BUMBLE BEE POLLEN FORAGING ON LUPINE (*LUPINUS*: FABACEAE):
WITHIN-WHORL DECISIONS

by

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We certify that we have read this study and that it conforms to acceptable standards of scholarly presentation and is fully acceptable, in scope and quality, as a thesis for the degree of Master of Arts.

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ABSTRACT

Bumble bee pollen foraging on lupine (*Lupinus*: Fabaceae): within-whorl decisions

Birgit Semsrott

Bumble bees (*Bombus*: Apidae) can maximize foraging efficiency in a resource-patchy environment by visiting mainly rewarding flowers and avoiding those that are either empty or less rewarding. This study investigated how bumble bees avoid unrewarding flowers of lupine (*Lupinus*: Fabaceae), a plant in which the pollen is hidden from view. I recorded whether bees left a whorl upon encountering various situations. Bumble bees clearly discriminated against flowers that showed unambiguous visual signs of being unrewarding. In the absence of any visual cues, bees made use of a presumably predictable spatial distribution of pollen within whorls. They were able to assess the amount of pollen collected per flower, and they departed upon encountering one or more unrewarding flowers. Bees did not use pollen scent to avoid unrewarding flowers, which cannot be identified by scent alone. By basing their foraging decisions only on the most reliable signals bumble bees may have maximized their pollen return.
ACKNOWLEDGMENTS

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INTRODUCTION

Bumble bees (Bombus Latreille) are confronted with floral resources that are unevenly distributed in space. The standing crop of pollen or nectar that is available in flowers is spatially patchy for several reasons, including differences in the rate of production and the depletion by foragers (nectar: Zimmerman 1981, Cresswell 1990, Waser and Mitchell 1990, pollen: Haynes and Mesler 1984, Harder 1990a). The distribution of pollen may be especially uneven since pollen, unlike nectar, is not replenished by flowers after its initial production. The prevailing spatial patchiness of floral resources challenges bees to adopt behavioral strategies that maximize their foraging efficiency. Presumably strategies that maximize the amount of pollen and nectar harvested from flowers will be selected because these resources affect colony fitness (Charnov 1976). Several studies have examined bumble bee behavior from this optimality perspective (nectar: Pyke 1979, Hodges and Wolf 1980, Hodges 1985; pollen: Haynes and Mesler 1984, Harder 1990a, Rasheed and Harder 1997a, b, Robertson et al. 1999).

A bee can maximize the amount of resources collected by visiting mainly rewarding flowers and avoiding those that are either empty or less rewarding. There are a number of ways in which a bee can assess the quality of a flower. In flowers with exposed nectar or pollen, a bee can make a direct visual assessment of the reward levels without landing (Cresswell and Robertson 1994, Zimmerman 1982). In flowers with resources hidden from view, it may be able to assess reward levels indirectly before landing if it can use a visual or olfactory cue that is correlated with the amount of nectar
or pollen available, such as age-related morphology (Pellmyr 1988), floral symmetry (Moeller 1995), color (Casper and Pine 1984, Gori 1989, Weiss 1991, Niesenbaum et al. 1999), or scent (nectar: Marden 1984, pollen: Dobson 1991). Even if bees cannot assess the quality of a flower before they land, and thus inevitably land on some empty or less rewarding flowers, they might still be able to enhance their overall foraging efficiency by taking advantage of a predictable spatial pattern of resource quantities within an inflorescence. For example, nectar-foraging bees have been shown to leave an inflorescence after some number of visits to flowers with below-average resource levels (see Hodges 1985), and to incorporate information from at least three previously visited flowers (see Dukas and Real 1993). While most research on foraging mechanisms has focused on nectar-collecting bees, little is known about the proximate rules that bumble bees use to make decisions when foraging for pollen.

This study examined bumble bee foraging behavior on the showy, pollen-rewarding flowers of lupine (*Lupinus*: Fabaceae), a plant that keeps its resources hidden from view. The flowers are arranged in whorls along vertical inflorescences that open sequentially from the bottom up. Bumble bees generally start foraging on one of the still-rewarding lower whorls and work their way up (Haynes and Mesler 1984, Harder 1990a). Since older flowers at the bottom of the inflorescence have received the most bee visits at any given time, the number of pollen grains per flower increases exponentially upwards (Harder 1990a). Within whorls, foragers may move from flower to flower until every one has been visited, or they may visit only one or a few flowers before moving onto the next whorl or inflorescence. How do bumble bees decide when to leave a whorl? Presumably
their seemingly random behavior reflects the pollen availability within whorls. The objective of this study was to describe the within-whorl foraging behavior of bumble bees.

There are two ways in which bumble bees can minimize the number of unrewarding lupine flowers they visit. First, if they are able to assess the amount of pollen collected from an individual flower, they could use the information obtained from that or from a succession of recently visited flowers to predict the quality of the next. Neighboring lupine flowers are of similar age, have received a similar number of bee visits, and therefore may be expected to have a comparable amount of pollen. Second, bumble bees may be able to cue in on some floral character that is correlated with the amount of pollen available. Lupine flowers vary in morphology at different developmental stages. Bees may use these differences in floral form as guides to rewarding flowers. Since lupine pollen is fragrant (see Dobson et al. 1996) foragers could also be attracted by scent.

