Anim. Behav., 1993, **45**, 37–41

Effects of nectar variance on learning by bumble bees

REUVEN DUKAS*‡ & LESLIE A. REAL†
*Department of Zoology, North Carolina State University, Raleigh, NC 27695-7617 U.S.A.
†Department of Biology, Indiana University, Bloomington, IN 47405, U.S.A.

(Received 22 November 1991; initial acceptance 30 January 1992; final acceptance 17 March 1992; MS. number: A6226)

Abstract. The variation in standing crops of nectar among flowers within a species is usually very high. This nectar variance may decrease a forager's ability to estimate the mean reward offered by alternative plant species. Consequently, learning performance under high nectar variance may be reduced. To examine this prediction, bumble bees were tested for their abilities to learn to discriminate rewarding from non-rewarding flowers under four levels of nectar variance. The learning rate of bumble bees was significantly lower with nectar variance than with no variance. However, learning rates did not differ among the three levels of nectar variance. The results suggest that under increasing nectar variance bees do not increase sampling period, but instead base their foraging decisions on the less reliable information from a certain maximum number of flowers sampled.

The standing crop of nectar varies considerably among flowers within a species as a result of variation in the rate of nectar production and the movement patterns of foraging nectarivores (Teuber & Barnes 1979; Zimmerman 1981, 1983; Pleasants & Zimmerman 1983; Southwick & Southwick, 1983; Real & Rathcke 1988; Cresswell 1990; Waser & Mitchel 1990). Bees and other animals are often sensitive to variance in reward distributions (reviewed by Real & Caraco 1986; Stephens & Krebs 1986); however, it is unclear how reward variance may affect the learning performance of foraging animals.

Variation in nectar means that bees encounter different nectar volumes in flowers within a species, from no nectar at all to relatively large nectar volumes per flower. Bees must estimate the means of nectar rewards offered by different floral types subject to this natural level of variation and uncertainty (Real 1981, 1991; Cartar 1991). Higher nectar variance may be associated with lower learning performance, because of the increasing difficulty of estimating the mean reward offered. The larger the variance, the greater the sample size required to accurately estimate the mean. Consequently, the learning task bees encounter under natural conditions may be substantially different from most learning studies on bees, where flowers of different types contain a fixed amount of nectar (e.g. Menzel et al. 1974; Heinrich et al. 1977; Couvillon & Bitterman 1980; Gould 1987). To ascertain the effect of resource variability on learning, we examined the abilities of bumble bees, *Bombus bimaculatus* Cresson, to learn to discriminate rewarding from non-rewarding flowers under four levels of nectar variance.

METHODS

Our experiments were conducted in a 120 × 120 × 18 cm wood enclosure placed indoors. The top of the enclosure consisted of a sheet of transparent Plexiglas, and the floor was pegboard with 0·5-cm holes, 2·5 cm apart. From 2116 possible positions we assigned random coordinates for 100 flowers. We placed a 4-cm plastic tube (0·5 cm in diameter; closed at its base) in each of the chosen coordinates. Every morning we filled the tubes with water and placed in each a fresh emasculated flower of *Abelia floribunda* (Caprifoliaceae). Flowers were washed and drained with a thin micropipette. Flowers did not secrete a measurable amount of nectar during the day. We dispensed either 30% sugar water (hereafter nectar) or 5 μl water into flowers using Hamilton syringes mounted in repeating dispensers. In each experiment we used two types of flowers: unchanged white flowers, and flowers painted yellow on two of the five petals (hereafter yellow flowers). One floral type contained nectar

‡Present address: Department of Mathematics, University of British Columbia, Vancouver, B.C., V6T 1Z2 Canada.

0003–3472/93/010037+05 $08.00/0

Table I. Nectar distribution of the rewarding floral type in the experiments with four variances*

Nectar variance (μl^2)	Number of nectar-filled flowers	Nectar volume† per flower (μl)	Number of bees tested
0	50	1	13
1	25	2	9
2	17	3	10
4	10	5	10

*All other flowers of the rewarding type and all flowers of the non-rewarding type contained water.
†Mean nectar volume in the rewarding floral type was held constant at 1 μl.

and the other type was non-rewarding. What we wished to ascertain was how increasing variance in reward in the rewarding floral type affected the rate at which experimentally naive bees learn to discriminate the rewarding from the non-rewarding floral type.

During the summer of 1990 we caged worker bumble bees, *B. bimaculatus*, individually in 30 × 18 × 15 cm plastic cages. Wild bees were caught early in the morning and deprived of food for 2 h. An experimental session consisted of six to seven trials and lasted for 2 days. In each trial each bee was allowed to forage on 30 flowers. We worked with two to eight bees in a session. In previous experiments the behaviour of one bee was not affected by the behaviour of the previous bee in the enclosure (Dukas & Real 1991). Sugar intake of bees (the major determinant of hunger level) was determined in earlier experiments (Dukas & Real 1991) and held constant across experimental sessions.

Learning Experiments

To increase nectar variance, we decreased the number of nectar-filled flowers of the rewarding floral type and increased the volume of nectar in each (Table I). We conducted experiments with nectar variances of either 0, 1, 2 or 4 μl^2, with corresponding coefficients of variation (CV) of 0, 100, 140 and 200%. All remaining flowers of the rewarding type contained 5 μl water each. The mean nectar volume in the rewarding floral type was held constant at 1 μl. All flowers of the non-rewarding type contained 5 μl of water. Bees rejected and left water-filled flowers immediately after probing.

