

BUZZING BEES (HYMENOPTERA: APIDAE, HALICTIDAE)  
ON *SOLANUM* (SOLANACEAE): FLORAL CHOICE AND  
HANDLING TIME TRACK POLLEN AVAILABILITY

TODD E. SHELLY<sup>1</sup>, ETHEL VILLALOBOS<sup>2</sup> AND STUDENTS OF THE FALL 1997 OTS-USAP<sup>2,3</sup>

<sup>1</sup>Present address: USDA-APHIS, P.O. Box 1040, Waimanalo, HI 96795  
and Hawaiian Evolutionary Biology Program, University of Hawaii  
Honolulu, HI 96822

<sup>2</sup>Organization for Tropical Studies-Undergraduate Semester Abroad Program  
Duke University, 410 Swift Avenue, Durham, NC 27705

<sup>3</sup>Students (in alphabetical order): Lisa Bell, Aisha Burden, Mark Fox,  
Ilmi Granoff, Nihara Gunawardene, Melisa Holman, Allison Hornor,  
Jane MacLeod, Julia Michalek, Casuarina McKinney-Richards,  
Adam Ruff, Aaron Smith, Darcy Thomas, and Olivia Watson

ABSTRACT

Flower selection and pollen-collecting effort were monitored for 3 species of bees that sonicate flowers of *Solanum wendlandii* Hook. for pollen in southern Costa Rica. Between 0700-0900 hours, *Bombus pullatus* (Fkln.), *Euglossa erythrochlora* Moure, and *Pseudaugochloropsis graminea* (Fabricius) foraged more frequently at new flowers (that had opened the day of observation) than old ones (that had opened at least 1 day before observation). Between 0900-1100 hours, however, this preference was no longer evident, and all 3 species visited new and old flowers with similar frequency. *E. erythrochlora* and *P. graminea* spent more time harvesting pollen during 1) initial (first or second) visits to new flowers than initial visits to old flowers and 2) initial visits to new flowers than final (seventh or later) visits to new flowers. Similar, although not statistically significant, trends were evident for *B. pullatus* as well. An experiment using pollinator exclusion bags revealed that the reduced foraging effort at individual flowers was resource-dependent and was not simply a time-dependent phenomenon.

Key Words: Apidae, buzz pollination, Costa Rica, foraging behavior, Halictidae, *Solanum*

## RESUMEN

Se monitoreó la selección de flores y el esfuerzo en recolectar polen de tres especies de abejas que frecuentan flores de *Solanum wendlandii* Hook. en al Sur de Costa Rica. Entre las 0700 y 0900 h, *Bombus pullatus* (Fkln.), *Euglossa erythrochlora* Moure y *Pseudaugochloropsis graminea* (Fabricius) forrajearon con mayor frecuencia flores nuevas (flores que abrieron el mismo día de hacerse la observación) que flores viejas (flores que abrieron por lo menos un día antes de hacerse la observación). Sin embargo, esta preferencia no se observó entre las 0900 y 1100 h, ya que las tres especies visitaron flores nuevas y viejas con la misma frecuencia. *E. erythrochlora* y *P. graminea* emplearon más tiempo cosechando polen en visitas iniciales (primera o segunda visitas) a flores nuevas que en visitas iniciales a flores viejas. Además, emplearon más tiempo en visitas iniciales a flores nuevas que en visitas finales (séptima visita en adelante) a flores nuevas. Se observó una tendencia similar (aunque no estadísticamente significativa) en *B. pullatus*. Un experimento empleando bolsas para excluir polinizadores demostró que la reducción en el esfuerzo por forrajear flores individuales estuvo determinada por la disponibilidad de alimento y no por el horario.

---

The manner in which nectar rewards influence flower selection and floral handling times has been well studied for a number of bee species (e.g., Cresswell 1990, Giurfa and Nunez 1992, Dukas and Real 1993). Early studies of nectar-collecting by bees (e.g., Waddington and Heinrich 1979, Pyke 1979) lent strong empirical support to the development of optimal foraging theory (Krebs and McCleery 1984). In contrast, fewer studies have investigated the foraging choices of bees harvesting pollen, and these have yielded mixed results regarding the ability of bees to assess pollen rewards from individual flowers. For example, Haynes and Mesler (1984) observed bumblebees foraging on inflorescences of a lupine species and found bees did not discriminate between old, pollen-poor flowers and younger flowers that contained greater pollen rewards. Based on patterns of turning frequency and directionality, Hodges and Miller (1981) similarly concluded that bumblebees were not adjusting their foraging movements in response to pollen availability.

