POLLEN TRANSFER:
PROCESSES AND CONSEQUENCES

by

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We accept this thesis as conforming to the
required standard

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ABSTRACT

The central theme of this thesis is the processes and patterns of pollen dispersal by hummingbirds. The main questions are: "To how many flowers past its source is pollen deposited?", and "What are the processes that influence the transfer and and deposition of pollen?".

Using powdered dyes as pollen mimics, I conducted laboratory experiments with rufous hummingbirds (Selasphorus rufus) and Indian paintbrush (Castilleja miniata) which showed that dye (and presumably pollen) is carried to many flowers past its source flower. The pattern of pollen deposition was complex and was related to variation in floral morphology. I hypothesized that variation in floral morphology has a strong influence on patterns of pollen transfer. The laboratory experiments also supported the hypothesis that pollen is deposited in partially overlapping layers on the pollinator.

A series of models showed that different sets of assumptions about pollen transfer could produce qualitatively similar patterns of pollen deposition, and that variability in deposition increased with the structural complexity of the model. In the model based on the laboratory experiments, variation in floral morphology (defined in terms of the locations where stigmas and anthers contact the pollen pool on the pollinator) had a significant influence on both pollen pool structure (layering) and on pollen carryover.

In field experiments on optimal outcrossing and pollen dispersal in Castilleja miniata, I found only very tentative
support for a within population optimal outcrossing distance. Variation between years was great: one year's experiment showed a peak of seed production at intermediate outcrossing distances, the other years data showed almost no effect of outcrossing distance. Pollen dispersal (as indicated by dye dispersal) is probably not limited by hummingbird territorial boundaries in this system, in the distance or direction of its movement.

I discuss the gap between knowledge of processes at the level of individual flowers and patterns at the level of populations of flowers, and suggest some experiments oriented towards trying to bridge that gap.
# TABLE OF CONTENTS

ABSTRACT ........................................................................................................... ii
LIST OF TABLES ..................................................................................................... v
LIST OF FIGURES .................................................................................................. vi
ACKNOWLEDGEMENTS ........................................................................................ viii

General Introduction .......................................................................................... 1
General System Introduction .............................................................................. 3

Laboratory Experiments On Pollen Transfer:

- Pollen Transfer Kinetics .................................................................................. 8
- Introduction ...................................................................................................... 8
- Materials and Methods .................................................................................... 11
- Results ............................................................................................................ 17
- Discussion ....................................................................................................... 35
  - Single Source Experiments .......................................................................... 35
  - Multiple Source Experiment ........................................................................ 38
  - Plant-Plant Transfer ..................................................................................... 40
  - Bumblebee-Hummingbird Comparison .......................................................... 41
  - Male Versus Female Function ...................................................................... 42
- Conclusions ....................................................................................................... 43

Alternative models of pollen transfer ................................................................. 45

- Introduction ...................................................................................................... 45
- On Building Models Of Pollination .................................................................. 48
- Alternative Models .......................................................................................... 49
- Model 1: Constant Proportion Of Pollen Grains Deposited: Exponential Decay .......................................................................................................................... 53
Model 2: Constant Proportion Of Pollen Grains Deposited: Exponential Decay With Blanks .......... 59
Model 3: Constant Number of Pollen Grains Dropped Off: Limited Stigmal Capacity ....................... 68
Model 4: 3-Dimensional Pollen Pool With Layering and Floral Variability ............................. 78
Discussion .................................................. 105
Implications For Mechanisms ........................... 105
Implications For Plant Populations .................. 107
Conclusions .................................................. 109

Optimal Outcrossing and Pollen Dispersal in Castilleja miniata ........................................... 111
Introduction .................................................. 111
Materials and Methods .................................... 114
Results ......................................................... 119
Optimal Outcrossing ...................................... 119
Dye Dispersal ................................................ 122
Discussion ..................................................... 129
Optimal Outcrossing Distance: Variability in Fitness ................................................................. 129
Dye Dispersal Distance: Is Pollen Dispersed Optimally? ......................................................... 132
Dye Dispersal Direction: Do Territorial Boundaries Restrict Pollen Movement? ......................... 133
Conclusions ..................................................... 135
General Discussion .......................................... 137
References ...................................................... 145
LIST OF TABLES

Table I. Means and extremes of dye carryover from laboratory experiments .................................. 24
Table II. Linear regressions of dye deposited on flower presentation sequence .................................. 27
Table III. Tests of dye deposition on different floral morphology classes ........................................ 28
Table IV. Comparison of single and multiple source trials - proportion deposited .................................. 34
Table V. Comparison of single and multiple source trials - mean and maximum carryover ......................... 35
Table VI. General characteristics of the models .......... 50
Table VII. Carryover statistics from models 2, 3, and 4 .. 67
Table VIII. Regression statistics for carryover on floral variability ....................................................... 103
Table IX. Degree of saturation of local flowers by dye from different sources ..................................... 125
Table X. Directional statistic R* ............................... 128
Table XI. Territory and meadow statistics for the dye dispersal experiment ......................................... 129
LIST OF FIGURES

Figure 1. Flowering stages in Castilleja, and drawing of a hummingbird feeding from Castilleja ....................... 5
Figure 2. Dye deposition on stigmas from the single source experiments ......................................................... 18
Figure 3. Dye deposition on anthers from the single source experiments ......................................................... 20
Figure 4. Dye deposition on stigmas and anthers of different morphological classes ................................. 22
Figure 5. Dye deposition on stigmas and anthers of different morphological classes - pooled data .......... 25
Figure 6. Dye deposited on flowers of different lengths, dye from one source ................................................. 29
Figure 7. The proportion of dye deposited on adjacent groups of 10 flowers .............................................. 32
Figure 8. Pollen deposition curves from model 1 ............... 55
Figure 9. Pollen deposition curves from model 2 at different per cent deposition ................................. 61
Figure 10. Frequency distributions of mean and maximum carryover from models 2, 3 and 4 ....................... 63
Figure 11. Mean and maximum pollen deposition from model 2 ................................................................. 65
Figure 12. Pollen carryover curves from model 3 .......... 70
Figure 13. Carryover from model 3 with varying stigmatic capacity ............................................................... 72
Figure 14. Variance in mean and maximum pollen carryover vs grains deposited per stigma - from model 3 ......... 75
Figure 15. Hummingbird with pollen pool from model 4 ...... 80
Figure 16. Pollen carryover curves from model 4 ............ 83
Figure 17. Pollen grains picked up vs. floral variability 90
Figure 18. Pollen grains deposited vs. floral variability 92
Figure 19. Proportion of pool occupied vs. floral variability ............................................. 94
Figure 20. Mean layering vs. floral variability .............. 96
Figure 21. Pollen carryover vs. floral variability .......... 99
Figure 22. Proportion of zero deposition vs. floral variability ..................................................101
Figure 23. Seed production under different pollination treatments ....................................................120
Figure 24. Proportion of dye dispersed to different distances away from the dye source ....................1,23
Figure 25. The proportion of observed flowers with dye at different dispersal distances ....................126
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"...with a little help from my friends."
"Thus science is much closer to myth than a scientific philosophy is prepared to admit. It is one of the many forms of thought that have been developed by man, and not necessarily the best. It is conspicuous, noisy, and impudent, but it is inherently superior only for those who have already decided in favor of a certain ideology, or who have accepted it without having ever examined its advantages and its limits."

Feyerabend (1975)

"Before I studied Zen, mountains were just mountains and rivers just rivers. While I studied Zen, mountains were no longer mountains and rivers no longer rivers. But once I achieved enlightenment, mountains were once again just mountains, and rivers once again just rivers."

Zen Saying
GENERAL INTRODUCTION

Plant-pollinator systems provide opportunities for studying the products of co-evolution of different groups of organisms. This is expressed in many dimensions for both the plants and the animals (Faegri and van der Pijl 1979; Grant and Grant 1968; Proctor and Yeo 1972). Early studies focussed on the structural aspects of these systems - the "fit" between pollinator and flower morphologies, and how this resulted in the pollination of the plants (summarized by Faegri and van der Pijl 1979; Proctor and Yeo 1972). There has been a gradual shift in emphasis to the more dynamic aspects of these systems e.g. pollinator behaviour (Gass et al. 1976; Gass 1979; Heinrich et al. 1977; Kodric-Brown and Brown 1978; Waddington and Holden 1979; Zimmerman 1979), patterns of nectar production and floral display (Cruden 1976; Corbet 1978a; 1978b; Feinsinger 1978; Willson 1979; Willson and Rathcke 1974; Augspurger 1980), and the structure of nectarivore communities (e.g. Colwell 1973; Feinsinger 1976; Carpenter 1978; Feinsinger and Colwell 1978; Pimm 1978; Wolf 1978; Montgomerie 1979).

I was initially interested in problems at the level of plant populations; gene flow, differentiation, and optimal outcrossing. However, I realized that to study these population problems in a predictive way, I would have to make poorly supported assumptions about pollen transfer processes at the level of individual flowers. These were
the same assumptions that I had found frustrating in the literature. For instance, it is commonly assumed that pollen picked up by a pollinator is deposited at only a few flowers after the one from which it is picked up (Levin et al. 1971; Frankie et al. 1976; Feinsinger 1978; Richards and Ibrahim 1979; Augspurger 1980; Schmitt 1980).

The main focus of this thesis is on patterns and processes of pollen dispersal at the level of individual flowers. My goal was to assess the validity of some commonly made assumptions and some alternatives to them. I did this first through laboratory experiments (chapter 3) and then through a series of simulation models that incorporated information from the experimental studies (chapter 4).

In chapter 5 I ask questions about mate quality and pollen dispersal at the population level. Specifically, I test the predictions that reproductive success will be maximized at an intermediate level of outcrossing, and that hummingbird territorial boundaries restrict the pool of potential mates available to the plants contained in the territory.

In the general discussion I assess whether my studies at the individual level will help in conducting research on population level patterns such as are discussed in chapter 5. I conclude with a discussion of the most profitable ways to follow up this work.
GENERAL SYSTEM INTRODUCTION

The *Selasphorus rufus* - *Castilleja miniata* system is good for studying plant-pollinator interactions. It is simple - one species of pollinator with two main food plants - and there had been nearly 10 years of ecological research on the system prior to the start of this study. Thus there is a context for studying hummingbird-plant interactions in which important ecological baseline information is already known.

Rufous hummingbirds (*Selasphorus rufus*) breed from southern Oregon to Alaska and defend temporary feeding territories in the subalpine meadows of northern California during their southward post-breeding migration (Gass 1974; Gass et al. 1976; Gass 1979). These studies were conducted at Grizzly Lake in the Salmon-Trinity Alps Primitive Area in northwestern California. Here rufous hummingbirds (primarily adult females and immatures) defend territories in densely flowering subalpine meadows from snowmelt in early July, until late August (Gass 1974; 1979; Gass et al. 1976). Their main sources of nectar are Indian paintbrush (*Castilleja miniata*) and columbine (*Aquilegia formosa*). Other flowers are fed on irregularly when these are not available or are rare.

The number of flowers defended by individual hummingbirds is regulated to provide their 24 hour energy needs (Gass et al. 1976; Gass 1979; Kodric-Brown and Brown 1978). Thus since *Aquilegia* produces nectar at roughly 4
times the rate of \textit{Castilleja} (per nectary), territories held in \textit{Castilleja} have many more flowers in them than territories held in \textit{Aquilegia} (Gass \textit{et al.} 1976). Excluding durations of less than one day, average tenure of hummingbirds in these meadows is 5.86 days, though one individual has been observed to stay for 21 days (Gass 1979).

My work focusses on interactions between \textit{Selasphorus rufus} and \textit{Castilleja miniata}. \textit{Castilleja} is a perennial herb that sets little seed when selfed (Perkins 1977, and chapter 5 on optimal outcrossing), or when hummingbirds are excluded (Carpenter 1981). Flowering is indeterminate and older flowers are lower on the inflorescence. New flowers emerge at the top of the inflorescence while the seeds are maturing below. Flowers are hermaphrodites, and though there is weak evidence for protogyyny, anthers and stigmas appear to mature at about the same time (Perkins 1977). Figure 1 diagrams \textit{Castilleja} flowers in various stages and shows the geometry of hummingbird feeding and pollination.

One plant may have many inflorescences, with an average of \(4.1 \pm 0.45\) flowers per inflorescence in the middle of the season. There are 0 to about 23 flowers per inflorescence, with an average of \(4.9 \pm 0.54\) in the middle of the season (excluding plants and inflorescences with 0 inflorescences or flowers, Perkins 1977).

Non-hummingbird pollinators rarely visit \textit{Castilleja} at Grizzly Lake (Perkins 1977, pers. obs.).
Figure 1. Flowering stages in *Castilleja* and drawing of a hummingbird feeding from *Castilleja*. Nectar is present and hummingbirds feed from stages 3-5. Anthers begin to dehisce at stage 4 and stigmas are receptive by stage 4. Flowering stages re-drawn from Perkins (1977). The hatched area on the hummingbird's forehead is, in addition to the proximate portion of the beak, the area contacted by the reproductive parts of the flowers - the area occupied by the pollen pool.

a = stigma
b = anthers
Details of the methods are particular to each section and will be described where they are used.
LABORATORY EXPERIMENTS ON POLLEN TRANSFER:
POLLEN TRANSFER KINETICS

Introduction

In the *Castilleja - Selasphorus* system, pollination involves the pickup of pollen by a hummingbird while it is feeding on the nectar in a flower, the transport of pollen in a pool on the hummingbird's forehead, and the deposition of the pollen on some of the flowers the hummingbird subsequently feeds from. The number of flowers past its source to which pollen is carried is referred to as pollen carryover. Pollen carryover is important because it determines whether a pollination event will result in outcrossing or selfing. The kinetics of pollen carryover are the shapes of pollen carryover or pollen deposition curves and the processes that give them their forms. Does pollen deposition decay exponentially? Does it decrease linearly? What underlying mechanisms are responsible for these patterns?

