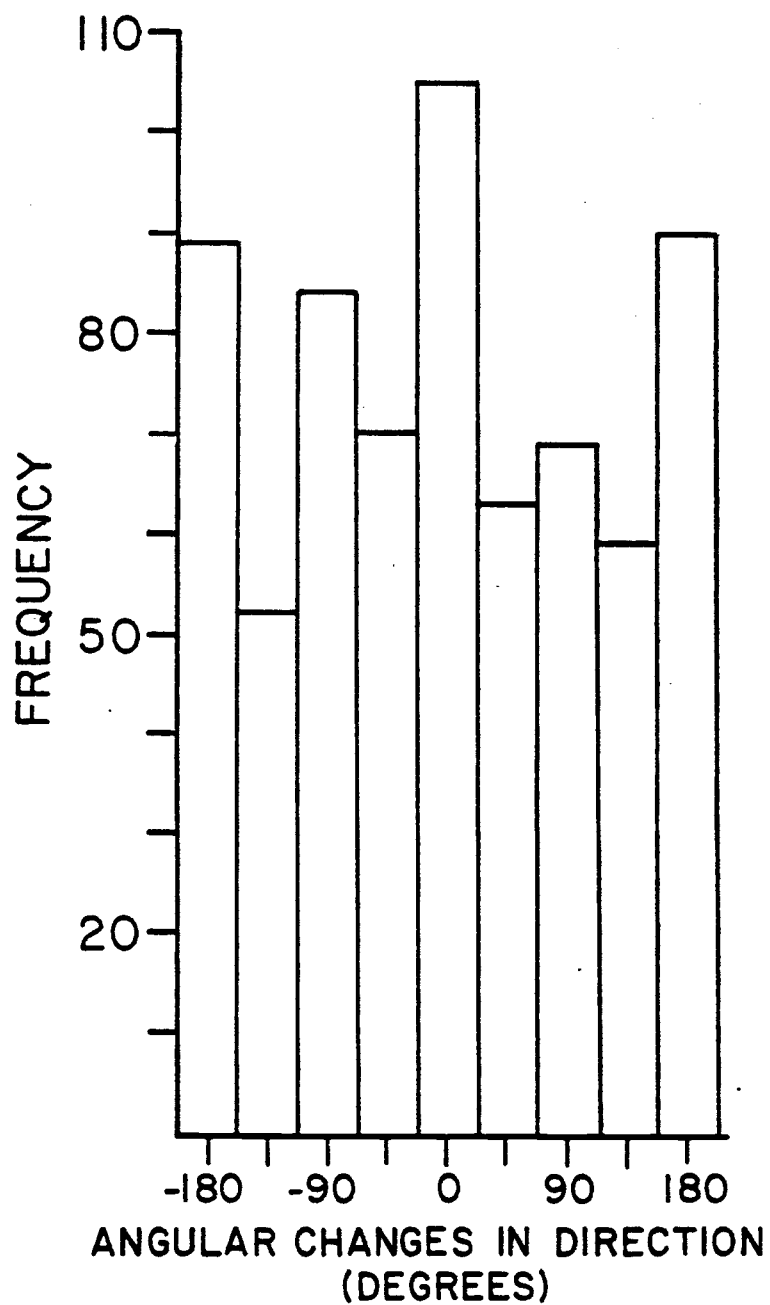


Figure 11. The distribution of angular changes in direction of bumblebees flying between plants. The distribution does not differ from a uniform distribution when tested with a Kolgomorov-Smirnov one sample test (N =number of flights=681, $p>0.2$).

Figure 11

CHANGES IN DIRECTION OF BUMBLEBEE FLIGHTS BETWEEN PLANTS



APPENDIX II

TABLE 9. Analysis of the number of bumblebees arriving at a plant per minute. The analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).

Source ^a	d.f.	MS	F	P <
B	1	0.909	10.61	0.001
S	2	0.378	1.23	NS b
E	2	0.015	0.19	NS c
BxS	2	0.307	3.58	0.03
BxE	2	0.079	0.92	NS
SxE	4	0.084	1.65	NS d
BxSxE	4	0.051	0.60	NS
Error	108	0.086		

a. B = Block, S = Size, E = Emasculation treatment

b. Effect of S tested against BxS

c. Effect of E tested against BxE

d. Effect of SxE tested against BxSxE

Table 10. Analysis of the proportion of a plant's flowers visited per minute. Analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).

Source ^a	d.f.	MS	F _c	P<
B	1	0.193	7.44	0.01
S	2	0.028	1.08	NS
E	2	0.018	0.70	NS
BxS	2	0.022	0.84	NS
BxE	2	0.011	0.44	NS
SxE	4	0.046	1.75	NS
BxSxE	4	0.035	1.33	NS
Error	108	0.026		

a. B = Block, S = Size, E = Emasculation treatment

b. Effect of S tested against (BxS + Error)
(Sokal and Rohlf, 1969: p.266)

c. Effect of E tested against (BxS + Error)
(Sokal and Rohlf, 1969: p.266)

d. Effect of SxE tested against (BxSxE + Error)
(Sokal and Rohlf, 1969: p.266).