On the other hand, several studies have demonstrated that bees do modify their foraging behavior in response to either anticipated or actual pollen rewards for individual floral visits. In the first demonstration of "distant assessment", Pellmyr (1988) showed that bumblebees assessed pollen rewards prior to alighting, based on age-dependent changes in floral shape, and rejected old flowers in favor of younger, pollen-rich flowers. He also showed that bumblebees adjusted their handling time with pollen availability and spent more time on younger flowers than older ones. Likewise, Gori (1989) experimentally removed pollen and found that bumblebees responded by visiting fewer flowers per inflorescence. Buchmann and Cane (1989) and Harder (1990) also reported a positive relation between pollen availability and handling time for individual floral visits, indicating immediate assessment of pollen returns.

The present study examined whether bees foraging on *Solanum wendlandii* Hook. selectively visited younger (pollen-rich) flowers over older flowers and also spent more time foraging on younger than older flowers. In addition, a pollinator exclusion experiment was conducted to assess whether, among young flowers, floral handling time varied between "virgin" vs. previously visited flowers.

## MATERIALS AND METHODS

Flowers of *S. wendlandii* have purple petals that fade with age (flowers probably last no more than 3 days). Five, large, tubular anthers are present with distal sections purple and basal parts yellow. Nectaries are absent, and flowers offer only pollen, which is released through 2 minute apical pores per anther (Michener 1962). Bees are able to gather the pollen efficiently only via sonication or buzzing of the anthers. Visiting bees grasp the anther cone and rapidly contract their indirect flight muscles (thus producing an audible sound or buzz), which transfers vibrations to the anthers and expels pollen onto the bee. Bees then groom and transfer the pollen to special structures (or corbiculae) on their hind legs for storage.

The study was conducted at the Las Cruces Biological Station of the Organization for Tropical Studies in southwestern Costa Rica. The patch of *S. wendlandii* observed was growing on a stone wall in the station clearing immediately adjacent to mixed primary and secondary pre-montane forest (elevation 1,100 m). Data were gathered from 18 September to 4 October, 1997, an interval falling toward the end of a 9-month rainy season. Observations were restricted to sunny days with air temperatures ranging between 20–23°C.

Visits by 3 species of buzz-pollinating bees were recorded continuously for individually tagged flowers of *S. wendlandii* between 0700-1100 hours over 4 days. Based on preliminary observations, the peak period of floral visitation occurred between 0800-1000 hours. Buzz-pollinators were never seen at the flowers prior to 0715 hours, and thus our data most likely describe complete sequences of bee visitation to the focal flowers. The 3 principal buzz-pollinating species included (in order of increasing body size) a halictid, *Pseudaugochloropsis graminea* (Fabricius), a euglossine, *Euglossa erythrochlora* Moure, and a bumblebee, *Bombus pullatus* (Fkln.). Several other *Euglossa* spp. were observed sonicating the flowers, but these were infrequent visitors.

On a given morning, 1-8 observers recorded the time of day, bee species, and duration of pollen collecting (to the nearest s) for individual foraging visits to 2-5 pairs of flowers, each pair consisting of a "new" (i.e., newly opened the same day as the observation) and an "old" (i.e., open for at least 1 day prior to the observation) flower. Operationally, the duration of pollen collection (here termed handling time) was equated with audible buzzing of the anthers. Flower age was determined by tagging stems 1 day before making observations with a small piece of green tape. Tags were placed below fully developed buds (set to open the following morning and be "new" flowers) and the closest (already) open flower. Paired new and old flowers were less than 30 cm apart in all cases.

We compared handling times of 1) "initial" visits (first or second visit observed over all bee species) to new vs. old flowers, 2) initial vs. "final" visits (seventh or later visit observed over all bee species) to new flowers, and 3) final visits to new flowers vs. initial visits to old flowers. Note that the terms "initial" and "final" refer to the sequential order of visits compiled over all bee species for particular flowers and not to inter-floral visitation sequences for particular bees. Counting visits independently of species identity provided only a rough index of pollen depletion, since possible interspecific differences in pollen removal were not documented. Note also that initial visits to old flowers refer, not to their first or second visits in absolute terms, but to the first or second visits recorded during our observations. Being at least 1 day old, old flowers had most likely been visited multiple times on the day(s) prior to our observations (a valid assumption given that 49 of the 52 new flowers we observed received 2 or more visits by sonicating bees).