Heretofore, most references to these processes have been implicit, and carryover has been assumed to be very short (Levin and Kerster 1967; 1968; 1969a,b; Levin and Berube 1972; Frankie *et al.* 1976; Feinsinger 1978; Richards and Ibrahim 1979; Augspurger 1980; Schmitt 1980). Levin and Berube (1972) found that carryover of heterospecific pollen
by Colias butterflies feeding on Phlox declined steeply and exponentially, with little carryover past the fourth flower in a five flower sequence. Assuming little flower-flower carryover, and a large number of flowers fed on per plant, Levin and Kerster (1969a) argued that there would be little carryover from one plant to another past the first few flowers.

This result of steep exponential decay has been generalized to other systems, e.g. Feinsinger (1978) for a tropical hummingbird-plant community, Augspurger (1980) for a tropical, mass flowering, insect pollinated shrub, and Frankie et al. (1976) for a tropical, mass flowering, bee pollinated tree. An inference of short gene dispersal distances is a consequence of these assumptions. To the extent that carryover does exceed these assumptions, the inference of restricted gene flow will be inaccurate.

Recent evidence suggests that this is an oversimplified and inaccurate description of pollen carryover kinetics in general. In contrast to Levin and Berube's (1972) result, Perkins (1977), working with rufous hummingbirds and emasculated Fuchsia flowers, found carryover to the end of her 20 flower sequences. Carryover was not smooth but was erratic. Thomson and Plowright (1980) found similar results for bumblebees (Bombus and Psithryus) feeding from Erythronium, Clintonia, and Diervilla. Pollen deposition declined roughly exponentially, but there was much variation in deposition, and carryover was common to 18 flowers (but
in one case pollen was deposited 54 flowers past the pollen source). Using powdered dyes as pollen mimics, Waser and Price (1981) found irregular deposition to 20 flowers past the dye sources (for Bombus feeding from Delphinium and Selasphorus platycercus feeding from Ipomopsis aggregata.

The first question this chapter addresses is: What is the shape of the pollen carryover curve for Selasphorus rufus feeding from Castilleja miniata? The null hypothesis is steep, smooth, exponential decay. Three qualitative predictions of this hypothesis are: 1) each flower visited has pollen deposited on it, 2) the amount of pollen deposited is a constant proportion of the pollen present on the bird (thus the amount deposited decreases with each flower visited), and 3) the constant proportion deposited is a substantial (i.e. > 0.2) proportion of what is present - carryover is to only a few flowers beyond the pollen source.

The second question is: What factors are responsible for the form of this relationship? Two general components that could be examined are the effects of hummingbird foraging behaviour (i.e. posture while feeding, response to nectar standing crop) and the characteristics of floral display. This section focusses on how variability in floral morphology influences pollen carryover by affecting the geometry of bird-flower contact.

The final question of this section is: to what extent is pollen from different flowers deposited on hummingbirds' foreheads in overlapping layers - can the pollen from one
flower cover up pollen from another? Although no one has addressed this question directly, the implicit assumption has been that layering of pollen does not occur (e.g. Waser 1978). Carryover is often thought of in terms of pollen deposition from independent individual flowers. However, pollen can potentially be both added to and removed from the pool of pollen on the hummingbird at each flower visited. Is the pollen in the pool from different flowers removed independently, or does the pollen from different flowers interact in some way that affects the probability of its deposition? If pollen deposited by later flowers in a sequence of visits covered up pollen from earlier flowers, it would make that pollen unavailable to subsequent stigmas. Pollen carryover curves generated under conditions where such layering can occur should decay faster than under conditions where layering does not occur. Note however, that if pollen was buried and subsequently uncovered, very long carryover could result.

Materials and Methods

In July of 1979 and 1980, two rufous hummingbirds were caught in the meadows around Grizzly Lake and kept in holding cages made of 3/4 inch mesh nylon seine netting. A solution of sugar, protein, and vitamin supplements was provided ad libitum from feeders.

In order to conduct experiments, the hummingbirds were trained to feed from Castilleja flowers presented in test
tubes. I tested different methods of presenting flowers. Presenting one inflorescence (several flowers) per test tube maintained the most realistic flower position, but made it difficult to score sequences of visits. For the following experiments, flowers were presented one per test tube, usually five tubes at a time, 5 cm. apart in a linear array. Before each experiment, flowers were drained with a micro-pipette and a constant amount (5 or 10 micro-liters) of imitation nectar added to each one (32% sucrose solution, the mean concentration of *Castilleja* nectar, L. Gass unpub. data).

In 1979 I used micronized, fluorescent, powdered dyes as pollen mimics (R-103-G-111, green; R-103-G-112, orange-yellow; R-103-G-119, blue; R-103-G-115, red; Radiant Color, 2800 Radiant ave., Richmond, California, see also Frankie 1973, Stockhouse 1976, Price and Waser 1979; Waser and Price 1981; Linhart and Feinsinger 1980). Thus these experiments studied dye carryover rather than pollen carryover.

Flowers for use in experiments were chosen from the meadows at random with the constraint that they were within the range of variation in shape and age that we had observed hummingbirds feeding from in the wild.

For the dye carryover experiments, a large, mature (dehiscing anthers, exposed stigma) flower was chosen from those collected, and injected with twice the amount of nectar that the dye recipients had. The stigma and anthers of the source flower were coated with dye, and the
hummingbird allowed to feed until a spot of dye was visible on its forehead. This sometimes took several visits. Then the remaining flowers were presented to the hummingbird. Trained hummingbirds almost always visited flowers in a linear sequence without revisits. Hummingbirds were frightened away if they attempted to revisit flowers. After the hummingbirds had fed, flowers were scored for stigma and anther position, and for the amount of dye on the stigmas and the anthers (dye on either stigmas or anthers indicates reproductively significant bird-flower contact). Stigma and anther position classes were distributed randomly in the presentation sequences (p<0.001; chi square). Flowers were usually scored within a few hours of conducting the experiment, but flowers from the multiple source experiment were scored over a period of several days.

The hummingbird's forehead was not cleaned between trials because cleaning had strong adverse effects on the hummingbird's subsequent behaviour. Dye was rarely visible from previous trials and between trial carryover was minimal. Dye color was changed from one trial to the next to minimize between trial effects.

For scoring the amount of dye deposited, I used an index based on the ease of seeing any dye that was present: visible by eye under daylight (8), visible with a ten power hand lens under daylight (4), visible by eye under ultraviolet light (2), visible with the hand lens under ultraviolet light (1), no dye visible under any conditions
The magnitudes of the numbers are my own subjective impression of the magnitude of the difference between the levels, and do not affect the conclusions: the tests I apply require at least ordinal but not ratio measures, and the general qualitative conclusions are not dependent on the absolute values of the numbers, but only on their relative values.

For the basic dye/pollen carryover experiments there was only one dye/pollen source. To test the hypothesis of layering of pollen or dye from different sources, I conducted an experiment with multiple dye sources. Before the first, and after every tenth dye recipient flower in a sequence, I presented a different dye source to the bird. Three colors of dye were used and there were thus 30 recipient flowers between dye sources of the same color. There were three cycles of 30 flowers giving 9 replicates, three for each colour of dye. This experiment was conducted on 16 Aug. 1979.

I predicted that if dye were deposited in overlapping layers, then carryover curves of individual flowers under multiple source conditions would be steeper and shorter than the curves from the single source trials. If there is no layering, spots of dye from different sources are independent, and the multiple source carryover curves should be the same as single source curves.

Because the amount of dye deposited at one flower is not independent of the dye deposited at other flowers, the
use of regression is not appropriate for analyzing these results. Instead, I calculated the proportion of the total index scores (for a run of 30 flowers) of dye deposited onto the first ten, the second ten, and the third ten flowers. Comparing these proportions controls for variation in the amount of dye deposited on the hummingbird. If layering occurs in the multiple source experiment, I would expect to see a greater difference in the proportion of dye deposited to sequential groups of ten flowers when compared to the single source carryover curves. This is the same as expecting curves with steeper slopes from the multiple source experiment. Implicit in this is the assumption that dye is not uncovered to a great extent once other dye has covered it up.

Two of the single source curves have less than 30 flowers. This will bias these tests towards the null hypothesis of no difference: estimates of carryover would be decreased relative to what they would have been had there been 30 flowers. This will decrease the perceived difference between the multiple source and single source values if the null hypothesis is indeed false.

Is it justified to assume that powdered dyes are good pollen mimics? This study focusses on qualitative properties of pollen carryover (e.g. every flower receives dye, exponential decay, etc.), and these properties should be consequences primarily of the geometry of bird-flower contact. Any small powdery substance should mimic the gross
behaviour of pollen, other things (e.g. stickiness) being equal. However, quantitative results should be interpreted conservatively. J. Thomson (pers. comm.) has found that while dyes slightly overestimated pollen carryover (for Bombus feeding on Erythronium), they did show the same qualitative behaviour as his trials with pollen. This result seems reasonable to generalize to the Selasphorus-Castilleja system.

In 1980 I conducted several tests using a Castilleja pollen source (virgin stigma and anthers), and emasculated recipients (virgin stigmas). I also ran trials using different color and size morphs of tulip pollen. Though Castilleja and tulip pollen trials differed quantitatively from each other and from the dye experiments, they were qualitatively similar. This lends credence to the use of dyes for preliminary explorations. The tulip and Castilleja pollen carryover experiments were not continued because of logistical problems.

In August 1980, 203 flowers were randomly chosen and photographed. Their reproductive state and morphological classes were recorded and these data were used to test for the independence of floral morphology and reproductive state. By projecting the photographs onto an Apple microcomputer graphics tablet, I was able to take measurements of floral variability that were used in the modelling chapter.
Results

In general, carryover was long and irregular. Some flowers received no dye at all while others following them received a great deal. The results of the basic dye carryover experiments are shown in figures 2 and 3. They are characterized by high variability in the amount of dye deposited, irregularly spaced "blanks" where no dye is deposited, and relatively long carryover. We can thus reject the null hypothesis of smooth, steep, exponential decay. Table I shows the means and extremes for dye carryover for the total number of flowers in the run, and for the run truncated to 23 flowers (the length of the shortest run). Notice that the mean dye carryover distance is dependent on run size.

A linear regression on the combined data from these runs has an x intercept at 36 flowers for dye deposited on stigmas, and 45 flowers for dye deposited on anthers (Table II). Linear regression should underestimate dye carryover if carryover does decay exponentially. These equations are included for comparison with other published values and will not be used in my analysis.

What role does flower variability play in the variability in dye dropoff? Figure 4 shows an example of the mean amount of dye deposited on stigmas and anthers of different morphological classes in one run (dye deposited from the spot of dye from one source). Some classes receive a disproportionate amount of dye. Even when the results of
Figure 2. Dye deposition curves from the 4 single source experiments - dye deposited on stigmas. See text for explanation of the index of the amount of dye deposited.
index of dye on stigmas

flower presentation sequence

n = 23

n = 24

n = 42

n = 41
Figure 3. Dye deposition curves from the 4 single source experiments - dye deposited on anthers. See text for explanation of the index of the amount of dye deposited.
flower presentation sequence

index of dye on anthers

n = 23

n = 24

n = 42

n = 41

flower presentation sequence
Figure 4. Dye deposition on stigmas and anthers of different morphological classes. Data from 1 flower sequence, i.e. dye originated from one source. Though floral morphology is related to floral age, the sequence of morphological classes presented here does not represent an aging sequence, i.e. class 8 stigmas are not necessarily older than class 7 stigmas.
Table I. Means and extremes of dye carryover from the laboratory experiments.

<table>
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<th>single source</th>
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<td>4.3 9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.3 21</td>
<td>6.8 18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.1 10</td>
<td>4.6 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.5 18</td>
<td>9.0 15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.5 9</td>
<td>5.9 18</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All runs are combined (Fig. 5), certain classes receive more dye than others. Table III summarizes tests of the independence of dye deposition and stigma and anther classes. Six out of 7 tests showed significantly non-random deposition of dye among these morphology classes.
Figure 5. Dye deposition on stigmas and anthers of different morphological classes. Combined data from 4 runs, i.e. dye originated from several sources.
mean amount of dye

another class

stigma class
Table II. Predictive linear regression equations (for pooled single source and pooled multiple source data) of dye deposited on flower presentation sequence. Entire runs used, see Table I for lengths

<table>
<thead>
<tr>
<th></th>
<th>single source</th>
<th>multiple source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dye on stigmas</td>
<td>dye on anthers</td>
</tr>
<tr>
<td></td>
<td>y = 1.16 - 0.03x</td>
<td>y = 2.78 - 0.06x</td>
</tr>
<tr>
<td></td>
<td>x intercept = 36</td>
<td>x intercept = 45</td>
</tr>
<tr>
<td></td>
<td>r = 0.24</td>
<td>r = 0.30</td>
</tr>
<tr>
<td></td>
<td>n = 130</td>
<td>n = 130</td>
</tr>
</tbody>
</table>

|                          | dye on stigmas         | dye on anthers         |
|                          | y = 0.72 - 0.03x       | y = 1.30 - 0.05x       |
|                          | x intercept = 28       | x intercept = 28       |
|                          | r = 0.26               | r = 0.33               |
|                          | n = 260                | n = 260                |

Based on the field measures of 1980, these stigma and anther classes based on morphology are not independent of stigma and anther classes based on reproductive state. The probability that shape classes and age classes are independent is $< 0.001$ for both stigmas and anthers. The contingency coefficient $C$ is 0.40 for the relation between stigma shape and stigma age classes and is 0.52 for anther shape and anther reproductive state classes (see Siegel 1956 for description of the contingency coefficient). Thus
Table III. Summary of Chi Squared tests for the independence of dye deposition on different stigma and anther classes. (data are from single source experiments)

<table>
<thead>
<tr>
<th>Stigma classes</th>
<th>Trial</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>( p = 0.001 )</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Accept Ho</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Expected values</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Too low for test</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>( p = 0.05 )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anther classes</th>
<th>Trial</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>( p = 0.001 )</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>( p = 0.02 )</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>( p = 0.05 )</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>( p = 0.001 )</td>
</tr>
</tbody>
</table>

flowers of different reproductive state have different likelihoods of receiving dye. Some of this is likely to be strictly due to geometric factors, and some due to factors such as the greater stickiness of receptive stigmas. Similarly, in the one run for which there are appropriate data, dye was not deposited randomly with respect to flower length \((p = 0.02; \chi^2 \text{ square, Fig. 6})\).