This study was designed to identify the proximate rules that bumble bees use to decide when to leave a lupine whorl. I determined whether bumble bees can assess the amount of pollen they have collected from individual flowers by testing the following two predictions: 1) Bumble bees visit fewer flowers on unrewarding whorls than on rewarding whorls, 2) Bumble bees are more likely to leave a whorl if the first flower encountered is unrewarding than if rewarding. Then, I tested whether bumble bees base their decision to continue foraging on a whorl on a) the presence of a pollen reward in the last-visited flower, b) the number of non-rewarding flowers already visited within a whorl
(exclusive of the last-visited flower), c) the appearance of the unvisited neighboring flower, and d) the pollen fragrance of this neighboring flower.
STUDY SPECIES AND STUDY SITES

Like other papilionoid legumes, lupine flowers are morphologically complex. The zygomorphic, 5-parted corolla consists of an upper “banner”, two lateral “wings”, and a lower “keel” (formed by the fusion of the lowest, innermost petals). The flowers do not offer nectar but protein-rich pollen to bumble bees, their main visitor. The pollen is hidden from view since the stamens and pistil are enclosed in the keel. During floral development, the pollen is moved into the tip of the keel where it exits through a small aperture upon pollinator manipulation. When a bumble bee lands on a flower, it pushes the banner up and the keel down, forcing the style with its hairy stigma through the pollen mass. Thus, the pollen is presented on the stigma. Each such manipulation may dispense less than 20% of the pollen originally present in the flower (see Harder 1990b). After a bee leaves a flower, the style slowly returns to its original position, and only after a recovery period of about twenty minutes can the flower be worked again for a maximal pollen return (Harder and Wilson 1994). Consequently, bumble bees rarely return to a flower immediately (Harder 1990a). After each visit, the bee grooms the pollen from its hairy body into specialized structures or pollen baskets (corbiculae) on the hind legs. Presumably the pollen baskets of bumble bees are similar to those of honey bees (Apis mellifera), which are equipped with hairs that signal whether and how much pollen has been collected (see Hepburn 1980).

Bee behavior was observed on Lupinus latifolius J. Agardh and L. andersonii S. Watson. The racemes of both species consist of more or less distinct whorls of 6 to 7 flowers. Generally, flowers open sequentially with age, one whorl per day. The two
species are similar in most respects, but differ in color and in the number of rewarding, open whorls. *L. andersonii* is a lighter purple than *L. latifolius*. At the study sites in 1999, *L. latifolius* generally had three open whorls, whereas *L. andersonii* had only two at any given time. The banner does not change color upon pollination in either species as it does in some lupines, making this potential visual clue unavailable to bumble bees (see Gori 1989).

In these species, two types of unrewarding flowers can be easily distinguished from rewarding ones (see Appendix 1). First, flowers that are about to open near the top of the raceme (referred to as “closed flowers” hereafter) are unrewarding, and can be distinguished from fully opened flowers by their distinct shape and morphology. The banner is curved upwards in open flowers, and downwards over the two wing petals in closed flowers. Second, spent flowers that no longer have pollen can be identified by their exerted stamens. Stamens protrude out of the flower either after all the pollen has been dispensed (stamens show at the top of the flower), or after the flowers have been robbed by solitary bees (stamens show at the bottom of the flower). Closed flowers are restricted almost exclusively to whorl 1, defined as the uppermost open whorl with more than half of the flowers having an operational pollen dispensing mechanism (whorls were numbered from the top down). Occasionally closed flowers were found in whorl 2. Spent flowers occur in every whorl, but increase in frequency from the top down.

Some fully open flowers contain pollen that cannot be harvested by bees. Since these flowers appear visually identical to rewarding flowers, they presumably cannot be identified as unrewarding by bees before they land. For example, if the dispensing
mechanism is repeatedly worked within a short time period, the flower does not release any pollen. The pollen tunnel created by the moving style needs to at least partially collapse in order for more pollen to be dispensed. Further, newly opened flowers in whorl 1 frequently do not have a functional dispensing mechanism either; probably due to stiff floral parts. While these flowers are only temporarily unrewarding, others continue to be so. For example, fly larvae that feed on the pollen inside the keel can permanently destroy the dispensing mechanism (Hayler 1999).

The study was carried out during July of 1998 and 1999. I conducted a series of observations, which are described in four parts below. Bumble bee foraging behavior was observed at three sites in the Horse Mountain area, Humboldt County, CA. Most observations (parts 1A, 3, and 4) were done in an expansive meadow complex (elevation 5000 ft) twelve miles south of Highway 299 on Titlow Hill Road. Two observations took place in smaller meadows approximately 3.0 miles (part 2) and 3.6 miles (part 1B) along the same road (elevation 4000 ft).

I observed the workers of six different bumble bee species. The following bumble bees were sampled in the meadow complex (percent of total bees observed in parentheses): *B. vosnesenskii* Radoszkowski (92% in 1998, 80% in 1999), *B. occidentalis* Greene (8% in 1998), *B. mixtus* Cresson. (15% in 1999), and *B. franklini* (Frison) (5% in 1999) for part 1A; and *B. vosnesenskii* (65%), *B. mixtus* (25%), *B. occidentalis* (<4%), *B. californicus* F. Smith (3%), *B. franklini* (3%), and *B. bifarius* Cresson (<1%) for part 3 and 4. In the two smaller meadows I sampled *B. vosnesenskii* (58%), *B. mixtus* (33%), *B. occidentalis* (5%), and *B. franklini* (4%) for part 1B and 2. Since there did not seem to be
obvious differences in foraging behavior, the data for all the species were combined. Other behavioral studies have combined the data for more than one bumble bee species in a similar fashion (Zimmerman 1982, Gori 1989, Pyke and Cartar 1992, Cresswell and Robertson 1994, Thomson 1996, Odell et al. 1999).
GENERAL METHODS

The following methods apply to all observations except those in part 1A.