As shown below, for 2 of the species there was a significant reduction in the time spent gathering pollen at new flowers through the morning, i.e., between early (0700-

0900 hours) and late (0900-1100 hours) observation periods. To determine whether this decrease was time-dependent (foraging rule: if late morning, spend less time per new flower) vs. resource-dependent (foraging rule: if pollen supply depleted, spend less time per new flower), we placed fine mesh bags on a total of 24 flower buds 1 day before opening. Buds were enclosed completely with nylon mesh screening secured to the stem with a wire clasp. Bags were sufficiently large that the exposed anthers of newly opened flowers were well below the screening, out of reach of potential pollinators. For each bagged flower, we tagged (but did not bag) a nearby (within 30 cm) fully developed bud. The following morning we removed the bags at 0900 hours and continuously recorded visits for the next 2 h; unbagged, new flowers were observed continuously from 0700-1100 hours. On a given day, an observer monitored visits continuously to 8 pairs of new flowers (i.e., 1 bagged, 1 unbagged). This experiment was completed over 3 mornings.

Upon completing behavioral observations, we collected flowers to estimate pollen supplies for (1) new, unvisited flowers (buds were bagged 1 day before opening and bagged flowers were collected the following day), (2) new, visited flowers (buds were tagged but not bagged 1 day before opening and flowers were collected the following morning), and (3) old, visited flowers (already open flowers were tagged and collected the following day). All flowers were collected at 1100 hours, and anthers were removed with a forceps, placed in a drying oven (55°C) for 24 h, and weighed to the nearest 0.001 g using a Mettler AE260 Analytical Balance.

Variation in floral handling times within and among bee species and anther weights among flowers was first analyzed using one-way ANOVA to detect significant inter-group variation overall and then the Tukey test to identify significant differences between specific groups ( $\log_{10}$  transformed values were used in both tests to control for the association between mean and variance; Zar 1996). However, data on floral visitation were not normally distributed (even after  $\log_{10}$  transformation), and consequently for this parameter within-species variation was first analyzed using the Kruskal-Wallis test (ANOVA by ranks) and then the non-parametric Dunn's test (Daniels 1990). In all cases, there was direct correspondence between the 2 types of tests: when ANOVA (or the Kruskal-Wallis test) detected (or, conversely failed to detect) significant variation overall, the Tukey test (or Dunn's test) also identified (or, conversely, failed to identify) specific, significant inter-group differences. Consequently, only the results of the Tukey tests (or Dunn's tests) are presented. For pairwise comparisons in the flower bagging experiment, we used the Mann-Whitney test, a nonparametric analogue of the Student's t test (Zar 1996).

## RESULTS

The 3 species displayed the same temporal pattern of abundance (Fig. 1). Few floral visits occurred before 0730 hours. Activity peaked between 0730-0830 hours and then declined steadily until the end of observations at 1100 hours. Despite frequent checks, no bees were seen at the flower patch in the afternoon or early evening. For all 3 species, approximately  $\frac{2}{3}$  of all floral visits occurred prior to 0900 hours.

The species also exhibited the same pattern of preference for floral age (Table 1). Individuals of all species preferred new over old flowers in the early morning but showed no such preference later in the morning. The early morning preference for new flowers was quite pronounced: new flowers were, on average, visited 3-4 times more often than old flowers. Reflecting the decline in overall activity, visitation rates to new flowers decreased significantly between early and late morning for all species. Visitation rates declined with time for old flowers as well, though this difference was not significant for any bee species.