These results suggest that dye (or pollen) transfer from one flower to another depends on congruence in the
Figure 6. Dye deposition on flowers of different lengths. Data from 1 flower sequence, i.e. dye originated from 1 source. Data are dye deposited on anthers.
The bar chart shows the proportion of corolla length classes (mm) with a dye.

The classes are:
- 12-14
- 14-16
- 16-18
- 18-20
- 20-22
- 22-24

The proportion with a dye is highest in the 16-18 class.
locations where the two flowers contact the hummingbird, which is dependent on similarity in their morphologies. Thus a short flower with its corolla tube bent to the right is unlikely to pollinate a long, straight flower with an erect stigma. Because of stochastic factors such as the wind blowing the flowers while the bird is feeding, and variation in the bird's behaviour (e.g. intruders feed much differently than territorial residents; per. obs.), this is not a simple deterministic correspondence, and flowers do occasionally have their pollen deposited on dissimilar types.

I compared the single source data with the multiple source data in three ways: comparing the difference in the proportion of the dye that was deposited on sequential groups of ten flowers, the flower to which the mean index unit of dye was carried, and the last flower in the run to receive dye.

In general, the data support the layering hypothesis. (for data see Fig. 7, for test results see Table IV). The comparisons of the proportion of dye deposited on adjacent groups of ten flowers were equivocal: they showed significant decreases in dropoff for dye deposited on multiple source stigmas, but not for dye deposited on anthers. However, when the differences between flowers 1-10 and 21-30 were compared, the differences between the multiple source values were significantly greater for both stigmas and anthers.
Figure 7. The proportion of total dye deposited that was deposited on the first, second, and third group of 10 flowers.
dye on stigmas  dye on anthers

Proportion of dye deposited

flower presentation sequence

single source  multiple source
Table IV. Comparison of differences in the proportion of total dye deposited, single source vs. multiple source experiments (Mann-Whitney U tests).

1) comparing the proportion deposited on flowers 1-10 with flowers 11-20:

<table>
<thead>
<tr>
<th></th>
<th>dye on stigmas</th>
<th>dye on anthers</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.055</td>
<td>0.230</td>
</tr>
<tr>
<td>ms &gt; ss</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2) comparing the proportion deposited on flowers 11-20 with flowers 21-30:

<table>
<thead>
<tr>
<th></th>
<th>dye on stigmas</th>
<th>dye on anthers</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.036</td>
<td>0.285</td>
</tr>
<tr>
<td>ms &gt; ss</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3) comparing the proportion deposited on flowers 1-10 with flowers 21-30:

<table>
<thead>
<tr>
<th></th>
<th>dye on stigmas</th>
<th>dye on anthers</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.024</td>
<td>0.055</td>
</tr>
<tr>
<td>ms &gt; ss</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ = approximate value because of ties.

Table V compares the means and extremes of pollen carryover for single and multiple source data. Single and multiple source curves did not differ in the mean carryover for stigmas, but differed significantly for anthers, carryover being longer for single source curves. The last flower in the sequence to receive dye was significantly farther in the sequence for single source runs for both stigmas and anthers.
Table V. Summary of tests of mean and maximum dye carryover comparing single and multiple source trials (Mann-Whitney U test).

Comparing means: single source (ss) vs. multiple source (ms)

<table>
<thead>
<tr>
<th></th>
<th>Stigmas</th>
<th>Anthers</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.107</td>
<td>0.036</td>
</tr>
<tr>
<td>ss = ms</td>
<td>ss &gt; ms</td>
<td>ss &gt; ms</td>
</tr>
</tbody>
</table>

Comparing extremes: single source vs. multiple source

<table>
<thead>
<tr>
<th></th>
<th>Stigmas</th>
<th>Anthers</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.021</td>
<td>0.003</td>
</tr>
<tr>
<td>ss &gt; ms</td>
<td>ss &gt; ms</td>
<td>ss &gt; ms</td>
</tr>
</tbody>
</table>

During the course of the experiment we were able to observe dye of different colors (and therefore from different sources) overlapping on the hummingbird's forehead, providing direct support for the layering hypothesis.

Discussion

Single Source Experiments

Dye deposition, and by inference pollen deposition, does not follow a simple exponential decay model, but decreases as a complex function of flower morphology, probably variability in hummingbird behaviour, and stochastic factors, such as the weather. This results in many flowers being "blanks" which do not interact with the
pool of pollen on the bird, and thus extends the number of flowers to which pollen is carried. Note that while a given flower may not receive any of the pollen that the hummingbird picked up from any other given flower, and thus constitutes a blank in that sequence, it may receive pollen that the hummingbird picked up from other flowers, and does not necessarily receive not pollen at all.

Increases in carryover may be very important to the plants. Obligately outcrossing plants that produce large numbers of flowers commonly have only a small proportion of their ovules setting seed, purportedly due to a flooding of the pollen pool with selfed pollen (see discussion below, and see Bawa 1974; Frankie et al. 1976; Schemske 1980). Because Castilleja is self incompatible and produces many flowers, and short between-flower flights by hummingbirds predominate in this system (Perkins 1977), increases in carryover will increase the likelihood of outcrossing, and thus increase plant fitness. The proportion of flowers that are blanks may be an important way that the plants are able to influence mate identity (i.e. self vs. non – self).

This does not imply group selection. One plant may have many flowers reproductive at a single time, and may represent a significant subset of the population of flowers that a hummingbird visits. If an individual plant's characters can increase its fitness by manipulating the proportion of blanks its pollen experiences, then those individuals will be selected for.
The proportion of blanks will probably change through a season since flower morphology classes are related to flower age classes, and the distribution of flower age classes changes as the season progresses (note though that the proportion of blanks is a function of the variance in flower morphology, and not morphology *per se*. This variance will probably increase to the peak of flowering and then decrease.). Flower density, the number of territories in a meadow, territory sizes, and the number of plants represented in a given territory change through the season also (Gass 1979), and it will be important to integrate all these seasonal changes in terms of their consequences for pollen flow between plants. Does a plant's optimal strategy of floral display change through the season, emphasizing maleness at one period and femaleness at another? If so, do plant's actual floral displays reflect this?

Thomson and Plowright (1980) observed much variation in pollen deposition by bumblebees, including some flowers that had no pollen deposited on them at all. They also attributed this to variation in floral geometry and pollinator posture, but suggested that such "haphazard" deposition is maladaptive because it might decrease the probability of successful transfer. This could be valid when a few (<30 or so) flowers are fed on. Patchy placement of pollen will decrease the likelihood of transfer since any given flower would be less likely to contact the animal in a location where pollen had been previously deposited.
However, if many flowers are visited (the usual situation for both hummingbirds and bees), the pollen pool as a whole becomes less patchy spatially, while retaining its heterogeneity in terms of pollen source identity. Thus the situation where patchy deposition of pollen is maladaptive is likely to be an uncommon special case.

**Castilleja** exhibits both precision in pollen placement, and variability between placements (compared to, for instance, *Aquilegia*, a self-compatible species). Both of these will be less important for self-compatible species. If selfing is not detrimental, then broad deposition of pollen and overlapping areas of floral contact on the bird will increase the probability of pollen transfer with no resulting loss in fitness when only a few flowers are visited.

**Multiple Source Experiment**

The hypothesis that pollen may be deposited in partially or totally overlapping layers is tentatively supported. What are the consequences of layering on pollen carryover? It has the opposite effect to flower variability in that it decreases carryover on the short term. However, on a longer time scale, it may lead to very long carryover since pollen that is covered up may be exposed subsequently by preening, or the sloughing off or deposition of upper layers (see discussion in next chapter). This could lead to extensive carryover between foraging flights.
Perkins (1977) concluded that between foraging flight carryover is probably the most important component in between genome pollen transfer. The length of time that pollen is viable once removed from the anthers may set limits to the benefits accruing from this uncovering process (pollen viability is sensitive to ultraviolet radiation (Percival 1965), relative humidity, and temperature (Stanley and Linskens 1974)). Frankie et al. (1976) have suggested that carryover of residual pollen from day to day may be important for outcrossing. In their system, day old pollen is viable and the self-incompatible trees they were studying set more seed than could be explained by their assumption of almost no carryover. Preliminary evidence suggests that layering occurs on bumblebees as well (J. Thomson, pers. comm.).

I have been referring to the pool of pollen on the hummingbird as if it were neat and highly structured. It probably is not. But it is distinctly three dimensional, and is probably heterogeneous with respect to pollen identity in all three dimensions. When I examined pollen pools on both captive and wild birds, they were irregular and often discontinuous, and they penetrated the three dimensional structure of the feathers. It took several applications of tape to the same place on a pollen pool in a bird's feathers to remove all the pollen.
Plant-Plant Transfer

Taking into account layering, and the probable overestimation of pollen carryover by dye carryover (see methods), we can assume, for speculative purposes, that a carryover limit of 15 to 20 flowers past the source is common in this system. In the average bout of foraging, a hummingbird will visit 21 flowers on 3-10 plants (Perkins 1977). Thus carryover to 2-9 plants past the source should be common, and for later plants in a flight, between bout carryover should be significant. Any carryover due to uncovering of buried pollen will be in addition to this.

This conclusion is in contrast to that of Levin and Kerster (1969 a) who concluded that carryover to 3-10 plants was a "gross overestimate" in a bee-plant system. Thomson and Plowright's (1980, and Thomson pers. comm.) estimates of bee carryover are between Levin and Kerster's (1969a) assumption for bees, Levin and Berube's (1972) findings for Colias butterflies, and my own values for hummingbirds. Waser and Price (1981) report pollen carryover values for bumblebees (Bombus) that are comparable to Thomson and Plowright's. They found higher carryover for hummingbirds (S. platycercus) than for bees, but not as high as I found for S. rufous.
Bumblebee-Hummingbird Comparison

Many experimental studies of pollinator-plant systems have involved bumblebees or hummingbirds (e.g. Pyke 1978; Price and Waser 1979; Thomson and Plowright 1980; Pleasants and Zimmerman 1980; Waser and Price 1981). There are important differences between these two classes of pollinators that have consequences for pollen transfer kinetics. Bumblebees often forage for pollen, hummingbirds do not, and bumblebees regularly remove pollen from its deposition sites on their bodies while grooming, and thus change the character of the pollen pool subsequent to the deposition of pollen (Thomson and Plowright 1980). While preening by hummingbirds may have the same effect as grooming by bumblebees, it is probably not as common while foraging, or as extensive.

Waser and Price (1981) report a stronger relationship between dye dropoff and flower visitation sequence for bumblebees than for hummingbirds (r=−0.44 vs −0.23, compare with Table II). Grooming by the bumblebees may reduce the effects of floral and behavioural variability on pollen carryover, leading to a stronger effect of flower sequence, and fewer "blanks". This could also explain the shorter, steeper, carryover curves that have been found for bumblebees. Because of these differences, the tendency to generalize quantitatively between these systems must be carefully reappraised.
Male Versus Female Function

A self-incompatible plant should minimize the transfer of pollen between its own flowers. One logical extreme of this could be accomplished by having only one flower mature at a time. This would maximize that flower's reproductive success through female function, but would probably not maximize the benefit to the plant as a whole. The more flowers a plant presents, the higher its success through male function - it will flood the pollen pool and should pollinate many other flowers. However, the more flowers a plant has simultaneously, the more likely it will be selfed. Augspurger (1980) describes a similar situation for a tropical mass-flowering shrub, where much selfing is expected due to "flooding" of the pollinators by pollen from the large number of flowers a single plant presents (see also Schemske 1980).

Freeman et al. (1980) suggest that in patchy environments plants will benefit from labile sexual expression, and that environmental determination of sexual expression is much more common than has generally been assumed. I suggest that in addition to the production of strictly male or female flowers, plants could achieve this kind of sexual tracking of their environment by subtle tuning of their floral display. Characters such as the number of flowers produced, variation in floral morphology, nectar production rate, nectar concentration, and the time courses of anther dehiscence and stigmal receptivity could
act together to make an apparent hermaphrodite functionally only one sex. When will it pay to ensure success through male function at the expense of success through female function? When will it pay to compromise at both and risk not doing well at either? How these different components of floral display interact with each other and with pollinator behaviour to result in patterns of pollination and fitness is the key problem facing pollination ecology in the near future.

**Conclusions**

Past analyses have tended to ignore variability in pollen transfer. Variability in floral morphology appears to play a crucial role in determining not only the length of pollen carryover, but to which individuals pollen is carried. In the future, variability should be considered as an important factor in itself, and not averaged out in the analysis. This is most true for species in which variation in pollinator - flower geometry is great, either due to characters of the pollinator, or of the plant.

Researchers study longer sequences of flowers than has been the norm in the past. Using short sequences biases the result towards short carryover. If long carryover is occurring, it will not be detected in short flower sequences.

These results are not conclusive, but suggest realistic alternative hypotheses for the shapes of pollen carryover
curves and the processes that generate them.
"... even when we have correct premises, it may be very difficult to discover what they imply. All correct reasoning is a grand system of tautologies, but only God can make direct use of that fact. The rest of us must painstakingly and fallibly tease out the consequences of our assumptions." Simon (1981)

Introduction

Transfer of pollen among flowers is a fundamental component of gene flow in many plant species. In the literature, pollen dispersal is often described by three components: pollinator flight distances, pollinator directionality, and pollen carryover. Pollinator flight distances have received much attention as estimators of pollen dispersal (Levin and Kerster 1969a,b; Levin et al. 1971; Beattie 1978; Beattie and Culver 1979; Schmitt 1980; Waser and Price 1981), and various aspects of pollinator behaviour, including inter-flight directionality of pollinators, have been studied by those interested in foraging (e.g. Pyke 1978; Heinrich 1979; Zimmerman 1979; Gass and Montgomerie 1981). However, little is known about
carryover, and assumptions about carryover are weak links in the chain of reasoning about gene flow.