In order to avoid sampling a bee more than once, every observed bee was marked on the prothorax with a permanent marker. Any unmarked bee was used for an observation, given the following conditions were met: 1) the whorl it visited included at least five flowers without a gap between them (produced by flowers that have fallen off), 2) its behavior was not strongly affected by external factors, such as wind, aphid-protecting ants, or other bumble bees visiting the same inflorescence, 3) it had been foraging primarily on lupine, as evidenced by a bright orange pollen mass on its corbiculae, and 4) it was “experienced” in collecting pollen from lupine (i.e., it had learned how to manipulate the pollen dispensing mechanism). Bees were classified as inexperienced if they crawled from flower to flower instead of flew; fell off flowers during flower manipulation; exclusively buzzed unrewarding flowers with exerted stamens; seemed to prefer foraging on mostly unrewarding flowers of lower whorls; or repeatedly tried tripping the dispensing mechanism of closed flowers.

I sampled bumble bee foraging behavior in whorl 1 and 2, and then combined the data for the two whorls. Even though foraging conditions in these two whorls might not have been quite the same due to differences in the distribution of pollen and in the number of closed and spent flowers, I expected bees to use the same types of foraging rules. The objective of this study was to determine general foraging rules, not to investigate the differences in behavior among whorls.

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In order to evaluate how bees respond to the absence of a pollen reward I needed to determine whether they had received any pollen during a visit. However, it was impossible to directly observe a bee getting pollen because bumble bees spent an equal amount of time foraging on rewarding lupine flowers as on unrewarding ones (Harder 1990a), and manipulate flowers and groom themselves quickly. Furthermore, unrewarding flowers do not always look different from rewarding ones. Therefore, I had to evaluate a flower after the departure of the bee and assume that its condition had not changed. The manipulation of the pollen dispensing mechanism in lupine can easily be duplicated by a hand (Harder and Wilson 1994), and I determined whether a flower had been rewarding by manually pumping the flower once immediately after the bee had left. If my follow-up manipulation dispensed pollen, I classified the flower as “rewarding”, and if it did not dispense pollen, as “unrewarding”. However, there were some problems associated with this type of assessment. If a bee took the last bit of pollen from a flower, my follow-up manipulation would have indicated that the flower was unrewarding when in fact it was not. In a similar fashion, if a bee visited a flower that had just received a number of other visits, it might have gotten the last available pollen before the pollen tunnel was in need of collapsing again. On the other hand, a newly opened flower might only dispense pollen after a repeated manipulation. Therefore, I needed to test the accuracy of this assessment of flowers. I worked the dispensing mechanism of a flower twice, and recorded whether the second manipulation produced the same result as the first. I tested 30 initially-rewarding flowers, and 30 initially-unrewarding in both whorls. Most second manipulations gave the same result as the first (Fisher’s exact test, both
whorls: $P << 0.001$). In whorl 1, the second manipulation disagreed only once (3.3%) with the first manipulation in both categories (for flowers that initially were either rewarding or unrewarding). In whorl 2, the second manipulation did not result in a reward one out of 30 times (3.3%) when the first did, and did result in a reward two out of 30 times (6.7%) when the first did not. Despite the problems and the small margin of error associated with this assessment of flowers, it seemed reasonable to use a follow-up manipulation to determine whether bees had gotten a reward from a flower.
SPECIFIC METHODS AND RESULTS

Part 1: The impact of a pollen reward

Methods.- A. If bumble bees can assess the amount of pollen collected from individual flowers they should visit fewer flowers on whorls that have been depleted of pollen than on those that have not been depleted. Furthermore, a higher percentage of bumble bees would be expected to depart after just one visit if the whorl is unrewarding than when it is rewarding.

I compared bumble bee foraging behavior on “experimental whorls” of 15 pairs of manipulated and control L. latifolius inflorescences. Members of a given pair were from the same plant, and each had the same number of open whorls (t=0.00, P=1.00) and open flowers per inflorescence (t= 0.36, P= 0.7). The location of each inflorescence pair varied throughout the large meadow. I observed nine pairs in 1998 and six in 1999. The pollen in the experimental whorl of manipulated inflorescences was removed by repeatedly working the dispensing mechanism. Great care was taken to handle the wing petals as little as possible so as not to cause wilting. Control whorls were not manipulated except to work the dispensing mechanism of each flower one time to ensure the presence of rewarding flowers and to control for handling.

The whorl with the greatest number of rewarding flowers was chosen to be the experimental whorl. This was usually whorl 2 (56%), followed by whorl 3 (25%) and whorl 1 (19%). The experimental whorl was the same for each member of the pair. There was a difference in the choice of the experimental whorl because the number of open
whorls per inflorescence was not the same in the two years. Therefore, the position of the most rewarding whorl slightly changed from one year to the next.

A bee was allowed to visit only one of the two experimental whorls. Observations of the same bumble bee on the same inflorescence pair was unlikely because bumble bees avoid re-visiting flowers immediately (see Heinrich 1979, Harder 1990a), and instead tend to follow plant-specific routes or “trap-lines” (Thomson et al. 1982, Thomson et al. 1987). Since bumble bees forage on an abundant pollen source for about 20 minutes before returning to the nest (B. Semrrott, observation of captive bumble bee colony) I thought it unlikely that bees using trap-lines would return to the same inflorescence within 30 minutes in a meadow as large as my study-site.