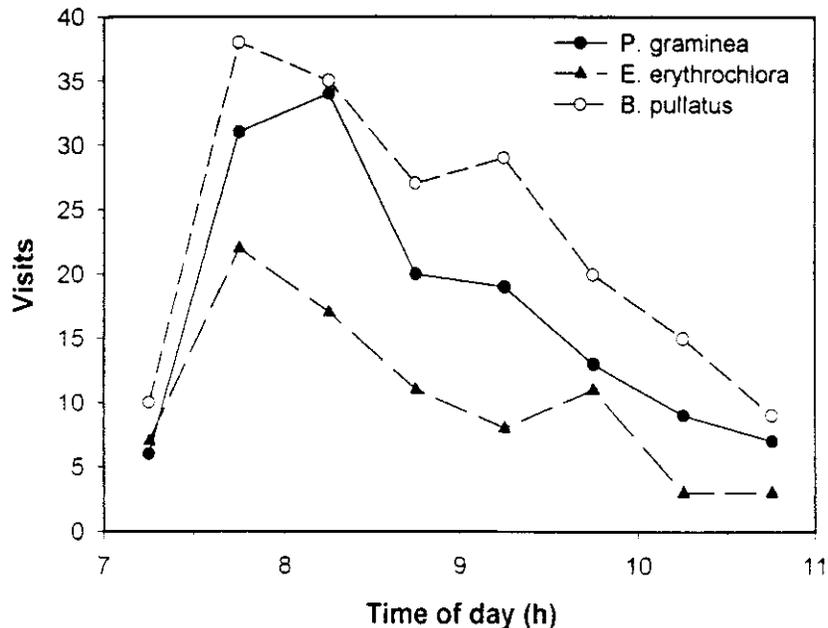


Fig. 1. Total numbers of visits recorded over all focal flowers (N = 52 pairs of new and old flowers) in relation to time of day.

During initial visits, *P. graminea* and *E. erythrochlora* spent significantly more time collecting pollen from new flowers than old ones (Table 2). Also, when visiting new flowers, these same species spent significantly more time collecting pollen during initial visits than final ones. These same trends were apparent for *B. pullatus* as well, although they were not statistically significant (Table 2). Handling times for initial visits to new flowers were similar to initial visits to old flowers in all 3 species (Table 2).

Regarding interspecific comparisons, *P. graminea* spent significantly more time collecting pollen during initial visits to new flowers than *E. erythrochlora* or *B. pullatus* (Table 2). Handling times did not vary among species for final visits to new flowers or initial visits to old flowers.

The incidence of initial and final visits to new flowers was not independent of the time of day, and the majority (51/69 = 74% over all species) of final visits to new flowers occurred after 0900 hours. Thus, as noted above, the decrease in buzzing durations between initial and final visits to new flowers noted for *P. graminea* and *E. erythrochlora* may have reflected a time-dependent, rather than a resource-dependent, shift in foraging behavior. However, in the flower bagging experiment mean handling times were greater for the initial visits to "virgin" new flowers than for the concurrent, final visits to unbagged, new flowers for all 3 species - *P. graminea*: 26 s vs. 11 s, respectively ( $n_1 = 11$ ,  $n_2 = 8$ ); *E. erythrochlora*: 15 s vs. 6 s, respectively ( $n_1 = 6$ ,  $n_2 = 6$ ); *B. pullatus*: 18 s vs. 8 s, respectively ( $n_1 = 17$ ,  $n_2 = 17$ );  $P < 0.05$  in all cases, Mann-Whitney test). Pollen-collection times for initial visits to previously bagged, new flowers were similar to those recorded for initial visits to unmanipulated, new flowers in the early morning for all 3 species ( $P > 0.05$  in all cases; Mann-Whitney test). Despite these

TABLE 1. VISITATION RATES TO NEW AND OLD FLOWERS DURING EARLY (0700-0900 HOURS) AND LATE (0900-1100 HOURS) MORNING. VALUES GIVEN REPRESENT MEAN NUMBER ( $\pm 1$  SD) OF VISITS PER FLOWER PER 2 H SAMPLING PERIOD; N = 52 FLOWERS IN ALL CASES. VALUES WITHIN A ROW FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT FOLLOWING THE DUNN'S TEST (P = 0.05).

	Early		Late	
	New	Old	New	Old
<i>P. graminea</i>	1.4a (1.4)	0.3b (0.6)	0.6b (1.0)	0.25b (0.6)
<i>E. erythrochlora</i>	0.8a (0.9)	0.3b (0.4)	0.4b (0.6)	0.2b (0.5)
<i>B. pullatus</i>	1.8a (1.5)	0.5b (0.5)	0.9b (1.0)	0.5b (0.5)

findings, bees visited virgin and previously available new flowers with similar frequency: over all 3 species, the mean numbers of visits recorded between 0900-1100 hours were 1.85 ( $\pm 1.2$ ) and 2.0 ( $\pm 1.3$ ) for virgin and unbagged new flowers, respectively (P > 0.05; Mann Whitney test).