For this discussion (as above) I will define pollen carryover as the number of subsequent flowers to which the pollen of one flower is carried. Carryover could also be defined in terms of numbers of plants. If pollen from one flower is carried over and deposited at more flowers than just the next one the pollinator visits, then pollinator flight distances will underestimate pollen transfer distances. Many times researchers have made relatively unsupported assumptions about the extent of pollen carryover (Levin et al. 1971; Frankie et al. 1976; Feinsinger 1978; Richards and Ibrahim 1979; Augspurger 1980; Schmitt 1980).

Many current ideas about pollen dispersal and the factors affecting it stem from the work of Levin and his colleagues (Levin and Kerster 1967; 1968; 1969 a,b; Levin and Berube 1972; Levin et al. 1971; Kerster and Levin 1968). In general, they observed that pollinator flight distances are leptokurtic and closely related to plant spacing distances, and inferred or assumed low pollen carryover. Some of these results appear to be general; for instance a leptokurtic distribution of pollinator flight distances is common in hummingbirds, bumblebees, hawkmoths, and butterflies (e.g. Perkins 1977; Price and Waser 1979; Waser and Price 1981; Beattie 1978; Schmitt 1980; Gass and Montgomerie 1981), although there may be significant differences in the degree of leptokurtosis between different
pollinators (Schmitt 1980).

Recent work on hummingbirds (Perkins 1977; Lertzman, this thesis; Waser and Price 1981) and bumblebees (Thomson and Plowright 1980; Waser and Price 1981) suggests that pollen carryover is longer and more variable than was previously indicated. This may be due to variation in both floral morphology (Lertzman, see last chapter) and pollinator behaviour (Thomson and Plowright 1980). If nuances of floral morphology or pollinator behaviour do strongly affect whether pollen is picked up by or dropped off from pollinators at any given flower, then little generalization between systems may be possible without taking these factors into account.

This chapter will describe alternative sets of assumptions about the processes involved in pollen transfer and will discuss their consequences in terms of pollen carryover. There are three objectives: to organize and state explicitly the common assumptions about pollen carryover, to assess the validity of these assumptions, and to suggest some alternatives. This discussion is based on a series of simulation modelling exercises. Before discussing specific models in detail, it is important to discuss the approach to modeling in general. The following section does this, and also introduces the structural framework of the models.
On Building Models Of Pollination

Models are useful as explicit statements of hypotheses. They allow formal evaluation of the consequences of given sets of assumptions, and are thus powerful deductive tools. I distinguish between pattern-oriented and statistical models, and process-oriented models.

Pattern-oriented models provide useful descriptions of broad relationships expected from theory or observed in nature, and to the extent that the patterns they generate are accurate, may be useful aids to understanding higher level phenomena. For example, models that generate patterns of pollen distribution can be used to examine the implications of these patterns for the neighbourhood structure of plant populations (Levin and Kerster 1969; Levin et al. 1971; Schmitt 1980). In my view, however, models can provide only limited insight into how patterns are generated unless they embody realistic biological processes. That is, the usefulness of models increases when they generate realistic biological patterns at one level through the simulation of realistic biological processes at a more detailed level (Clark and Holling 1979). Such hierarchically structured models are doubly vulnerable to logical or experimental invalidation, and therefore doubly powerful as heuristic tools. Not only can their output fail to show reasonable patterns, but their explicit assumptions about the underlying mechanisms can be shown to be wrong.

There are three essential components to process models
of pollination: pickup of pollen by a pollinator from the anthers of flowers, transport of pollen in a pool of pollen on the pollinator, and deposition of pollen on the stigmas (or anthers!) of other flowers. Alternative models may be distinguished by the number of sub-components into which these basic three are disaggregated, by the assumptions that are made about those sub-components, and by the ways in which interactions between these components are organized.

The central feature of each model is a visit by a pollinator to a single flower: the "unit interaction". Population-level consequences of assumptions about the unit interaction are generated by iterating through it many times and collecting information from each iteration about pollen picked up or deposited, or about the structure of the pollen pool. By allowing variation in the factors that affect the components of this interaction, for instance variation in floral morphology, we are able to study how the distribution of traits in populations of flowers is reflected in population-level patterns of pollen dispersal.

**Alternative Models**

Models 1 through 3 were programmed in BASIC on Apple microcomputers. Model 4 was written in "C" and run on a Digital PDP-11/45. Copies of all programs are available on request.

The models are arranged in order of increasing realism of both process and pattern and increasing structural
complexity (Table VI). Model 1 is pattern oriented, with

Table VI. General characteristics of the models.

<table>
<thead>
<tr>
<th>character</th>
<th>model 1</th>
<th>model 2</th>
<th>model 3</th>
<th>model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>stigmatic capacity</td>
<td>unlimited</td>
<td>unlimited</td>
<td>limited</td>
<td>limited</td>
</tr>
<tr>
<td>anther capacity</td>
<td>limited</td>
<td>limited</td>
<td>limited</td>
<td>limited</td>
</tr>
<tr>
<td>pollen picked up from every flower</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>pollen from each source deposited at every flower</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>number of pollen grains picked up, when not 0</td>
<td>constant</td>
<td>constant</td>
<td>constant</td>
<td>variable</td>
</tr>
<tr>
<td>number of pollen grains deposited, when not 0</td>
<td>constant proportion of the pollen remaining from each source</td>
<td>constant number randomly chosen from the pollen pool remaining from each source</td>
<td>variable: function of number and distribution of pollen remaining from each source</td>
<td></td>
</tr>
</tbody>
</table>

little underlying realism. It is simple, historically significant, and represents commonly held assumptions. I treat it as an initial null hypothesis for comparison with other models and experimental data. Model 2 is a simple
modification of model 1 that produces more reasonable patterns of pollen dispersal, but still lacks a realistic mechanism. Models 3 and 4 make more explicit statements about pollen pool structure and how it affects pollen deposition. Model 4 incorporates stochastic variation in floral morphology that determines from where in the pollen pool pollen will be deposited or picked up.

These models assume that the pollinator deposits pollen on a flower's stigma prior to picking up pollen from its anthers. Though this refers to the temporal sequence of the mechanics of pollen transfer, it implies an assumption about plant breeding systems. Thus the models will apply most directly to systems where flowers rarely if ever receive their own pollen.

Spatial structure is absent from these models. Flowers are unique, and are visited only once. They have no pre-defined association as plants. Superposition of spatial factors and pollinator movement on top of these models is the next step in the modelling of pollen transfer, but it is not covered here.

The pollinator starts out at the beginning of a model run with no pollen on it - an empty pollen pool. As it feeds, pollen is added to and taken away from the pool according to the rules for that model. To measure pollen carryover, one flower is chosen from each run and the deposition of it's pollen is followed. In models 1 and 2 there is no effect of pool size on the probability of
deposition of individual pollen grains, and I studied carryover from the first flower of the sequence. In models 3 and 4, deposition probabilities vary with pool size, so I studied carryover from the 11th and 20th flowers in each sequence respectively.

I used 50 flower sequences (i.e. checking 50 flowers past the source for possible pollen deposition) to generate the output for comparison between all four models. Data presented are from up to 60 runs of a model in order to generate a distribution of possible outcomes from a given set of assumptions. The restriction to a maximum of 50 flowers per run was set by space limitations on the Apple microcomputers. Model 4 is also discussed with more flowers per run for the more detailed analysis of its behaviour.

The output variables are mean and maximum carryover. Mean carryover is calculated by summing the sequence numbers of the flowers that receive pollen and dividing that number by the number of flowers that received pollen. For instance, a sequence in which pollen was deposited on the 5th, 16th, and 24th flowers past the source would have a mean carryover of: 5 + 16 + 24 / 3 = 15 flowers. The maximum carryover is the sequence number of the last flower in a sequence to receive pollen. Unless specified otherwise, "carryover" refers to both of these statistics. Grand means and mean maxima are the means of these values for a number of sequences.
Model 1: Constant Proportion Of Pollen Grains Deposited: Exponential Decay

To study gene flow or to make inferences about the genetic structure of plant populations often requires a knowledge of pollen dispersal distances (i.e. Levin and Kerster 1968; 1969 a,b; Richards and Ibrahim 1978; Schmitt 1980; Waser and Price 1981). Because this is difficult to measure, observed pollinator movement distances are often used to estimate pollen transfer distances (e.g. Beattie and Culver 1979). This correspondence is perfect if there is no pollen carryover. Levin and Kerster (1969a) used exponential decay to model carryover to produce a correction factor for pollinator flight distances so as to better estimate pollen transfer distances. They found that, given the assumption of exponential decay, their estimates of pollen transfer distances were insensitive to their assumptions about carryover (i.e. the values of the parameters of the model). Since exponential decay characterizes much of the literature, I will start with it as the initial null hypothesis for pollen transfer.

Exponential decay is produced if a pollen pool is decremented by a constant proportion of its current size at each flower visited. When this proportion is large, the decay curve is steep. Thus, in this model, a constant number of pollen grains is picked up at each flower visited by the pollinator, and a constant proportion of the pollen remaining from each previously visited source is deposited
at each subsequent flower. Pollen from different sources is assumed not to interact in the pollen pool on the pollinator, and is equally available for deposition. There is no limit to the number of grains that can be deposited on stigmas.

The amount of pollen deposited declines rapidly with the number of flowers after the pollen source (Fig. 8). When the proportion of the remaining pollen deposited from each source is high (0.4), no pollen is deposited after 11 flowers. When the proportion is very low (0.05), small amounts of pollen are deposited up to 46 flowers past its source.

There are three key qualitative attributes of this model that we can compare with data: 1) all flowers receive pollen and the curve is thus smooth. 2) a constant proportion of the remaining pollen is deposited at each flower, so that we should observe deposition as a decreasing function of the flower visitation sequence, and 3) if the proportion deposited is large, the curve drops steeply. Based on experimental results from bumblebees (Thomson and Plowright 1980; Waser and Price 1981), and hummingbirds (Perkins 1977; Lertzman, this thesis), each of these points can be rejected. In each of these experiments pollen deposition was variable; some flowers received no pollen, and later flowers in a sequence often received more pollen than earlier flowers. However, some of these results must be accepted cautiously and qualitatively. Lertzman (this
Figure 8. Pollen deposition curves from model 1: constant proportion deposited, simple exponential decay. Each curve indicates the pattern of pollen deposition from source flowers expected under model 1 if 40%, 20%, 10%, or 5% of the pollen from each source remaining on the pollinator were deposited on each flower visited. The ordinate scale is arbitrary, indicating that 100 grains were picked up from the source flower.
POLLEN GRAINS DEPOSITED

FLOWERS PAST SOURCE

10 20 30 40

5% 10% 20% 40%
thesis) and Waser and Price (1981) used fluorescent powders as pollen mimics, and they assumed that the dyes mimic pollen at least qualitatively. However the correspondence between pollen carryover and dye carryover is not well established (see discussion in chapter on laboratory experiments).

While this model may seem something of a straw man, it is implicit in much current thinking about pollen carryover. It is corroborated by Levin and Berube's (1972) data on Phlox and Colias butterflies, but their results are probably not general. For instance, Levin et al. (1971) cautioned that systems in which pollen is transported on a proboscis will show lower carryover than those in which pollen is carried on the body of the pollinator because probosci are more efficient at depositing pollen.

The paper by Levin and Berube (1972) has had a large impact on assumptions about pollen carryover, and pollen flow is probably sensitive to these assumptions (even though Levin and Kerster (1969a) showed that pollen flow was insensitive to parameter changes given the exponential decay model, this does not necessarily hold for the assumptions about the underlying model itself). It is therefore valuable to discuss their results in detail. One factor that may have led to the view of very efficient pollen deposition from probosci is that Levin and Berube (1972) experimentally prevented the butterflies in their experiments from retracting their probosci between flowers.
Normal coiling and uncoiling could displace pollen from its sites of initial deposition, which would lead to a more patchy distribution of pollen. A spatially patchy distribution of pollen on the pollinator probably results in longer carryover than a uniform and contiguous distribution (Thomson and Plowright 1980; Lertzman, this thesis). Additionally, Levin and Berube (1972) used short (5 flower) sequences in their experiments; therefore they could not have observed long carryover even had it occurred. Observed carryover is very sensitive to the length of the sequence of flowers that is used to measure it. For instance, when we increased the number of flowers in a sequence from 30 to 100 (in model 4), the proportion of the total pollen transferred that was deposited more than 19 flowers past the source increased from about 10% to about 30%.

The effect of sequence length on observed carryover seems an obvious point, but there are no experimental measures of carryover with realistically large numbers of flowers. In the wild, some hummingbirds need to feed from an average of 3700 flowers per day (Gass et al. 1976), and we have no idea whether short to moderate (e.g. 30 flowers) experimental sequences (e.g. Thomson and Plowright 1980; Waser and Price 1981; Lertzman, this thesis) give any where near a realistic picture of pollen transfer when such large numbers of flowers are involved.

What can be inferred about carryover from the pollen loads on stigmas observed in nature? Based on the highly
variable pollen loads found on the stigmas of adjacent flowers and plants, Levin and Kerster (1967; 1968) inferred low carryover. Alternatively, these variable pollen loads could have resulted from longer but more variable patterns of pollen deposition. In this case, adjacent flowers would not necessarily receive similar pollen loads. How could this variability originate? The following models examine some possibilities.

Model 2: Constant Proportion Of Pollen Grains Deposited: Exponential Decay With Blanks

Without explicitly modelling the processes that generate variability, the exponential decay model can be made to produce somewhat more realistic results by assuming that some flowers do not interact with the pool of pollen on the pollinator (Lertzman, this thesis): no pollen is picked up from their anthers and/or none is dropped off to their stigmas. In nature, this could be produced by variation in floral morphology, in the reproductive state of the anthers and stigmas, and in pollinator behaviour (e.g. the orientation of relevant parts of the body while contacting the flowers). These factors could act in either or both of two ways. They could influence the probability that flowers will contact the pollinator, or they could influence the probability of successful pollen transfer if that contact is made. I will refer to these flowers that create gaps in pollen deposition sequences as "blanks" (see chapter on
laboratory experiments).