I observed bumble bee foraging behavior on the two inflorescences simultaneously for 30 minutes. The number of visits to the experimental whorl of both inflorescences was recorded for each bee into a tape recorder. I counted the same number of bees for each member of the inflorescence pair. On average, the first two bees were included in the analysis. For each inflorescence I calculated the proportion of bees leaving the experimental whorl after one visit. The data for both the number of flowers visited and the percentage of bees leaving the whorl were analyzed with paired t-tests.

Methods.- B. I tested whether bumble bees are more likely to depart after just one visit if the first flower encountered is unrewarding by observing bee behavior on whorl 1 (n=69) and whorl 2 (n=73) of unmanipulated L. latifolius inflorescences. The first flower visited within a whorl was noted, and then marked after the departure of the bee. I recorded whether bees made additional visits on the same whorl. Whether the initial visit
was rewarding or not was determined by working the dispensing mechanism once after the bee had left. Of all first flowers visited, 52% were rewarding in whorl 1, and 40% were rewarding in whorl 2. A Fisher's exact test was used to determine if the proportion of bumble bees leaving the whorl after the initial visit was greater when the first visit was unrewarding than when it was rewarding.

Results.- Bumble bees visited significantly more flowers on whorls with pollen than on those without (t= -3.92, P<0.001, Fig. 1). Depleted whorls received 1.51 ± 0.65 visits, and control whorls 3.72 ± 2.42 visits. The percentage of bees leaving the whorl after one visit was significantly higher on emptied than on control whorls (t= 2.98, P=0.005, Fig. 2). An average 64% of bees (± 40) left depleted whorls, whereas an average of 26% of bees (± 37) left rewarding whorls after one visit. Likewise, the proportion of bumble bees leaving the unmanipulated whorls after just one visit in part 1B was greater when the first visit was unrewarding than when it was rewarding (P<<0.0001, Fig. 3). Only 4% of the bees left the whorl when the first flower was rewarding. However, there was almost an equal chance (53%) of a bumble bee staying or leaving the whorl when the first flower was not rewarding.
Fig. 1. The average number of flowers visited by bumble bees on control whorls (of rewarding flowers) and depleted whorls (of unrewarding flowers). The box plot displays the 10th, 25th, 50th, 75th, and 90th percentiles. The dots represent outliers.
Fig. 2. Percent bumble bees leaving control whorls (of rewarding flowers) and depleted whorls (of unrewarding flowers) after just one visit to the whorl. The box plots display the 10th, 25th, 50th, 75th, and 90th percentiles. The median of the control whorl is 0.
Fig. 3. Percent bumble bees staying or leaving upon encountering either a rewarding or an unrewarding flower as their first flower within that whorl. The sample sizes for each group are given above the columns.
Part 2: The impact of the appearance of the neighboring flower

**Methods.** - If the appearance of a flower affects decision-making, a bumble bee should be more likely to leave a whorl upon encountering a spent or closed flower. I tested this prediction by observing bumble bee foraging behavior on whorl 1 and 2 of *L. andersonii*. I recorded whether bumble bees stayed or left the whorl after just one visit, and noted the appearance of the two “neighboring flowers”. A neighboring flower was defined as adjacent to the last-visited flower, and as having not yet received a visit. Flowers were classified as “attractive” if they were open, and as “unattractive” if they were either closed or spent. I sampled an equal number of bees staying and leaving with a total of 84 observations per whorl. Since the lack of a reward in the first visited flower strongly affected bee behavior I included only those observations in which bumble bees landed on a rewarding flower. The study site included approximately 40 plants, each with 6-12 inflorescences. A Fisher’s exact test was used to determine if the proportion of bumble bees staying in the whorl after their first visit was greater when both of the neighboring flowers were attractive than when at least one of them was unattractive.

**Results.** - The appearance of the neighboring flower strongly affected a bee’s decision to leave a whorl. The proportion of bumble bees staying in the whorl after their initial visit was greater when both of the neighboring flowers were attractive (89%) than when at least one of them was unattractive (P<< 0.0001, Fig. 4). The few times bumble bees encountered two unattractive neighboring flowers (0.06%) they invariably left the whorl. Since 80% bees left the whorl upon encountering one unattractive flower, bumble
Fig. 4. Percent bumble bees staying or leaving the whorl upon encountering either an attractive or an unattractive flower. The sample sizes for each group are given above the columns.
bees seemed to visually assess both of the neighboring flowers, and seemed to be deterred by the presence of just one closed or spent flower.

Part 3: The impact of prior experience within a whorl

Methods.- Bees obviously use the presence of pollen and the appearance of neighboring flowers when they decide whether to continue foraging on a whorl. This experiment was designed to determine a) whether prior experience on a whorl also affects departure, and b) how these three variables are incorporated during decision-making. For example, experience might to some extent override the influence of reward and appearance. Bees that have encountered several rewarding flowers on a whorl may be less apt to depart upon encountering an unrewarding or unattractive flower on the basis of this experience.

Observations were carried out in a large meadow with many hundreds of *L. latifolius* plants (each with 5-10 inflorescences). The exact sequence of bee movements within whorl 1 and 2 was noted by marking the first and last flowers visited immediately after the bee had left. Then, I recorded if the flowers visited were attractive and rewarding. I also noted the appearance of the neighboring flower(s) adjacent to the last flower visited. I sampled 490 bees on whorl 1 and 385 bees on whorl 2. A bumble bee was observed foraging on one whorl only. Since a bee's decision to leave a whorl may depend on the number of flowers visited within that whorl, I analyzed bumble bee behavior at different "positions". For example, position 1 was the first flower visited, and position 2 was the second flower visited in a whorl. Bumble bees leaving the whorl at
position 1 had the option of going to two adjacent flowers. In this case the neighboring flower was classified as “unattractive” if at least one of the two fit that category. Occasionally a bee foraging within a whorl did not fly to the nearest flower, but skipped one or more in between. In those cases, I only included positions prior to skipping except for the one immediately preceding it. For example, if a bee visited three flowers next to each other, but then skipped one before going to the remaining flowers within that whorl, I analyzed their behavior at positions 1 and 2 only.