Anther (mg dry) weights were significantly greater for new, unvisited flowers ( $\bar{x} \pm 1$  SD = 149  $\pm$  14 mg) than either new, visited (131  $\pm$  12 mg) or old, visited (125  $\pm$  9 mg) flowers (P < 0.05 in both cases; Tukey test using transformed [ $\log_{10}$  x] data). Among visited flowers, anthers from new flowers weighed more than those from old flowers, although this difference was not statistically significant (P > 0.05; Tukey test using transformed [ $\log x_{10}$ ] data).

#### DISCUSSION

Foraging choices frequently involve "non-energetic" benefits (Rasheed and Harder 1997). That is, although their collection requires energy expenditure, the resources do not provide energy directly to the forager but serve other functions. For bees, pollen harvesting yields non-energetic benefits: pollen serves primarily as a protein source for developing larvae, while nectar is the chief energy source for flight and other ac-

TABLE 2. HANDLING TIMES AT NEW AND OLD FLOWERS DURING INITIAL (FIRST OR SECOND) AND FINAL (SEVENTH OR GREATER) VISITS TO INDIVIDUAL FLOWERS. MEAN VALUES TO NEAREST S ( $\pm 1$  SD, N) ARE PROVIDED. VALUES WITHIN A ROW FOLLOWED BY SAME LOWERCASE LETTER ARE NOT SIGNIFICANTLY DIFFERENT; VALUES WITHIN A COLUMN FOLLOWED BY THE SAME UPPERCASE LETTER ARE NOT SIGNIFICANTLY DIFFERENT FOLLOWING THE TUKEY TEST (USING TRANSFORMED [ $\text{LOG}_{10}$  X] DATA; P = 0.05).

	New		Old
	Initial	Final	Initial
<i>P. graminea</i>	37aA (24, 34)	15bA (10, 24)	10bA (6, 19)
<i>E. erythrochlora</i>	18aB (11, 19)	8bA (4, 14)	6bA (4, 14)
<i>B. pullatus</i>	14aB (13, 45)	9aA (6, 31)	8aA (6, 29)

tivities (Heinrich 1979). In addition to the fact that nectar is an easily measured and manipulated resource, the research emphasis on nectar foraging by bees derives largely from the working assumption that energy is an appropriate fitness "currency" for foraging animals. Nonetheless, energy-mediated constraints on bee activity might be expected to generate a common pattern of foraging behavior regardless of the type of resource collected (i.e., energy- or non-energy-based). In fact, Rasheed and Harder (1997) found that pollen-gathering bumblebees foraged in a manner qualitatively similar to that reported for nectar-gatherers, i.e., in both cases, bees maximized foraging efficiency or benefit-to-metabolic cost ratio.

The present study provides additional evidence that pollen-foraging bees modify their behavior in response to anticipated and actual pollen returns from individual flowers. All 3 species studied preferred new over old flowers in the early morning. As petal color changed greatly with age, it seems likely that bees used reflectance patterns (in the visual or ultraviolet spectra) as long-distance cues of pollen supplies. By late morning, however, the bees did not differentiate between new and old flowers, and, as the measurements of anther weight suggest, this shift reflected an increased similarity in the pollen abundance of new and old flowers. Thus, the bees presumably could distinguish between new and old flowers late in the morning but, owing to reduced pollen levels in the new flowers, "ignored" this distinction. Additional data on approach and rejection probabilities for flowers of different ages are required to confirm color-based discrimination in the early morning.

In contrast, bees were apparently unable to make long-range assessment of pollen availability among new flowers. In the bagging experiment, bees visited virgin and unbagged new flowers with equal frequency. This finding was not unexpected, since the pollen is concealed within minute pores on the anther (Michener 1962). Potential cues, such as pollen odor (Buchmann 1983) or "bruise marks" left on the anthers by previous visitors (J. Cane, pers. comm.), were presumably either absent or weak. Consistent with other studies, therefore, our data suggest that long-distance assessment of nectar (Neff and Simpson 1990) or pollen (Pellmyr 1988) rewards may depend exclusively on "gross" features of floral morphology, such as overall shape or color.

Upon alighting, bees clearly adjusted their harvesting effort to match pollen availability. In the bagging experiment, handling times for all 3 species were significantly longer for virgin, new flowers than for unbagged, new flowers. Observations for *P. graminea* and *E. erythrochlora* showed that initial visits to new flowers were significantly longer than either initial visits to old flowers or final visits to new flowers. Other studies (Pellmyr 1988, Buchmann and Cane 1989, Harder 1990) report this same trend, supporting the general observation that, as bee visitation continues in a flower patch, individual foragers encounter diminishing pollen returns per flower and therefore spend less time at individual flowers.