Model 2 has two kinds of blanks; anthers that don't deposit any pollen on the pollinator, and stigmas that receive no pollen from the pollinator. Missed stigmas increase the carryover of previously deposited pollen (compare Figs. 8 and 9). Missed anthers decrease the observed mean carryover because zero values from "non-participating" anthers are included in the population statistics (Fig. 10; Table VII).

As expected, both mean and maximum carryover decrease as the proportion of the remaining pollen deposited at each flower increases (Fig. 11). For comparison of this model with the following two models (Table VII and Fig. 10) I use a proportion of 0.3 as representative.

This model assumes that there is no interaction between pollen from different sources, and that stigmas have unlimited capacity for pollen. Although the deposition of pollen is still a decreasing function of the number of flowers visited previously, the number of grains stigmas receive is variable because not all previously visited flowers contribute pollen to the current flower.

This model of exponential decay with blanks is primarily of value as a more reasonable null hypothesis than simple exponential decay; it mimics the variability of the real world without specifying causal mechanisms. Thus it provides a basis for comparison with the following models, which make explicit statements about the processes that
Figure 9. Pollen deposition curves from model 2: constant proportion deposited, exponential decay with blanks. Symbols and scales are as in Figure 1, except that the abscissa extends to 50 flowers past the source flower. Based on field measurements of reproductive state in *Castilleja miniata* 26% of the stigmas, and 27.5% of the anthers, randomly chosen, did not interact with the pollen pool on the pollinator i.e. were blanks.
POLLEN GRAINS DEPOSITED

FLOWERS PAST SOURCE

5% 10% 20% 40%

10 10 20 30 40
Figure 10. Frequency distributions of mean and maximum pollen carryover from models 2, 3, and 4. Sample sizes (number of runs of the model) are given in Table 2. Data from model 2 with 0.3 of the remaining pollen deposited at each flower, data from model 3 with 10 pollen grains picked up and 3 grains deposited at each flower.
Figure 11. Mean and maximum pollen deposition from model 2. Closed circles and solid lines indicate means of 30 runs. Open circles and broken lines indicate the 25 of those runs in which some pollen was dropped off (i.e. excluding zero deposition values). Vertical lines indicate 95% confidence intervals.
Table VII. Carryover statistics for models 2, 3 and 4.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th>model 3</th>
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<th>model 4</th>
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<td>16</td>
<td>5</td>
<td>14</td>
<td></td>
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<tr>
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<td>9</td>
<td>15</td>
<td>0</td>
<td>14</td>
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<td>1.2</td>
<td>43.3</td>
<td>85.5</td>
<td>98.2</td>
<td></td>
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<tr>
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<td>17</td>
<td>32</td>
<td>7</td>
<td>17</td>
<td></td>
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<tr>
<td>median extreme carryover</td>
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<td>18</td>
<td>35</td>
<td>0</td>
<td>16</td>
<td></td>
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<tr>
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<td>30</td>
<td>60</td>
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zero depos. = zero deposition

generate variability in pollen deposition.
Model 3: Constant Number of Pollen Grains Dropped Off:

Limited Stigmal Capacity

In his models of competition between plants for pollination, Waser (1978) assumed that a constant number of pollen grains was picked up from each flower visited, and that a constant number of grains, randomly chosen from the pool, was deposited at each flower. Each flower had a limited number of grains on its anthers, and a limited amount of space available on its stigma. Model 3, based on Waser's assumptions, assumes that pollen grains from early flowers in visitation sequences are just as likely to be chosen for deposition as pollen from more recently visited flowers. Thus there is again no interaction between pollen from different source flowers (in contrast, model 4 explicitly models interactions among pollen grains in the pollen pool). Following Waser (1978), in model 3 the pollinator deposits 3 randomly chosen pollen grains on the stigma of each flower and picks up 10 pollen grains from the anthers of each flower. Grains are drawn independently and without replacement from the pool of pollen on the pollinator.

There are two differences between this model and Waser's (1978). Whereas he simulated a pollinator moving through an array of flowers, and flowers could be revisited, in this model flowers have no spatial identity and are only visited once. Similarly, while a total of 3 grains was deposited per flower per visit, these were counted towards a
total of 5 per flower as a maximum stigmal capacity. In model 3, since revisits do not occur, the 3 grains deposited on one visit are equivalent to the stigmal capacity. The difference between 3 and 5 grains might be important if I was also directly modelling gene flow, but since here I am primarily interested in the consequences of the pollen deposition rules, the distinction is not important.

Model 3 produces long and irregular carryover (Fig. 10, Fig. 12, and Table VII) that is similar to some experimental results (Thomson and Plowright 1980; Lertzman, this thesis). This variability most likely results from the limited stigmal capacity. Stigmal capacity is less than the number of grains available for deposition. Thus even though each stigma is saturated, only a small proportion of the pollen grains available can be deposited on any given stigma. For a given pollen source, many blanks exist. When the model is driven with increasing stigmal capacity, mean carryover decreases and maximum carryover shows a peak between 3 and 5 grains per stigma (Fig. 13). This peak of maximum carryover occurs in the range of pollen pickup to deposition ratios commonly observed in real systems (Waser 1978).

The shorter carryover at the two extremes of stigmal capacity results from different causes. The only situation in which zero deposition values occurred (i.e. when all pollen from a particular source remained on the pollinator and was never deposited) was when only one grain was deposited per stigma. Thus at the lowest stigmal capacity,
Figure 12. Three representative pollen deposition curves from model 3: constant number of grains deposited. 1, 2, or 3 pollen grains could be deposited per stigma.
POLLEN GRAINS DEPOSITED
Figure 13. Mean (closed circles and solid lines) and maximum (open circles and broken lines) pollen carryover from 30 runs of model 3 at each of several levels of stigmal capacity. Vertical lines indicate 95% confidence intervals.
carryover was lowered because pollen stayed on the pollinator and was not deposited. The probability that a given pollen grain will be deposited is sensitive to the current size of the pollen pool on the pollinator. After many flowers have contributed pollen to the pool the probability that pollen from any given flower will be chosen is quite small: \[ p = \frac{1}{10N - N}, \] where \( N \) is the number of flowers fed on previously. The reason that carryover decreases at high ratios of deposition to pickup is that as the number of pollen grains deposited per stigma approaches 10, pollen is being deposited from the pollinator almost as fast as it is being picked up. When 10 grains were deposited by anthers and 10 picked up by stigmas carryover was to one flower past the source.

Carryover (grand means and mean maxima) is both longer and more variable in model 3 than in results from model 2 (Table VII, Fig. 10). Carryover in model 2 could be made longer by having a higher proportion of "blank" stigmas, but I suspect that the variance would not increase. It is important to note that this is variance not only in pollen deposition in individual sequences of flowers (i.e. Fig. 12), but in the population parameters associated with the distribution of that variability (i.e. Table VII, Fig. 10). Variability in model 3 output is highest at low ratios of deposition to stigmas to pickup from anthers (Fig. 14). This is consistent with the decreased probability that a given pollen grain will be deposited
Figure 14. Variances of mean (closed circles and solid lines) and maximum (open circles and broken lines). Pollen deposition distances from the same 30 runs of model 3 as Figure 6.
under these conditions.

The pollen pool itself is assumed to be a randomly organized bin of pollen grains: selection of pollen for transfer to stigmas is random, without regard for either the characteristics of the current flower or the sequence in which pollen was deposited in the pool. This is difficult to imagine in terms of underlying biological processes. Although grooming by bumblebees or preening by hummingbirds could conceivably randomize pollen in the pool with respect to visitation sequence (or obliterate it altogether), I would still expect some non-random pollen pool structure. In other words, I would expect precedence for deposition from the pollinator based on the time since last grooming or preening.

An alternative model might retain limited stigmal capacity but incorporate precedence for deposition based on visitation sequence. For instance, the probability that pollen will be deposited could be made dependent on the number of grains remaining from a flower and the number of flowers visited since they were picked up. This probability could be calculated using arbitrary scaling factors to produce reasonable patterns, but I chose instead to model a hypothetical causal mechanism. The next step, then, is to explicitly model pollen pool structure and its input/output processes. When comparing model 4 with model 3, the question that should be asked is "How much understanding is gained by adding these additional components and their
attendant complexity, and at what cost?"

**Model 4: 3-Dimensional Pollen Pool With Layering and Floral Variability**

Other than limited stigmatic surface area, what factors could result in the "blanks" of model 2? Thomson and Plowright (1980) and Lertzman (this thesis) suggested that variation in the orientation of pollinator and the flower during contact results in patchy distribution of pollen in the pollen pool. A possible mechanism for this is that variation in floral morphology, for instance in flower length or lateral displacement of stigmas and anthers, would result in pollen from different flowers being deposited in different locations in the pollen pool. Flowers of different lengths would contact pollinators in different places, and hence pollen transfer between them would be unlikely.

This model rests on the following assumptions:

1. Pollinator-flower geometry during contact determines where pollen will be deposited on pollinators.

2. Pollinator-flower geometry is driven by variability in floral morphology (one of several possible mechanisms).
3. If different flowers deposit pollen in the same place on the pollinator, it will be deposited in layers, or last-in-first-out stacks.

These ideas are based on laboratory experiments with rufous hummingbirds (*Selasphorus rufus*) and Indian paintbrush (*Castilleja miniata*) flowers (Lertzman, this thesis), and probably apply most directly to hummingbird-plant systems.

In this model, the pollen pool is a 3-dimensional array 14 units in width by 36 units in length (Fig. 15). The dimensions are based on measurements of pollen loads on freshly netted wild *Selasphorus rufus* that held territories in *Castilleja miniata* meadows, and on laboratory birds that had fed from known numbers of flowers. The third dimension, depth, is allocated dynamically as space is needed (Fig. 15). Whether model anthers and stigmas contact this pool or not, and where they contact it, if they do, depends on their length and lateral displacement from the midline of the flower. Both anthers and stigmas are constant in size, but anthers have areas 6 times larger than stigmas (4 x 6 vs. 2 x 2 pollen pool map units, based on measurements of *Castilleja miniata*).

I assume that variation in two floral characters, lateral displacement and corolla tube length, is normally distributed (Fig. 15). Real *Castilleja miniata* flower lengths deviate significantly from normality (they are both skewed and leptokurtic), but the lateral displacement of
Figure 15. Drawing of an immature male rufous hummingbird (traced from a photograph) showing the hypothetical bivariate normal distribution of contact between the pollinator and floral reproductive organs. The two dark rectangles indicate the relative sizes of Castilleja miniata stigmas (small) and anthers (large). Bivariate normal distribution from Sokal and Rohlf (1969; Fig. 15.1, p. 501).
stigmas and anthers does not deviate significantly from normality (data are from the measurements of photographs described in the section on laboratory experiments).

In unit interactions between pollinators and flowers, anthers and stigmas are chosen randomly from normal distributions of a given mean and variance. Flowers are thus most likely to contact the center of the pool rather than the edges. Flowers in the tails of the distributions are likely to contact the pool only partially if at all, and will have less surface area available for pollen transfer. If there is no pollen at a location in the pool when a stigma contacts it, then no pollen is deposited on that stigma. Stigmas receive pollen from the top layer of the pool only, and once the top layer at a location is removed, the remaining layers are pushed up one step closer to the surface. They are pushed back down if pollen is added on top of them.

This model produces highly variable carryover (Fig. 16, Table VII). Many flowers receive no pollen, and those that do receive variable amounts. In these 50-flower runs, there are many cases where no pollen from a flower is deposited on other flowers. The frequency distributions of mean and maximum carryover show a high proportion of zero deposition values and have long tails representing less frequent long carryover (Fig. 10).

Zero deposition could result either from pollen being buried by other pollen and never being uncovered, or from
Figure 16. Three representative pollen deposition curves from model4: 3-dimensional pollen pool. 0, 1, 2, 3, or 4 pollen grains could be deposited per stigma.
pollen being deposited at locations where other flowers are unlikely to contact it (it could also result from no pollen being picked up by the pollinator from that flower, see below). Because I assume the pollen pool is rectangular, and that flower variability follows a bivariate normal distribution, the corners of the pollen pool have a low likelihood of contacting flowers. If a uniform distribution of flower variability is assumed, and zero deposition values are still present, then zero deposition is not a result of the distribution of flower variation, but more likely a result of the covering up of pollen. This appears to be the case; when I run model 4 assuming uniformly distributed variation in floral morphology, many zero values still occur. If the runs had been longer, say 120 flowers, pollen from these sources may eventually have been deposited, but I have not observed this.

Two kinds of modifications to model 4 would make it more likely that buried pollen would resurface:

1. The "coal scoop" hypothesis suggests that stigmas are dragged through the pollen pool, rather than touching on its surface as we have assumed so far. This has two new consequences: pollen from more than the surface layer is available for deposition on stigmas, and unit interactions create 3-dimensional grooves through the pollen pool rather than simply lifting the top layer of pollen from
an area of their own size. Both of these consequences would make it much more likely that stigmas contact pollen in a patchy or sparsely filled pool, and that buried pollen would resurface. This would decrease the incidence of zero deposition and increase mean carryover.

2. In nature, differential stickiness of pollen to other pollen, to stigmas, and to anthers may keep pollen pools from building up as much as in this model. Additionally, periodic sloughing of patches of pollen, induced by rain, grooming or preening by the pollinator, wind resistance during flight, or weak binding of pollen in the pool may "reset" the pool and expose deeply buried pollen. There is evidence that in many anemophilous species of plants, pollen commonly occurs in clumps of 2 - 9 grains (Anderson 1970, in Stanley and Linskens 1974). Pollen - pollen binding of this sort would facilitate the sloughing off of larger masses of pollen, both onto stigmas and during preening.

Overall mean carryover for model 4 is much less than for model 3 (Table VII), and the incidence of zero deposition is much greater (Fig. 10). However, if mean carryover is calculated excluding the zero deposition values (i.e. if we only consider carryover of pollen that was
deposited on stigmas), then carryover in model 4 increases substantially. Both mean and maximum carryover in model 4 have greater variances than in model 3. This difference is maintained even when zero deposition values are excluded.