I used multiple logistic regression analyses to determine which of the following three independent variables affected a bee’s decision to leave the whorl at position 1 through 4: 1) the number of unrewarding flowers already visited within a whorl (exclusive of the last-visited flower), 2) the lack of a pollen reward in the last-visited flower, or 3) the appearance of the neighboring flower. Position 1 was analyzed for only two independent variables since it is the first flower visited and bumble bees, therefore, could not yet have experienced unrewarding flowers within that whorl. The appearance of the neighboring flower (“appearance”), and the absence or presence of a pollen reward in the most recently visited flower (“reward”) are dichotomous variables. The number of unrewarding flowers already visited (“experience”) is dichotomous for position 2, and continuous for positions 3 and 4. I included all interaction terms in the analysis, because a bee’s decision to depart may depend on a combination of two factors. For example, a bee may leave a whorl upon encountering several unrewarding flowers only if the last flower visited has also been unrewarding. I used a backward selection model for the analysis, which starts out with all variables (main effects and interaction terms), and then one by
one removes any that do not have an effect on the dependent variable, until only those remain that are statistically significant. I used odds ratios to report the results of the analyses. Odds ratios larger than 1 imply an effect of the tested variable.

**Results.-** The best logistic models for all the positions tested are summarized in Table 1. Every model correctly classified at least 82% of the cases (position 1: 91.09%, position 2: 85.16%, position 3: 82.41%, position 4: 82.63%). Figures 5 and 6 depict how bees responded to varying combinations of the treatment variables.

The appearance of the neighboring flower was by far the best predictor of whether a bee continued to forage on a given whorl. Almost without exception, bees left a whorl if they encountered an unattractive adjacent flower, regardless of whether the last visited flower was rewarding or whether previously visited flowers in the whorl had been rewarding. When these two other variables ("reward" and "experience") were held constant, the estimated odds of leaving the whorl were 24-174 times greater (depending on the position) when the neighboring flower was unattractive than when it was not.

Bees also based their foraging decisions on the presence of pollen in the last-visited flower and on their prior experience within that whorl. For example, the odds of leaving the whorl at position 1 were 24 times greater when the last-visited flower was unrewarding than when it was not, given that the neighboring flower was attractive. While bees sometimes departed upon encountering an unrewarding flower regardless of their prior experience (position 2), their behavior was usually affected by the number of unrewarding flowers visited. The odds of leaving the whorl in the absence of a reward in the last-visited flower increased by a factor of 4 and 3 for each one-unit increase in the
Table 1. Logistic regression models for the effects of a) the number of unrewarding flowers visited (“experience”), b) the lack of a pollen reward in the last-visited flower (“reward”), and c) the appearance of the neighboring flower (“appearance”) on a bumble bee’s choice to depart. All three interaction terms were included in the analysis. The sample size (n) is given for each position.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression Coefficient</th>
<th>Standard Error</th>
<th>Odds Ratio*</th>
<th>95% C.I.</th>
<th>P</th>
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<td></td>
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<td>0.74</td>
<td>174.12</td>
<td>40.95- 740.44</td>
<td>&lt;&lt; 0.0001</td>
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<tr>
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<td>0.39</td>
<td>23.67</td>
<td>10.95- 51.15</td>
<td>&lt;&lt; 0.0001</td>
</tr>
<tr>
<td><strong>Position 2</strong></td>
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<td></td>
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<tr>
<td>n=674</td>
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<tr>
<td><strong>Intercept</strong></td>
<td>-3.91</td>
<td>0.62</td>
<td></td>
<td></td>
<td>&lt;&lt; 0.0001</td>
</tr>
<tr>
<td><strong>Appearance</strong></td>
<td>4.24</td>
<td>0.63</td>
<td>69.13</td>
<td>20.04- 238.41</td>
<td>&lt;&lt; 0.0001</td>
</tr>
<tr>
<td><strong>Reward</strong></td>
<td>1.44</td>
<td>0.24</td>
<td>4.22</td>
<td>2.64- 6.75</td>
<td>&lt;&lt; 0.0001</td>
</tr>
<tr>
<td><strong>Appearance*Experience</strong></td>
<td>0.71</td>
<td>0.25</td>
<td>2.04</td>
<td>1.26- 3.31</td>
<td>0.0039</td>
</tr>
<tr>
<td><strong>Position 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=506</td>
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<tr>
<td><strong>Intercept</strong></td>
<td>-2.38</td>
<td>0.47</td>
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<td></td>
<td>&lt;&lt; 0.0001</td>
</tr>
<tr>
<td><strong>Appearance</strong></td>
<td>3.22</td>
<td>0.50</td>
<td>24.92</td>
<td>9.33- 66.59</td>
<td>&lt;&lt; 0.0001</td>
</tr>
<tr>
<td>*<em>Appearance <em>Reward</em></em></td>
<td>1.39</td>
<td>0.29</td>
<td>4.03</td>
<td>2.30- 7.06</td>
<td>&lt;&lt; 0.0001</td>
</tr>
<tr>
<td><strong>Reward*Experience</strong></td>
<td>-0.85</td>
<td>0.24</td>
<td>2.34**</td>
<td>1.47- 3.74</td>
<td>0.0004</td>
</tr>
<tr>
<td><strong>Position 4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=236</td>
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<td></td>
</tr>
<tr>
<td><strong>Intercept</strong></td>
<td>-3.55</td>
<td>0.82</td>
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<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><strong>Appearance</strong></td>
<td>5.03</td>
<td>0.84</td>
<td>152.50</td>
<td>29.47- 789.02</td>
<td>&lt;&lt; 0.0001</td>
</tr>
<tr>
<td><strong>Reward*Experience</strong></td>
<td>1.06</td>
<td>0.36</td>
<td>2.88**</td>
<td>1.43- 5.79</td>
<td>0.0031</td>
</tr>
<tr>
<td>*<em>Appearance <em>Experience</em></em></td>
<td>-0.80</td>
<td>0.27</td>
<td>2.23**</td>
<td>1.31- 3.81</td>
<td>0.0032</td>
</tr>
</tbody>
</table>