TABLE 11. Analysis of the number of flowers visited per bumble-bee on a plant. The analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).

Source ^a	d.f.	MS	F	P	
B	1	128.4	2.43	NS	
S	2	770.0	4.62	NS	b
E	2	32.7	0.28	NS	c
BxS	2	166.7	3.16	0.05	
BxE	2	115.0	2.18	NS	
SxE	4	32.7	0.22	NS	d
BxSxE	4	152.4	2.89	0.03	
Error	91	52.7			

- a. B = Block, S = Size, E = Emasculation treatment
b. Effect of S tested against BxS
c. Effect of E tested against BxE
d. Effect of SxE tested against BxSxE

TABLE 12. Analysis of the proportion of a plant's flowers visited per bumblebee. The analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).

Source ^a	d.f.	MS	F	P	
B	1	0.004	1.70	NS	
S	2	0.006	15.29	0.06	b
E	2	0.006	2.12	NS	c
BxS	2	0.000	0.15	NS	
BxE	2	0.003	1.30	NS	
SxE	4	0.001	1.00	NS	d
BxSxE	4	0.001	0.61	NS	
Error	91	0.002			

a. B = Block, S = Size, E = Emasculation treatment

b. Effect of S tested against BxS

c. Effect of E tested against BxE

d. Effect of SxE tested against BxSxE

TABLE 13. Analysis of the number of stems visited per bumblebee on a plant. The analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).

Source ^a	d.f.	MS	F	p <	
B	1	6.21	1.70	NS	
S	2	78.32	19.33	0.05	b
E	2	0.11	0.33	NS	c
BxS	2	4.10	1.22	NS	
BxE	2	5.27	1.57	NS	
SxE	4	0.57	0.17	NS	d
BxSxE	4	4.39	1.31	NS	
Error	91	3.36			

- a. B = Block, S = Size, E = Emasculation treatment
 b. Effect of S tested against BxS
 c. Effect of E tested against BxE
 d. Effect of SxE tested against BxSxE

TABLE 14. Analysis of the proportion of a plant's stems visited per bumblebee. The analysis was a 5-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).

Source ^a	d.f.	MS	F	P <	
B	1	0.151	11.53	0.001	
S	2	0.177	2.30	NS	b
E	2	0.001	1.00	NS	c
BxS	2	0.077	5.92	0.004	
BxE	2	0.001	0.10	NS	
SxE	4	0.002	0.40	NS	d
BxSxE	4	0.005	0.35	NS	
Error	91	0.013			

a. B = Block, S = Size, E = Emasculation treatment

b. Effect of S tested against BxS

c. Effect of E tested against BxE

d. Effect of SxE tested against BxSxE

TABLE 15. Analysis of the number of pollen grains per stigma. The analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).

Source ^a	d.f.	MS	F	P <	
B	1	11285.3	4.52	0.05	
S	2	10956.8	5.11	NS	b
E	2	52173.2	5.29	NS	c
BxS	2	2144.7	0.86	NS	
BxE	2	9862.2	3.95	0.025	
SxE	4	3329.8	1.35	NS	d
BxSxE	4	373.0	0.15	NS	
Error	702	2496.8			

a. B = Block, S = Size, E = Emasculation treatment

b. Effect of S tested against BxS

c. Effect of E tested against BxE

d. Effect of SxE tested against (BxSxE + Error)
(Sokal and Rohlf, 1969: p.266).

TABLE 16. Analysis of seed-set per flower. The analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).

Source ^a	d.f.	MS	F	P <	
B	1	11.99	6.15	0.01	
S	2	53.25	7.12	NS	b
E	2	66.49	1.63	NS	c
BxS	2	7.48	3.84	0.02	
BxE	2	40.94	21.00	0.0001	
SxE	4	37.32	2.40	NS	d
BxSxE	4	15.54	7.97	0.001	
Error	1782	1.95			

a. B = Block, S = Size, E = Emasculation treatment

b. Effect of S tested against BxS

c. Effect of E tested against BxE

d. Effect of SxE tested against BxSxE

TABLE 17. Analysis of seed weight. The analysis was a 3-way mixed model ANOVA (Block: random effect; Size and Emasculation treatment: fixed effects) (Sokal and Rohlf, 1969).

Source ^a	d.f.	MS	F	P <	
B	1	10.74	88.22	0.0001	
S	2	0.91	0.77	NS	b
E	2	0.63	2.29	NS	c
BxS	2	1.18	9.68	0.001	
BxE	2	0.28	2.27	NS	
SxE	4	0.72	1.71	NS	d
BxSxE	4	0.04	3.49	0.01	
Error	342	0.12			

a. B = Block, S = Size, E = Emasculation treatment

b. Effect of S tested against BxS

c. Effect of E tested against BxE

d. Effect of SxE tested against BxSxE