Thus models 3 and 4 both show regular long carryover (Fig. 10), but model 4 has a higher variance associated with carryover and a much higher frequency of zero deposition. When I decreased the ratio of stigmal pickup to anther deposition in model 3 from 3:10 toward 1:10 (Figs. 13 and 14), model 3's behaviour approached that of model 4. However, it remained intermediate and did not reach the level of model 4. For instance, model 3 produced 13% zero deposition when 1 grain was deposited per stigma, compared to 65% in model 4. Model 4 has potentially a much smaller stigma:anther capacity ratio and the remaining difference between model 3 and model 4 could be due to this. However I suspect that it is due to a combination of the large number of grains in the pollen pool relative to stigmal capacity and the likelihood that many of those grains will be permanently covered by other pollen. As long as the rate of covering is greater than the rate of uncovering, the possibility of layering will enhance the effect of limited stigmal capacity relative to anther deposition and produce this effect of many zero deposition values.

Though there is some experimental evidence suggesting layering of pollen on hummingbirds, (Lertzman, this thesis), it is far from conclusive. Because of the potential that
layering profoundly affects carryover by increasing the likelihood of zero deposition (which may just be very long carryover), it is important to assess the occurrence of layering in real systems.

The role of variation in floral geometry was one of the main results of the laboratory experiments, and is the prime distinguishing feature of model 4. What are the effects of increasing or decreasing the amount of variability in floral characters?

I accomplished this by varying the coefficients of variation for floral contact in both the x and y dimensions. In the previous discussion of model 4 results, floral variability was normally distributed in the x and y dimensions of the pollen pool with a coefficient of variation of 1. In the following discussion these coefficients of variation vary from 0.2 to 4.0. This is analogous to an experiment where a pollinator fed from populations of flowers that varied from being highly stereotyped in form to being highly variable. Floral characters measured in the field had coefficients of variation from about 0.2 to 2.0. For instance two measures of flower length had coefficients of variation of 0.291 and 0.345, and two measures of lateral displacement (of stigmas and anthers) had coefficients of variation of 1.29 and 1.80.

There are three types of output variables with which I will examine the effects of driving model 4 with the amount of floral variability: 1) total pollen pool input and
output; the total number of pollen grains picked up and deposited by the pollinator, 2) pollen pool structure at the end of an 85 flower run; the proportion of the pool occupied in the x,y dimensions, and the mean depth of the layers in the pool, and 3) the pollen carryover statistics, mean and maximum carryover.

Pollen pickup decreases with increasing floral variability (Fig. 17). As flowers are more and more variable, a greater proportion of them occur in the tails of the distribution that extend over the edge of the pollen pool. The pollinator receives no pollen from these flowers. This explains many of the zero deposition values (actually zero pickup values) that occur at high floral variability. However, even at the lowest level of floral variability, when zero pickup is very unlikely, there was 20% zero deposition.

The total number of pollen grains deposited first increases slightly and then decreases with increasing floral variability (Fig. 18). Why is less pollen deposited at very low floral variability than at slightly higher levels of variability? At very low variability, only a small proportion of the area of the pool is occupied (Fig. 19), and most of the pollen is concentrated in a highly layered central area (Fig. 20). All flowers are contacting the pool in almost exactly the same location, and since more pollen is put into the pool with each flower than is taken out, pollen is only exposed on the surface and available for
Figure 17. Total number of pollen grains picked up by the pollinator while feeding from 85 flowers, output from model 4 driven with changing floral variability. Coefficients of variation in both the x and y dimensions were stepped from 0.2 to 4.0 in increments of 0.2. There were 20 runs of the model (i.e. 20 sequences of 85 flowers) at each level of floral variability.
Figure 18. Total number of pollen grains deposited from the pollen pool while the pollinator feeds from 85 flowers, output from model 4 driven with changing floral variability. The method for generating these results was as described for Fig. 17.
Figure 19. The proportion of the pollen pool occupied in the x and y (surface) dimensions at the end of runs of 85 flowers, output from model 4 driven with changing floral variability. The methods for generating these results was as described for Fig. 17.
Figure 20. Mean layering in the pollen pool at the end of runs of 85 flowers, output from model 4 driven with changing floral variability. The method for generating these results was as described for Fig. 17.
deposition for a few flowers before it is covered up. Hence the high degree of layering. There are two reasons why total pollen deposited decreases at higher floral variability. First, because less pollen is picked up to begin with (Fig. 17), there is less available to be deposited. Second, the pollen pool is much sparser at high levels of floral variability and it is less likely that any given flower's stigma will contact the pool at a location where pollen has been previously deposited. Figure 19 shows that the proportion of the pool occupied is low at both low and high variability. However, at low floral variability, floral contact is generally restricted to that small area, while at high variability, contact is spread throughout the pollen pool.

Regressions of mean and maximum pollen carryover on floral variability have a significant negative slope (Table VIII and Fig. 21), i.e. there is apparently longer carryover with lower floral variability. However, if the zero carryover values are removed, the regression of mean carryover on floral variability has a significant positive relationship, and that for maximum carryover has no significant relationship (note however, that in all cases the r squared is quite small). The proportion of zero deposition values increases with floral variability (Fig. 22). This accounts for the negative slope of the regression of carryover on variability when zero values are included. Thus at high levels of floral variability,
Figure 21. Means and 95% confidence intervals for mean and maximum carryover of pollen when model 4 is driven with changing floral variability. The method for generating these results was as described for Fig. 17.
Figure 22. The proportion of zero deposition when model 4 is driven with changing floral variability. Zero deposition could have resulted from either no pollen being picked up or no pollen being deposited. The method for generating these results was as described for Fig. 17.
Table VIII. Regression statistics for mean and maximum carryover on floral variability, with and without zero deposition values.

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<th>Regression equation</th>
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<th>r squared</th>
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<td>$p &lt; 0.001$</td>
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<td>Maximum carryover without zero's</td>
<td>$y = 24.7 + 2.39x$</td>
<td>$p = 1.0$</td>
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carryover is likely to be long, if any pollen is deposited at all. But there is an increased likelihood that no pollen will be picked up, and that if any pollen is picked up, none will be deposited. At low levels of floral variability, carryover is shorter, but it is much more likely that some pollen will be deposited. The high incidence of zero deposition at high floral variability is probably due to the decreased pickup of pollen by the pollinator under those conditions.

The optimum level of floral variability in terms of pollen carryover is thus intermediate. It is the result of
a balance between decreased carryover at low floral variability and the increased incidence of zero pickup and deposition at high floral variability.

Driving model 4 with floral variability is useful in 2 ways. First, it shows that, both pollen pool structure and pollen carryover are sensitive to the amount of floral variability, given the model's assumptions. It thus provides an opportunity, in a model system, for exploring how floral characters can drive pollen pool structure, and how pollen carryover is related to pollen pool structure. Second, it provides testable predictions of model 4.

I would be surprised if the detailed predictions of model 4 accurately reflect real world phenomena. While model 4 does represent an educated guess at the processes of pollen transfer, there is much that it leaves out (e.g. movement of stigmas and anthers through the pollen pool, sloughing off of pollen, etc.). It is a first approximation at modelling pollen pool structure, and as such is more useful than the previous models. However, its main point is to stimulate ideas and suggest experiments. It is more a statement about ideas than a conclusion about the real world.
Discussion

Implications For Mechanisms

Much pollination research has attempted to study broad population level patterns of pollen and gene flow, but has been limited by incomplete knowledge of pollen transfer processes at the individual level. Historically, as in this discussion, the focus has moved from description of patterns toward the consideration of processes, with some accompanying increase in understanding of how the patterns are generated. Each alternative model I have discussed added components to the determination of pollen transfer: "blanks", limited stigmal surface area, variability in flower morphology producing a spatially structured pollen pool, and the third dimension of the pollen pool - depth. These additional components and the ways that they interact should profoundly affect the conceptual context in which pollination research is conducted, but we need critical experiments to assess their importance and generality.

The most important and general conclusion to be drawn from these model results is that simple and often implicit assumptions about pollen pool structure and the rules for pickup and deposition of pollen can have major consequences in terms of pollen carryover. One example of this is the complex pattern of pollen deposition that resulted from assuming random selection of pollen grains and limited stigmal capacity in model 3. This kind of result
illustrates and reinforces the importance of making assumptions about carryover as explicit as possible.

Models 3 and 4, which make explicit statements about the processes that generate variability in pollen deposition, predict longer and more variable carryover than has often been assumed. If carryover is much lower (or higher) than suggested here, either the processes modelled are invalid, or there are other processes acting which influence carryover. Some possible factors are: 1) pollen grains stick together and come off in large clumps, 2) the flowers and the pollinators contact each other in more stereotyped ways than we assumed, or 3) stigmal capacity is high relative to the amount of pollen picked up by the pollinator. Any of these could lower flower to flower carryover, even if the processes described by these models were in operation. Note that the first possibility reflects a structural characteristic of the natural system and would require a structural change in the model, whereas the others are merely quantitative (that is, they would be modelled simply by changing parameter values in the model).

Increasing the complexity of model structure resulted in an increasingly complex pattern of model output. While this has great heuristic value in terms of understanding the consequences of assumptions, increasing complexity does not necessarily imply increasing realism. We need more knowledge of real systems to assess both the hypothetical mechanisms on which the models are based and the patterns
they generate. These models will be worthwhile if their results can be used to focus future research.

Implications For Plant Populations

Based on an average of about 5 flowers visited per plant by hummingbirds on single bouts of foraging (Perkins 1977), mean and maximum pollen carryover values of both models 3 and 4 would lead to plant to plant carryover in the range suggested by Levin and Kerster (1969a) and Levin et al. (1971) - about 3 to 10 plants, depending on the number of flowers visited per plant. However, these models suggest that while much of the pollen will be deposited near its source plant, a smaller but substantial proportion will be carried many flowers past its source. The tail of the distribution of carryover distances is long; both models 3 and 4 show infrequent, but regular deposition of pollen 40 to 50 flowers past the source. To the extent that the processes described in these models represent real systems, we need to be asking about the importance of such rare events in nature.

If, after pollination, real pollen grains from different sources compete for a limited number of ovules, or if reproductive success of ovules (i.e. seed number, seed quality, or seedling quality) varies with pollen dispersal distance in the manner suggested by Price and Waser (1979), Waser and Price (1981) and the following chapter, then a small proportion of the pollen transfers could contribute
disproportionately to reproductive success. This possibility can only be assessed with reference to the pollen transfer mechanisms and optimal outcrossing distances of particular systems, so it would be premature to conclude that long distance pollen transfers do generally have disproportionate fitness consequences. However, I can conclude that mean values of pollen carryover are not sufficient for assessing the importance of rare events. In fact, it is logically impossible in general to understand anything about the importance of rare events by examining measures of central tendency.

These analyses have been oriented towards male function; the primary focus has been on how many flowers beyond its source pollen is deposited. The complementary analysis would also be worthwhile, asking: "what is the distribution of identities of pollen grains received by a stigma?". Certainly, for any understanding of how selection acts on factors influencing carryover we will have to be able to integrate consequences of pollen dispersal events in terms of both male and female function. For example, the finding that seed set is maximized at a particular pollen dispersal distance (female function; Price and Waser 1979; next chapter) must be interpreted in terms of the factors that determine the actual distances to which pollen is carried (male function; this study). These two kinds of factors clearly are not independent. For instance, flooding a pollen pool with one's own pollen should increase success
as a male, but decrease success as a female (Schemske 1980; Lertzman, previous chapter). There is a growing literature on the tradeoffs and constraints involved in male vs. female expression and the tactics appropriate for each (e.g. Willson and Rathcke 1974; Willson 1979; Charnov et al. 1976; Charnov and Bull 1977; Janzen 1975; Hancock and Bringhurst 1980; Lertzman, previous chapter). It will be most important in the future to tighten the link between our interest in such fitness consequences of different patterns of pollen dispersal and our knowledge of the processes that act in producing these patterns.

Conclusions

Process-oriented models that include the distribution of individual variability are more likely to be useful in understanding pollination systems than deterministic pattern-oriented models that are primarily sensitive to mean values.

As components of pollen transfer are modelled more explicitly, pollen transfer is less predictable from flower visitation sequences alone and pollen is carried farther and more variably beyond its source.

Detailed analysis of a model based on the laboratory
experiments of the previous chapter showed that both pollen pool structure and pollen carryover are sensitive to the amount of floral variability, given the models assumptions. Comparison of these models suggests that increasing structural complexity can provide increasing heuristic power, but I caution against making the conclusion that this also implies increasing realism.

Critical experiments in natural systems are needed to assess the contribution of rare events (i.e. pollen transferred in the tails of the dispersal distribution) to plant reproductive success.
OPTIMAL OUTCROSSING AND POLLEN DISPERSAL IN CASTILLEJA MINIATA

Introduction

It is a commonplace that an animal's fitness is related to the quality of its mate (e.g. Coulson 1966; 1968; Coulson and White 1958; Orians 1969; Downhower and Armitage 1971; Cox and LeBoeuf 1977; Weatherhead and Robertson 1977). Recently, similar ideas of mate selection and mate quality have been applied to plants (e.g. Janzen 1975; Willson 1979; Charnov 1979). Two types of factors lead to differential mate quality; phenotypic characters such as competitive or foraging ability and territorial aggressiveness, and more directly genotypic characters such as genetic similarity (Waser and Price 1981). In plants, pollen is the only representation of the male that is available for assessment by the female. Assessing mate quality based on phenotypic characters requires that genes favoring the sporophyte are either expressed in or correlated with traits of the gametophyte (Waser and Price 1981). Though this has been documented (Mulcahy 1971), it is less plausible than differential mate quality based on genetic similarity as a factor of general importance for plants.

Genetic similarity of mates is often dichotomized as inbreeding versus outbreeding. While the depression of reproductive output due to inbreeding is common (Falconer 1960; Wright 1977), decreased reproductive success due to
outbreeding depression is less well known (Falconer 1960). The products of interpopulation crosses first show heterosis and then increasing incompatibility as the distance (or time) separating the populations increases (Kruckeberg 1957; Oliver 1971; Hughes and Vickery 1974; Vickery 1978). Hughes and Vickery (1974) suggested that the maximum heterozygosity that will result in heterosis is the same as the level at which partial crossing barriers start to occur. Presumably, the mating of more distant individuals, adapted to different microhabitats, leads to the mixing or breakdown of gene complexes favorable to one or the other sites and thus a lower fitness of the offspring in either site. Thus, at an inter-population spatial scale, an intermediate level of outcrossing should result in the highest reproductive success.