* odds ratio > 1 implies an effect of the tested variable on a bumble bee's choice to leave the whorl
** odds per one-unit increase in the variable
RA: flower rewarding, both of the neighboring flowers attractive
UA: flower unrewarding, neighboring flowers attractive
RU: flower rewarding, at least one of the neighboring flowers unattractive
UU: flower unrewarding, at least one of the neighboring flowers unattractive

Fig. 5. Percent bumble bees staying or leaving the whorl after encountering one of four situations at position 1. Each situation is abbreviated with a three-letter code. The first letter represents the "reward" variable, the second the "appearance" variable. The sample size for each category is written above each column.
**Fig. 6.** Percent bumble bees staying or leaving the whorl after encountering one of eight situations—each abbreviated by a three-letter code—at positions 2, 3, and 4. The first letter represents the "experience", the second the "reward", and the third the "appearance" variable. "Experience" is modified into a dichotomous variable in positions 3 and 4, but is continuous in the logistic regression analysis (see text). The sample sizes for each category are placed above each column.

**RRA:** (all previously visited) flower(s) rewarding, last flower visited rewarding, neighboring flower attractive

**URA:** (at least one) flower previously visited unrewarding, last flower visited rewarding, neighboring flower attractive

**RUA:** (all previously visited) flower(s) rewarding, last flower visited unrewarding, neighboring flower attractive

**UUA:** (at least one) flower previously visited unrewarding, last flower visited unrewarding, neighboring flower attractive

**RRU:** (all previously visited) flower(s) rewarding, last flower visited rewarding, neighboring flower unattractive

**URU:** (at least one) flower previously visited unrewarding, last flower visited rewarding, neighboring flower unattractive

**RUU:** (all previously visited) flower(s) rewarding, last flower visited unrewarding, neighboring flower unattractive

**UUU:** (at least one) flower previously visited unrewarding, last flower visited unrewarding, neighboring flower unattractive
number of previous unrewarding flowers at positions 3 and 4 respectively. Therefore, prior experience only had an impact on decision-making when the last-visited flower was unrewarding. However, prior experience also influenced departure decisions independent of a reward in the last-visited flower. The odds of leaving the whorl (in the presence of an attractive neighboring flower) increased by a factor of 2 for each additional unrewarding flower encountered at positions 2 and 4.

**Part 4: The impact of pollen fragrance**

*Methods.*- The objective was to determine whether bees use pollen scent as a guide to rewarding flowers. If pollen fragrance affects decision-making, bees should not only leave a whorl upon encountering unattractive or unrewarding flowers, but also upon encountering neighboring flowers without pollen fragrance. Since lupine pollen has scent (see Dobson *et al.* 1996), the presence of pollen within the flower was taken as an indication of fragrance. Study site and methods were the same as in part 3, except that in addition to noting the conditions associated with the other variables, I determined whether there was any pollen inside the keel of the neighboring flower. I restricted my observations to whorl 1, because only there were the presence of a pollen reward and the visual attractiveness of a flower independent of each other. While an unattractive flower with exerted stamens does not contain any pollen, an unattractive closed flower does. The neighboring flower was classified as “pollen-containing” if both of the adjacent flowers fit that category. The sample size of position 1 (n=192) was the only one large enough for the analysis. Therefore, I was not able to include the “experience” variable. A multiple
logistic regression analysis with a backward selection model was used to determine which of the following three independent variables affected the bee’s decision to leave the whorl at position 1: a) the lack of a reward in the last-visited flower b) the appearance of the neighboring flower, or c) the presence of pollen inside the keel of the neighboring flower. In addition to these three main effects, all three interaction terms were included in the analysis.