In sum, our data indicate that pollen-collecting bees are sensitive to varying resource levels within individual flowers and respond by selecting and intensively handling more rewarding flowers. Thus, while the nature of the rewards differ, pollen- and nectar-foraging bees appear similar in attempting to maximize the rate of resource collection. Instead of maximizing the rate of fuel (nectar) intake, however, pollen-collecting bees forage in a manner that increases the rate of pollen delivery to developing larvae.

#### ACKNOWLEDGMENT

We thank Luis Diego Gomez for weather data and for identifying the plant, Raul Rojas for much logistical support, Jim Ackerman for supplying a key for euglossines,

the staff of the Instituto Nacional de Biodiversidad for assistance in identifying the bees, and Emma and Miranda Shelly for assistance with data collection. We also thank Jim Cane for supplying references and encouragement and Jack Neff for helpful comments on an earlier draft.

## REFERENCES CITED

- BUCHMANN, S. L. 1983. Buzz pollination in angiosperms, pp. 73-113 in C. E. Jones and R. J. Little (eds.). Handbook of experimental pollination biology. Van Nostrand Reinhold, New York.
- BUCHMANN, S. L., AND J. H. CANE. 1989. Bees assess pollen returns while sonicating *Solanum* flowers. *Oecologia (Berl)* 81: 289-294.
- CRESSWELL, J. E. 1990. How and why do nectar-foraging bumblebees initiate movements between inflorescences of wild bergamot *Monarda fistulosa* (Lamiaceae). *Oecologia (Berl)* 82: 450-460.
- DANIELS, W. W. 1990. Applied nonparametric statistics. PWS-KENT Publishing, Boston.
- DUKAS, R., AND L. A. REAL. 1993. Effects of recent experience on foraging decisions by bumble bees. *Oecologia (Berl)* 94: 244-246.
- GIURFA, M., AND J. NUNEZ. 1992. Foraging by honeybees on *Carduus acanthoides*: pattern and efficiency. *Ecol. Entomol.* 17: 326-330.
- GORI, D. F. 1989. Floral color change in *Lupinus argenteus* (Fabaceae): why should plants advertise the location of unrewarding flowers to pollinators? *Evolution* 43: 870-881.
- HARDER, L. D. 1990. Behavioral responses by bumble bees to variation in pollen availability. *Oecologia (Berl)* 85: 41-47.
- HAYNES, J., AND M. MESLER. 1984. Pollen foraging by bumblebees: foraging patterns and efficiency on *Lupinus polyphyllus*. *Oecologia (Berl)* 61: 249-253.
- HEINRICH, B. 1979. Bumblebee economics. Harvard University Press, Cambridge, MA.
- HODGES, C. M., AND R. B. MILLER. 1981. Pollinator flight directionality and the assessment of pollen returns. *Oecologia (Berl)* 50: 376-379.
- KREBS, J. R., AND R. H. MCCLEERY. 1984. Optimization in behavioural ecology, pp. 91-121 in J. R. Krebs and N. B. Davies (eds.). Behavioural ecology: an evolutionary approach. Blackwell Scientific Publications, London.
- MICHENER, C. D. 1962. An interesting method of pollen collecting by bees from flowers with tubular anthers. *Rev. Biol. Trop.* 10: 167-175.
- NEFF, J. L., AND B. B. SIMPSON. 1990. The roles of phenology and reward structure in the pollination biology of wild sunflower (*Helianthus annuus* L., Asteraceae). *Israel. J. Bot.* 39: 197-216.
- PELLMYR, O. 1988. Bumble bees (Hymenoptera: Apidae) assess pollen availability in *Anemonopsis macrophylla* (Ranunculaceae) through floral shape. *Ann. Entomol. Soc. America* 81: 792-797.
- PYKE, G. H. 1979. Optimal foraging in bumblebees: rules of movement between flowers within inflorescences. *Anim. Behav.* 27: 1167-1181.
- RASHEED, S. A., AND L. D. HARDER. 1997. Foraging currencies for non-energetic resources: pollen collection by bumblebees. *Anim. Behav.* 54: 911-926.
- WADDINGTON, K. D., AND B. HEINRICH. 1979. The foraging movements of bumblebees on vertical "inflorescences": an experimental analysis. *J. Comp. Physiol.* 134: 113-117.
- ZAR, J. H. 1996. Biostatistical analysis. Prentice-Hall, Upper Saddle River, New Jersey.