Recently it has been proposed that similar processes occur within populations. Price and Waser (1979) and Waser and Price (1981) suggested that reproductive success will be maximized at intermediate outcrossing distances that may be quite small (e.g. 1 - 10 m for Delphinium nelsoni and Ipomopsis aggregata). This assumes a strong inverse correlation of relatedness and distance, and thus requires extensive genetic differentiation of plant populations on a small spatial scale. Such differentiation could result from either strong selection, highly restricted gene flow, or both. Ehrlich and Raven (1969) suggested that local differentiation might be much more common than had
previously been believed, and that selection might overcome the homogenizing effects of gene flow in these populations. Since then it has been shown that genetic differentiation of populations can occur quite locally, and that in some cases it can be maintained by selection even in the presence of extensive gene flow (Antonovics 1971; Endler 1973; 1977; Schaal 1975; Adams 1977; Keeler 1978; Silander 1979).

In this study I ask whether Castilleja miniata, an outbreeding perennial hermaphroditic herb, exhibits a within population intermediate optimal outcrossing distance. I then compare this information to estimates of actual outcrossing distances based on the dispersal of dye by its hummingbird pollinators. This system is similar in many ways to the only other systems for which there is information of this type (Waser and Price 1981). It differs in that Castilleja miniata appears to be more specialized on hummingbirds as pollinators than either of the other plant species studied so far (Delphinium nelsoni and Ipomopsis aggregata). This Castilleja – Selasphorus rufus system has also been the subject of a study of the influence of hummingbird territoriality on pollen flow (Perkins 1977).

I use the dye dispersal data to address the question of the influence of hummingbird territoriality on the pattern of pollen movement in a meadow. Linhart (1973) concluded that because most of the foraging flights of a territorial pollinator are within its territory, plants within the territory will have a pool of potential pollen donors and
recipients that is mostly restricted to other plants in the territory. However, Perkins (1977) concluded that though this may be true on the short term, on the long term (i.e. through a flowering season), this effect is swamped. Because territorial boundaries fluctuate in time, plants have a much larger pool of potential mates than those sharing the same territory at any one point in time. I ask whether territorial boundaries restrict pollen movement on the short term, and whether they affect the direction of pollen dispersal from plants near the boundaries.

**Materials and Methods**

All experiments were carried out in meadow 2 of the Grizzly Lake study area (Gass et al. 1976). *Castilleja miniata* is the predominant hummingbird pollinated flower in this meadow. Only a few *Aquilegia formosa* are present, and they are only there early in the season. Meadow 2 is bounded on the south by a talus slope, on the north by coniferous forest, and on the top and bottom by short cliffs (about 5-15 m). It has a slope of 35-40 degrees and an eastern exposure. Of the 3 main study meadows at Grizzly Lake, meadow 2 is the most homogeneous in terms of the distribution of flowers. The one major discontinuity is a small drainage running from the top to the bottom that only carries water early in the season (just after snowmelt) before much *Castilleja* is in bloom. Though micro-environmental features have not been measured, there appears
to be a substantial gradient of moisture from the wetter areas below the cliffs at the top of the meadow to the drier exposed areas at the bottom of the meadow. This is based on direct observation of soil moisture, and of the other vegetation growing in the meadow.

To assess the reproductive value of pollen from different distances, I hand pollinated flowers with pollen that originated from various pre-determined distances away (after Price and Waser 1979). In 1979 the hand pollination treatments were: selfing, and pollen from 0.5, 2, 10, and 30 m away from the experimental plant. In 1980 the treatments were selfing, and 0.5, 1, 5, 10, and 30 m.

Pollen recipients (experimental plants) were located throughout the meadow, and treatments were assigned to them randomly. To obtain pollen, the treatment distance was measured away from the experimental plant and flowers were searched at that distance until enough pollen could be obtained on a wooden toothpick (a new toothpick for each pollen treatment). This sometimes required several flowers on more than one plant. This pollen was then carried to the experimental plant and applied to all receptive stigmas but one. The length of time in transit was standardized for pollen from all distances to control for loss of viability. All experimental stigmas were coated with pollen as completely as possible. Flowers were then covered in mesh bags that were fine enough to exclude hummingbirds and bumblebees. A long lepidopteran proboscis (e.g. of
hawkmoths) could probably fit through the mesh, but it would have been difficult for the insect to reach the nectar and I have not observed lepidopterans feeding from *Castilleja* at Grizzly Lake. The flowers in each bag (each inflorescence) left unpollinated were controls for non-experimental pollination. If these flowers set seed above the mean for selfed flowers, then data from that bag was discarded. Seeds were collected when the pods were near dehiscence, and the "normal" seeds per pod (in size and appearance) were counted under a dissecting microscope.

Treatments have unequal sample sizes because a high incidence of seed predation by insect larvae reduced sample sizes unequally.

To estimate pollen dispersal, I used the same fluorescent powders described in the laboratory experiments on pollen carryover. Four dye source plants were chosen in a single territory, the boundaries of which were monitored before and during the experiment. The territorial boundaries approached the bottom edge of the meadow (east side), and followed the meadow boundary at the talus on the south side. The boundaries extended into the center of the meadow at both the top (west) end of the territory and the north side of the territory. The territory overlapped much of the area that was occupied by the focal individual described by Gass (1979). One dyed plant was at the edge of the territory and at the edge of the meadow (orange), one plant was at the edge of the territory and at the center of
the meadow (green), and two plants were in the center of the territory near the center of the meadow (red and blue, see Fig. 26). Four flowers were chosen on each of four inflorescences on each of these plants and had dye applied to them. An excess of dye was applied by dipping the stigmas and anthers into a test tube containing dye. A different colour of dye was applied to each plant.

Dye was applied on the evening of 26 July. On the night of the third day following (29 July), the green and red plants were censused. On the following night (30 July), the blue and red plants were censused. It would have been preferable to do all the plants on the same night, but it took the full night to do the first two plants. We censused flowers by extending a tape measure a given distance out from the plant, and walking a circle with a radius of that distance. All flowers of all plants that contacted that circle were examined by eye under ultraviolet light for dye particles. Only dye actually on stigmas, anthers, or in the corolla tube near the anthers were counted as having dye, i.e. dye on non-reproductive structures was ignored.

Some precision was lost by using presence/absence sampling as opposed to picking flowers and examining them later under a dissecting microscope where dye grains could be counted (e.g. Price and Waser 1979; Waser and Price 1981). However, this would have caused a major perturbation to the meadow—removal of about one fourth of the flowers—that I was not willing to make. Also, presence/absence
measures make less rigid assumptions about how well the dyes mimic the behaviour of pollen than actual counts of dye grains.

I divided the area around each experimental plant into eight equal pie sections and recorded the direction from the source for each flower censused. Thus dye dispersal information is two dimensional; presence/absence at a given distance and in a given direction. For each distance and direction (number of distances = 5, number of directions = 8), I calculated the per cent of the total number of flowers with dye on them that occurred there, and divided that number by the number of flowers that occurred there. This provides a statistic that is corrected for the greater number of flowers sampled in circles of larger radius. This is the statistic used in the test described below. However, the uncorrected proportion of dye at each distance is presented in figure 24 because the proportions make more intuitive sense.

If territorial boundaries do restrict pollen movement on a day-to-day basis, then plants near territorial boundaries should show a net movement of pollen or dye in toward the center of the territory. Plants in the center of the territory should show a more homogeneous dispersion of pollen in all directions. To test the null hypothesis that pollen was distributed uniformly from each plant (as opposed to directionally), I used a modification of the Rayleigh test that takes into account the magnitudes of the vectors
in each direction (Moore 1980). This test makes the assumption that any departure from uniformity will be unimodal, and thus may give misleading results if the data are multi-modally distributed (Batschelet 1965). This assumption seems reasonable in that there are no a priori reasons to expect multimodal distributions of pollen dispersal vectors in this system if they represent a large number of flights. Since this territory contained an average of 856 flowers during this experiment, and all or most had to be visited by the hummingbird each day (Gass et al. 1976; Gass 1979), the assumption that these distributions represent a large number of flights is justified.

Results

Optimal Outcrossing

The results show much variability both within and between treatments and between years (Fig. 23). While in 1979 there was a peak in seed production at the 2.0 m treatment, there was no such trend in 1980, when the mean seeds per pod were almost identical between treatments. In both years selfed flowers set almost no seed.

If the 1979 data are grouped into selfed, close (0.5 and 2.0 m), and distant (10.0 and 30.0 m) treatments, the close group sets significantly higher numbers of seeds than either the selfed or distant groups (Mann-Whitney U test; p
Figure 23. Seeds produced under different hand pollination treatments, means and 95% confidence intervals.
The diagram shows the number of seeds per pod across different pollination treatments and years. Each plot represents a different year:

- **1979**
  - Pollination treatment ranging from 0 to 30 meters.
  - Number of seeds per pod at each treatment: 3, 6, 7, 8.
  - Sample size (n) for 1979: 7.

- **1980**
  - Pollination treatment ranging from 0 to 30 meters.
  - Number of seeds per pod at each treatment: 7, 13, 9, 7.
  - Sample size (n) for 1980: 14.

- **1979 + 1980**
  - Pollination treatment ranging from 0 to 30 meters.
  - Number of seeds per pod at each treatment: 6, 10, 13, 16, 15.
  - Sample size (n) combined for 1979 and 1980: 21.

The diagram indicates a trend where seed production increases with increasing pollination distance, until a certain point where it begins to decrease.
= 0.002 for close vs. selfed, p = 0.001 for close vs. distant).

When 1979 and 1980 data are combined (Fig. 23), this trend is maintained; while (except for selfing) adjacent treatments do not differ significantly, the 2 m sample is significantly higher than either the selfed or the 30 m samples (p < 0.001, p < 0.25 respectively, Mann-Whitney U test).

Dye Dispersal

In general, much of the dye was deposited on or near the source plants, with smaller proportions being carried longer distances (Figs. 24 and 25). However, within this pattern there was a great deal of variability between plants. For example, 41% of the flowers observed with dye from the red plant were 2 m away from the dye source plant, but only 9% of the flowers with dye from the green dye plant were 2 m away (Fig. 24).

With such a small sample it is difficult to make a conclusion about the effects of territorial and meadow edges on the distance that dye is transferred. However there appear to be no consistent differences between the edge and center plants in dye dispersal distance.

The comparison of dye dispersal distances for these plants is confounded by variation in plant density (Table IX). The edge of the meadow was the least dense area, and also, because the orange plant lay at the meadow edge, much
Figure 24. The proportion of the total observed dye that was dispersed to various distances away from the source plant. There was no 10 m sample for the green dye plant.
Table IX. Degree of saturation of local flowers by dye from different sources.

<table>
<thead>
<tr>
<th>source</th>
<th># flowers</th>
<th># flowers with dye</th>
<th>% flowers with dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>green</td>
<td>280</td>
<td>33</td>
<td>11.8</td>
</tr>
<tr>
<td>red</td>
<td>192</td>
<td>48</td>
<td>25.0</td>
</tr>
<tr>
<td>blue</td>
<td>191</td>
<td>52</td>
<td>27.2</td>
</tr>
<tr>
<td>orange</td>
<td>73</td>
<td>23</td>
<td>31.5</td>
</tr>
</tbody>
</table>

of the area sampled was actually outside the meadow and therefore contained no plants. The result that dye (or pollen) from plants in areas of lower density is carried longer distances (Table IX) is in agreement with previous findings of density dependent pollinator flight distances and pollen dispersal distances (Levin and Kerster 1969 a,b; Beattie 1976). Even though the lowest density plant had the highest per cent saturation of local flowers with dye (Table IX), fewer flowers within 10 m actually received dye.

The directional trends of dye movement are illustrated in Figure 25. Though it appears that the edge plants have a distribution more skewed directionally than the center plants, none of the plants deviated significantly from a uniform circular distribution (Table X). However, while they did not reach statistical significance (i.e. alpha = 0.05), the edge plants have higher (more significant) values
Figure 25. The directional vectors of dye dispersal and territorial boundaries on the day before the beginning of the dye dispersal experiment. The length of the lines for each direction indicate the amount of dye that was dispersed in that direction and do not indicate distance.
one unit of: \[ \frac{\% \text{ total pollen}}{\text{number of flowers}} \]

territory boundary

edge of meadow
Table X. Directional statistic R* for each plant in the dye dispersal experiment.

<table>
<thead>
<tr>
<th></th>
<th>orange</th>
<th>green</th>
<th>red</th>
<th>blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>R*</td>
<td>0.40</td>
<td>0.41</td>
<td>0.16</td>
<td>0.23</td>
</tr>
<tr>
<td>p</td>
<td>&gt;0.5</td>
<td>&gt;0.5</td>
<td>&gt;0.9</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

of R* than do the center plants (Table X). Thus this result is suggestive, but far from conclusive.

The territorial boundaries fluctuated a great deal during the course of the dye dispersal experiment (Table XI). In the 6 days from the first census to the final one the area contained within the boundaries increased and then decreased to less than the original value. The number of flowers contained in the territory doubled and then decreased to less than the original value. This followed the pattern of flowers in the meadow as a whole.
Table XI. Territory and meadow statistics for the dye dispersal experiment.

<table>
<thead>
<tr>
<th>date</th>
<th>25 July</th>
<th>28 July</th>
<th>31 July</th>
</tr>
</thead>
<tbody>
<tr>
<td>area of focal territory</td>
<td>491</td>
<td>764</td>
<td>350</td>
</tr>
<tr>
<td>perimeter of focal territory</td>
<td>101</td>
<td>124</td>
<td>81</td>
</tr>
<tr>
<td># Cast. in territory</td>
<td>675</td>
<td>1295</td>
<td>590</td>
</tr>
<tr>
<td># Aqu. in territory</td>
<td>-</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td># Cast. in meadow</td>
<td>3324</td>
<td>4133</td>
<td>2346</td>
</tr>
<tr>
<td># Aqu. in meadow</td>
<td>41</td>
<td>36</td>
<td>5</td>
</tr>
<tr>
<td># territories in meadow</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Cast. = Castilleja miniata

Agu. = Aquilegia formosa

Discussion

Optimal Outcrossing Distance: Variability in Fitness

The optimal outcrossing experiment was fairly inconclusive. This population of Castilleja miniata may have an intermediate optimal outcrossing distance of between 1 and 10 m (tentatively around 2 m), but if it does, its
expression is quite variable.