Results. - The presence of pollen in the neighboring flower did not affect a bumble bee’s decision to leave a whorl, and the variable was not included into the model (Table 2). The model correctly classified at least 89.53% of the cases. However, after having visited a rewarding flower, relatively more bees departed upon encountering an attractive, neighboring flower without pollen than upon encountering one with pollen (Fig. 7). The lack of pollen scent from the neighboring flower may have affected bee departure in some way, even though this was not statistically significant.
Table 2. Logistic regression model for the effects of a) the lack of a reward in the last-visited flower ("reward"), b) the appearance of the neighboring flower ("appearance"), and c) the presence of pollen inside the keel of the neighboring flower ("scent") on a bumble bee's choice to leave position 1 in whorl 1. The backward selection procedure did not select "scent" into the model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression Coefficient</th>
<th>Standard Error</th>
<th>Odds Ratio*</th>
<th>95% C.I.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position 1 n=192**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-3.00</td>
<td>1.02</td>
<td>&lt; 0.0035</td>
<td></td>
<td>&lt; 0.0035</td>
</tr>
<tr>
<td>Appearance</td>
<td>3.75</td>
<td>1.07</td>
<td>42.50</td>
<td>5.23-345.13</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Appearance*Reward</td>
<td>2.91</td>
<td>0.66</td>
<td>18.35</td>
<td>5.05-66.73</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

* odds ratio > 1 implies an effect of the tested variable on a bumble bee's choice to leave the whorl
** n= sample size
RAS: last flower visited rewarding, neighboring flower attractive and with pollen scent
RAN: last flower visited rewarding, neighboring flower attractive and without pollen scent
UAS: last flower visited unrewarding, neighboring flower attractive and with pollen scent
UAN: last flower visited unrewarding, neighboring flower attractive and without pollen scent
RUS: last flower visited rewarding, neighboring flower unattractive and with pollen scent
RUN: last flower visited rewarding, neighboring flower unattractive and without pollen scent
UUS: last flower visited unrewarding, neighboring flower unattractive and with pollen scent
UUN: last flower visited unrewarding, neighboring flower unattractive and without pollen scent

Fig. 7. Percent bumble bees staying or leaving whorl 1 after encountering one of eight situations—each abbreviated by a three-letter code—at position 1. The first letter represents the "reward", the second the "appearance", and the third the "scent" variable. The sample sizes for each category are placed above each column.
Discussion

Bumble bees respond to the distribution of pollen within inflorescences on at least two levels. They clearly discriminate against flowers that show unambiguous visual signs of being unrewarding. There is also evidence that bees can assess the amount of pollen available in flowers they visit, and that they depart upon encountering one or more unrewarding flowers. On the other hand, bees do not use pollen scent to avoid unrewarding flowers.

By judging the appearance of a nearby flower, bumble bees were able to avoid obviously unrewarding flowers without having to land on them first. They almost invariably left the whorl upon encountering an unattractive flower. Bees seemed to be able to distinguish between three classes of lupine flowers by cueing in on the morphological characters of open, closed, and spent flowers. Since pollen is not constantly replenished as it is with nectar, age-related floral attributes are often correlated with the amount of pollen available. For example, bumble bees differentiate between different age-related floral shapes of *Anemonopsis macrophylla* (Pellmyr 1988). In lupine, however, the age of a flower is not always indicative of the amount of pollen dispensed. Since attractive, open flowers do not always offer a reward, bumble bees cannot entirely rely on the appearance of flowers to avoid less rewarding sources. Bumble bees were able to assess the pollen quantity they had collected from a single lupine flower. This confirms the findings of Harder (1990a) and Buchmann (1989) that bumble bees somehow are able to gauge the collected amount of this non-ingested resource. The ability to make foraging decisions based on individual flowers may be a
fundamental mechanism underlying energy efficiency in pollen foraging (see Rasheed and Harder 1997a,b). It still needs to be shown on what scale bumble bees are able to measure quantities collected, and whether they use some type of departure rule when foraging on pollen. If their behavior is similar to nectar-foraging bees, they would be leaving an inflorescence upon encountering a "bad flower"—one with a pollen quantity below a certain threshold value (see Hodges 1985). In flowers with hidden resources, such as lupine, basing foraging decisions on such threshold values may be an effective way of avoiding less rewarding flowers.

In the presence of an attractive neighboring flower, bumble bees rarely based their foraging decisions only on the condition of the last-visited flower. While bees often departed when the last-visited flower was unrewarding, they usually did not entirely disregard their prior experience within that whorl, like nectar-foraging bees do on wild bergamot, *Monarda fistulosa* (see Cresswell 1990). Instead, they based their departure on the reward levels of all the flowers visited. Apparently bees were able to integrate the information from at least three flowers when collecting lupine pollen, just as they have been shown to do during nectar-foraging (see Dukas and Real 1993). Unless a bee is foraging on the top or bottom whorls, where either closed or spent flowers predominate, it probably is more beneficial to make a prediction of a neighboring flower based on the resource level of more than one flower.

Bumble bees did not seem to use olfactory cues to avoid unrewarding flowers. While they have a developed sense of smell, and are often attracted by volatile floral compounds (see Marden 1984, Dobson 1991), the presence or absence of pollen inside
the neighboring flower did not affect a bee’s choice of departure. It has been suggested that they may only be able to smell lupine pollen if it is present at the tip of the keel, that is, outside the flower (see Dobson et al. 1996). However, pollen was never at the tip of the keel at my site. Pollen and other floral fragrances may not play an important role in foraging on lupine because the presence of pollen inside a flower does not guarantee that pollen will be dispensed during the next visit. The presence of a pollen reward is not only dependent on the total number of received visits or the age of the flower, but also on the quantity and frequency of recent visits, and the time that has passed since the last visit (which determines whether the pollen tunnel has had a chance to collapse; see Harder and Wilson 1994). For example, a rewarding flower that has received multiple bee visits within a short period is likely to become unrewarding. Only after some time has passed without visits, and the pollen tunnel has had time to collapse, will the flower become rewarding again. Therefore, bees cannot identify unrewarding flowers by scent alone. Only if there is no pollen in the neighboring flower is this olfactory cue reliable, and bees may have occasionally used scent in the absence of other cues, i.e. unrewarding or unattractive flowers.

While the decision to leave a whorl often depended on more than one factor, bumble bees seem to base it on the most reliable signal. A spent or closed flower was sufficient reason to leave a whorl, regardless of the quality of any previously visited flowers. Bees only considered the reward levels of previously visited flowers if the neighboring flower was attractive, because a within-whorl spatial correlation of pollen reward was probably not as reliable a signal as obviously unrewarding flowers. Bees did
not base their foraging decisions on the pollen scent of neighboring flowers, and thereby avoided the least reliable cue.

There are likely to be other factors affecting bumble bee foraging decisions. For example, bees avoid flowers that carry the scent from previous visitors (see Goulson et al. 1998, Stout et al. 1998, Williams 1998). Also, foraging decisions may not only depend on external factors since bumble bee learning varies with size and age (Laverty 1994), their foraging efficiency decreases with age (Thomson et al. 1982), and their behavior can be modified by endoparasitic fly larvae (Schmid-Hempel and Schmid-Hempel 1991, Schmid-Hempel and Stauffer 1998).

Bumble bees seemed to respond in varying degrees to a pollen reward. More bumble bees left depleted and control whorls after just one visit in part 1B (Fig. 2) than in the equivalent situations in part 3 (Fig. 5). The discrepancy in departure rate is especially high between the pollen-depleted flowers in part 1B and the situations in part 3, in which bees were confronted with an unrewarding last flower and an attractive neighboring flower. Maybe bees were affected by changes caused by handling the flowers. The repeated manipulation of the keel may have harmed the pollen dispensing mechanism. If bees can sense such differences in the flexibility of the keel, they might use this as yet another cue for less rewarding flowers.

One of the largest limitations of parts 2, 3, and 4 is that bumble bees are unlikely to make foraging decisions based on a simple dichotomous system of "pollen reward present" vs. "pollen reward absent". Instead, they are likely to be able to assess the pollen content of individual flowers on a finer scale than that, since bumble bees foraging on
lupine spend more time on flowers of high-pollen content (of previously bagged inflorescences) than on average flowers (Harder 1990a). Since methods of estimating the pollen collected from individual flowers by bumble bees have not been developed yet, the dichotomous choice system seemed adequate. However, some inconsistencies in their behavior could be due to type of assessment. For example, some bumble bees may have left the whorl after just a few visits, even though all of the flowers encountered were rewarding and attractive, because of a low pollen reward in some or all of the visited flowers.

Future research needs to determine whether bumble bee foraging decisions within lupine whorls are indeed founded in the resource distribution of pollen. Only if there is a predictable spatial pattern of pollen quantities, is it sensible to depart after visiting one or more unrewarding flowers. Further, if the pollen quantity dispensed per visit is not correlated within a whorl, bees could just skip an unattractive flower instead of leaving the whorl altogether.

In summary, within the patchy resource environment of lupine inflorescences, bumble bees have several ways to avoid unrewarding flowers. Since the pollen is hidden from view in lupine, bumble bees use indirect ways of assessing the quality of a nearby flower. By cueing in on the appearance of flowers and leaving the whorl after some number of unrewarding flowers, bumble bees may maximize their pollen return while keeping their energy costs as low as possible.


Gori DF (1989) Floral color change in Lupinus argenteus (Fabaceae): why should plants advertise the location of unrewarding flowers to pollinators? Evolution 43: 870-881


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Hayler KF (1999) Pattern, frequency, and fitness consequences of pollen feeding by fly larvae. Master’s thesis, Humboldt State University, Arcata, California


Laverty TM (1994) Bumble bee learning and flower morphology. Anim Behav 47: 531-545


Pyke G (1979) Optimal foraging in bumblebees: Rule of movement between flowers and inflorescences. Anim Behav 27: 1167-1181


Rasheed SA, Harder LD (1997b) Foraging currencies for non-energetic resources: pollen collection by bumblebees. Anim Behav 54: 911-926


Appendix 1

I determined whether petal color in *L. latifolius* is linked to floral reward levels, *i.e.*, the amount of pollen dispensed per visit. The flowers of some species change color upon pollination after their resources have been exhausted (see Casper and Pine 1984, Gori 1989, Weiss 1995, Niesenbaum *et al.* 1999). The flowers of both *L. latifolius* and *L. andersonii* change or lose color as they age, and while there is a distinct difference in color between whorls, there is often one among flowers of the same whorl as well. However, it was not apparent whether this difference in color between flowers of the same whorl was correlated with the amount of pollen dispensed per visit.

I tested whether flower color was indicative of the available pollen by randomly sampling the pollen mass dispensed per visit of 15 flowers in whorl 2 for each of the following three color categories: 1) purple, 2) off-white (often with some purple or brown spots), and 3) mostly brown to completely brown (possibly with some signs of wilting or drying). The pollen quantity received per bumble bee visit was estimated by manually working the pollen dispensing mechanism once and receiving the pollen on a small piece of waxed paper (~20 mm x 30 mm). The waxed paper was then folded and stored in envelopes. The pollen dried in a more or less single layer of pollen grains. The number of pollen grains was counted using a light microscope with an inserted grid in one ocular. Pollen grains were counted individually unless they were equally distributed within a patch. Then the number of pollen grains was counted in one cell, and multiplied by the number of cells they occupied with a similar density. An analysis of variance showed that the number of pollen grains dispensed per visit indeed did not significantly differ in the
three color groups (F = 1.42, P = 0.26). Therefore, petal color within whorls was not
 correlated with the pollen quantity available to bees.