Allard and Workman (1963) found sharp seasonal changes in the adaptive values of different genotypes in experimental lima bean populations grown in a controlled environment. It is likely that in seasonally variable alpine environments the selective significance of given genotype combinations would change from year to year even more than in such studies under controlled conditions.

Weather in the two years of this study differed greatly. In 1979 the snow melted off of the meadows early, and the ice was gone from Grizzly Lake before 8 July. The date we first observed territorial behaviour by hummingbirds in the meadow was 16 July, and it rained 18% of the days between 8 July and 1 Sept. In 1980, the snow did not melt from the meadows till quite late, and the ice was not gone from Grizzly Lake till the third week in July. The date of first observed territoriality was 31 July and it did not rain at all between 9 July and 28 Aug.

In a long lived perennial plant it is hard to imagine that the genetic structure of the population would respond to such changes on a season to season basis (unless many plants were killed by catastrophic events; e.g. Gass and Lertzman 1980), but it is easy to picture drastic fluctuations in the adaptive value of given traits, and thus in the value as a mate of the individuals that carry those traits. In such plants the population structure probably reflects long term trends or invariants in the environment.
However, measurements in a given year may be strongly dependent on short term fluctuations that have little to do with the ultimate fitness of these individuals. In their study of allele frequencies in experimental lima bean populations, Allard and Workman (1963) could not predict from any pair of generations either the mean selective value of any allele or its variance over 11 generations. This reinforces the importance of long term studies of population dynamics and genetics in a known ecological context. It would be valuable to follow microhabitat changes, changes in optimal outcrossing distance, changes in recruitment, and changes in genetic structure over several seasons representing the range of seasonal variability. Such a long term study would be difficult and labor intensive, but should yield very valuable information.

Variability in optimal outcrossing results both within and between years could be an artifact of hand pollination methods. It seems likely that this is at least partially true for the within year, within treatment variation. It is less likely to be the cause of the changes in pattern between years, i.e. I expect quantitative, but not qualitative errors. Similar between year variation has been found in other studies (Price and Waser 1979; Waser and Price 1981; N. Waser and M. Price pers. comm.). Increased sample sizes and improved methods of hand pollination will increase the confidence that can be placed in results such as these.
Another reason for the lack of a clear pattern may be that I only measured seed production, and have ignored possible effects of outcrossing distance on seed quality. This is clearly important and needs to be studied before firm conclusions about optimal outcrossing in this system can be reached.

**Dye Dispersal Distance: Is Pollen Dispersed Optimally?**

How do achieved pollen dispersal distances compare with a possible optimal dispersal distance of about 2 m? The dye dispersal experiment suggests a fairly good correspondence. A higher proportion of dye was transported to the optimal distance (1-2 m) in this system than has been found for *Delphinium* or *Ipomopsis* (Price and Waser 1979; Waser and Price 1981). However, these comparisons are confounded by variation in plant density. Differences in the density of flowers will be reflected in dye dispersal distances (Levin and Kerster 1969 a,b; Beattie 1976), and between population comparisons must take this into account.

We should not necessarily expect close correspondence between actual and optimum pollen dispersal distances (Price and Waser 1979; Waser and Price 1981). I have defined optima in terms of the plant's needs alone, and realized pollen dispersal results from interactions between plants and pollinators. Co-evolution does not imply co-operation.

It is not known yet to what extent plant characters are able to manipulate pollinator behaviour. While Price and
Waser (1979) suggest that plants could only exert minor effects on pollination in this manner, Thomson and Plowright (1980) found a significant relationship between pollen deposition and nectar standing crop. This suggests that plants may be able to exert a great deal of influence on pollination through their influence on pollinator behaviour.

**Dye Dispersal Direction: Do Territorial Boundaries Restrict Pollen Movement?**

Though none of the four plants showed a statistically significant deviation from a uniform directional distribution of pollen, the territory edge plants showed more directional movement of pollen than the territory center plants. This difference may be real, and only a larger sample or better resolution of territorial boundaries and better choice of plants is needed for statistical significance. Alternatively, territorial boundaries may have have little restrictive influence on the pollen dispersal of most plants even on the short term. If territorial boundaries are only vaguely defined or shift on a short time scale (i.e. such as the period of this study) then they may have little meaning for pollen dispersal. The major changes in both the area occupied by the focal territory, and the number of flowers it contained combined with the lack of significant directionality in the dye dispersal from territory edge plants lends support to this hypothesis. A plant that one day is on the edge of a
territory and might have directionally skewed dispersal of its pollen, could be several meters inside the territory the following day. A plant that is outside the territory on one day and therefore potentially excluded from the plants in that territory as a mate, may be inside the territory the following day (see Perkins 1977). Thus this system contrasts strongly with the one studied by Linhart (1973) which is apparently more temporally stable to know whether other systems will be more similar to one or the other of these examples will require a knowledge of the dynamics of territoriality in those particular systems.

Do plants at loosely defined boundaries receive enhanced pollen dispersal by visits from both adjacent resident hummingbirds? The opposite may be happening: plants at disputed boundaries probably receive fewer visits than other plants. The green dye plant, in the most dense area studied, had pollen deposited on fewer flowers than either of the center territory plants, and of the flowers within 5 m, the percentage with dye was less than half of the value for the two center territory plants. The territory boundary next to the green dye plant changed the most of any boundary in the study; from 1 m away from the green dye plant on the day before dye was introduced to 5 m away 2 days following, to 4 m away on the day before the final census. If plants in such disputed areas are visited less frequently, but by both territorial residents, then a non-directional pattern of dye dispersal such as I observed
would result.

Indistinct territorial boundaries are likely to occur only in a relatively homogeneous meadow such as meadow 2. In a much more heterogeneous meadow (e.g. much of meadow 3) where territories may be separated by areas without flowers, territorial boundaries will be much more distinct and may act more like reflecting boundaries for pollen dispersal. This prediction of a low visitation frequency to plants on disputed territorial boundaries should be easily testable.

**Conclusions**

*Castilleja miniata* may have an intermediate optimal outcrossing distance on the order of 2 m. But if so, it is variably expressed from year to year. This variation may be related to climatic and microhabitat changes.

Dye dispersal distances show much variability between plants, but in general, approximate optimal outcrossing distances fairly well. A higher percentage of dye was transported to the purported optimal distance than in other studies. I suggest that this might be due to *Castilleja miniata* specializing on hummingbird pollinators while *Delphinium nelsoni* is pollinated extensively by both hummingbirds and bumblebees.

Dye dispersal directions do not deviate significantly from a uniform circular distribution for both territory center and territory edge plants. This is probably related to changes in territory boundaries over the course of the
study. This supports Perkins' (1977) conclusion that territory boundaries in this system do not restrict gene flow.

Both the optimal outcrossing experiment and the dye dispersal experiment suffered from small sample sizes. The conclusions are tentative at best.
GENERAL DISCUSSION

The laboratory studies showed that pollen carryover is complexly patterned, and suggested that these patterns are related to morphological characters of flowers. Variation in floral morphology was implicated as a key factor influencing carryover because of its influence on the number of "blank" flowers in a sequence, and on layering of pollen in the pool on the pollinator.

Modelling exercises showed that such patterns can be produced by different sets of assumptions. In exploring one set of assumptions in detail (model 4), I found that both pollen pool structure and pollen carryover were sensitive to the amount of variation in flower morphology.

The two field experiments gave interesting results but are not well linked to the laboratory and modelling studies. *Castilleja miniata* probably has a within population optimal outcrossing distance that may or may not be expressed in any given year. A significant proportion of the pollen probably reaches this distance, and hummingbird territoriality probably does not restrict pollen dispersal significantly.

The missing link between the laboratory and modelling studies and the field studies involves how processes at individual flowers are distributed over space by the interaction between hummingbird movement rules and the spatial distribution of energy (nectar). A statistical relation between plant dispersion and pollinator movement, and gene flow has been known since 1969 (Levin and Kerster...
1969 a,b), but a causal relation is still vague, despite the recent profusion of research on pollinator behaviour.

Is it valuable to expend effort to make this mechanistic connection between the level of individual flowers and populations of flowers? For some purposes one can adequately describe pollen dispersal without knowing its underlying mechanisms. Given statistical expectations of carryover (in numbers of flowers), flowers per plant, the distribution of plants in space, and pollinator movement, we can make a prediction for mean pollen dispersal distances. For instance, if one's goal is to estimate plant population neighborhood sizes (e.g. Levin and Kerster 1971; Augspurger 1980), then this information is probably adequate — unless rare events play an important role in the system under study. However, if it is specifically the dynamics of a pollinator—plant relationship that one is interested in, the statistical description is inadequate.

Dynamics refer to changes in time in which the characters of the system's state at one point in time feedback to influence its future states. The study of dynamics necessarily involves an understanding of the feedback mechanisms — not just a description of their results. Just as knowing the numbers of clouds in the sky does not lead to a predictive theory of weather (Clark and Holling 1979), knowing the locations and identities of pollen grains is inadequate for predicting either how they got there or what kinds of fitness consequences they will
have in those locations. Whether or not detailed processes studies are worthwhile depends on ones ultimate research goals.

If it is important to find the causal relationships in pollen dispersal, what are the best ways to go about it? The prime focus should be process oriented experiments - with at least as much emphasis on field experiments as in the laboratory. Models are useful for exploring ideas generated by experiments (as in this thesis), but the empirical base is too weak at this point to support large, predictive, dynamic models (e.g. Gilbert et al. 1975; Clark and Holling 1979). Similarly we don't know enough yet to build theory with a sound empirical base.

What experiments should be done next? The focus is on characters of the plants that influence pollinator behaviour to produce patterns of pollination, and on the fitness consequences of these patterns for the plants.

1. Laboratory and Field Experiments on Pollen Carryover
a) How does pollen carryover vary with the amount of floral variability? The role of floral variability was a key issue in both the laboratory and modelling studies. An experiment should be performed that studies how changing the amount of variability in a population of flowers influences pollen carryover. This parallels the sensitivity
analysis of model 4 to floral variability, this experiment requires a lab system where both carryover and floral variability can be measured easily. I attempted this last summer but could not complete it due to logistical problems. This experiment is important both as the logical follow up of the previous laboratory experiments, and as a test of the predictions of model 4.

b) How does pollen carryover vary with the amount of nectar per flower? Nectar production rate (and the concentration of sugar in the nectar) is one of the most obvious ways that plants might influence pollinator behaviour (Heinrich and Raven 1972), and is at least partially heritable in some species (Shuel 1952; Walker et al. 1974). The amount of time that a pollinator spends at a flower is sensitive to the amount of nectar in the flower, and Thomson and Plowright (1980) found that flowers with more nectar received more pollen. This experiment needs to be repeated in other systems and in more detail. Nectar standing crops and nectar production rates are typically highly variable (Cruden 1976; Corbet 1978; Feinsinger 1978; Pleasants and Zimmerman 1979; Montgomerie et al. in prep), and pollinators appear to be sensitive to the amount of variation
in nectar in their food sources (Caraco et al. 1980). What is the influence of this variability in nectar on pollen carryover?

c) How do these laboratory studies relate to the real world? The laboratory experiments should be done in parallel with field experiments on the same questions. This will be difficult and labor intensive, but worthwhile. For instance nectar per flower, plant density, and flowers per plant could be easily manipulated. The effect of these variables on pollen carryover and dispersal distance is baseline information for understanding the connection between plant phenotypic characters and plant fitness.

2. Laboratory and Field Studies of Pollen Pool Structure
All the above experiments focus on pollen transfer from individual sources. It is important to study the context in which pollen transfer occurs; i.e. the pollen pool. How many flowers have pollen represented in the pollen pools that are observed on birds in the field? Does a pollen pool reach a steady state of inputs and outputs after some number of flowers? Is carryover different from a pollen pool in the buildup
process than from a pool at steady state? I performed pilot studies on these questions last summer. These questions could be answered by a combination of laboratory studies where pollen pool structure is measured after the pollinator has fed from various numbers of flowers, and measures of pollen pool structure from wild birds.

3. Field Experiments on Plant Reproductive Success
The purpose of these experiments is to correlate the results of the field experiments on pollen carryover and dispersal distance with consequences in terms of plant fitness. The same manipulations of nectar standing crop, plant density, and flowers per plant that I suggested for studying pollen carryover could be continued through the season and the subsequent seed production measured. The connection between proximate "behavioural" patterns and reproductive output is weak in most studies of foraging or reproductive tactics. These experiments provide an opportunity to make a strong connection.

4. Laboratory Experiments On Pollinator Behaviour
The key questions here are how the pollinators respond to different distributions of energy in
space - in particular how they deal with spatial and temporal variability. Detailed laboratory studies are needed to understand how the pollinators end up performing in a certain way. It would also be valuable to monitor pollinator behaviour in conjunction with the field manipulations discussed above. Thus I am suggesting an integrated series of studies where, with the same set of manipulations of floral characters, one would look at pollinator behaviour, pollen carryover and dispersal distance, and plant reproductive success.

5. Heritability
Any evolutionary interpretation of the results of these experiments would require either knowledge or assumption of the heritabilities of the plant characters under consideration. Current knowledge is rudimentary (e.g. nectar production rate is to some degree heritable; Shuel 1952; Walker et al. 1974). This question takes on major significance in the light of the burgeoning literature on how plant characters influence pollinator behaviour.

Pollinator - plant systems are dynamic, in both a proximate and an evolutionary sense. To understand them requires a research program that integrates characters of both the plants and the pollinators, and the processes
through which they interact. I think this integration is possible, but only through much work with real systems, and a re-orientation of research away from testing optimality theory towards building up some more empirically based theory.
REFERENCES