In each experiment, equal numbers of each of the two floral types (rewarding or non-rewarding) were randomly distributed in the enclosure. In the first trial of all experiments, both floral types contained 1 μl of nectar, and bees were tested for any initial preference for either of the two types. In subsequent trials of each experiment, the nectar distribution in the 50 flowers of the rewarding type differed according to experiment (Table I). In all experiments, the 50 flowers of the non-rewarding type contained 5 μl water per flower. In each trial, we allowed one bee at a time to forage on 30 flowers in the enclosure and monitored her number of visits to flowers of the rewarding type. The nectar-filled flowers that were visited were then refilled with nectar, and a subsequent bee was introduced into the enclosure. A trial, therefore, consisted of 30 visits by each bee used in this particular experimental session. Every other trial, we changed the positions of nectar-filled flowers of the rewarding type, so bees could not learn their location. The experimental session was terminated when bees visited more than 85% of the flowers of the rewarding type; this occurred after five to six learning trials. Bees were then marked with a model paint and released.

We repeated each experimental design in two separate series. In the first series, the rewarding floral type was white and the non-rewarding was yellow. In the second series, the rewarding type was yellow and the non-rewarding type was white.

Comparison of Learning Rates

To compare learning rates of bees among the four experiments, we used a non-linear learning model

$$N = 30 - 15 \times e^{-\delta v}$$

where N is the number of bee visits to flowers of the rewarding type per trial, 30 is the total number of bee visits per trial, 15 is the expected number of visits to flowers of the rewarding type per trial prior to learning, δ is the learning rate, and v is the cumulative total number of bee visits to flowers. This model described bee learning in our previous experiments (Dukas & Real 1991). We used non-linear regression analysis (NLIN; SAS Institute 1985) to estimate learning rates (δ). F-values for tests of significance among learning rates were calculated from the corresponding partial sums of squares.

RESULTS

Floral Choice and Revisitation

In the first trial of each experiment, we compared the number of visits to flowers of the rewarding and non-rewarding types. Floral preference of bees did not differ significantly between the four experiments in each of the two series (Kruskal–Wallis test, $df=3$, $P>0{\cdot}1$ for both series). Bees apparently did not distinguish between nectar-filled and water-filled flowers of the rewarding type before probing. This is because the proportion of visits to nectar-filled flowers equalled the proportion of these flowers in the enclosure ($\chi^2=1{\cdot}1$, $df=1$, $P>0{\cdot}25$ for variance $= 1\ \mu l^2$; $\chi^2=0{\cdot}16$, $df=1$, $P>0{\cdot}5$ for variance $=2\ \mu l^2$; $\chi^2=0{\cdot}08$, $df=1$, $P>0{\cdot}75$ for variance $=4\ \mu l^2$). This result suggests that bumble bees did not have remote perception of flower quality in our experiments.

Revisitation of bees to flowers of the rewarding type did not differ significantly between experiments ($F=2{\cdot}75$, $df=3, 34$, $P=0{\cdot}06$). The mean ($\pm$SE) percentage of revisitation was $13\pm3{\cdot}8\%$ ($N=13$ bees), $16{\cdot}3\pm3{\cdot}2\%$ ($N=9$), $17\pm2{\cdot}7\%$ ($N=10$) and $13{\cdot}7\pm5{\cdot}6\%$ ($N=10$) in experiments 1, 2, 3 and 4, respectively.

The Effect of Nectar Variance on Learning Rates

The bees showed a significant decrease in the rate of learning to visit flowers of the rewarding type between zero and non-zero variance experiments ($F=15{\cdot}2$, $df=1, 259$, $P<0{\cdot}001$ for the difference between experiment 1 and experiments 2, 3 and 4; results of ANOVA test show similar difference; $F=8{\cdot}8$, $df=1, 249$, $P<0{\cdot}005$; Figs 1 and 2). However, the learning rates of bees were not significantly different among variances 2, 3 and 4 μl^2 ($F=0{\cdot}17$, $df=2, 183$, $P>0{\cdot}5$; Figs 1 and 2). Learning rates did not differ significantly between series 1 and 2 in each experiment ($F=0{\cdot}03$, $df=1, 260$, $P>0{\cdot}5$).

DISCUSSION

The Effect of Nectar Variance on Learning Rates

Variation in nectar reward was associated with a lower rate of learning by bumble bees, suggesting that nectar variance may reduce the ability of bees to estimate the mean reward offered by flowers. We only varied nectar volumes in one floral type in order to isolate the effects of nectar variance on learning from its effects on floral choice resulting from risk-averse behaviour (Real & Caraco 1986; Stephens & Krebs 1986; Cartar 1991; Real 1991). Learning rates of bees might be even lower under field conditions, where several floral types offer varying nectar quantities. As a result, bees may have to visit a larger number of flowers of different species before making foraging decisions. This difficulty of learning about the quality of alternative nectar sources may significantly decrease the value of frequent sampling by bumble bees.

The standing crop of nectar in flowers also varies over time (Real & Rathcke 1988; Cresswell 1990), additionally reducing the ability of bees and other nectarivores to learn about the quality of alternative floral types. Temporal variation in nectar standing crops may further reduce the value of the information learned, because the characterization of nectar standing crops is bound to be short-lived (Stephens & Krebs 1986). The attendant difficulties in learning due to spatial and temporal variation, coupled with the limited value of the information learned, may partially explain bees' tendencies to restrict visits to only one or a few floral species after an initial sampling period (Heinrich 1976, 1979). In addition, learning and memory constraints may affect the abilities of bees to forage simultaneously on different floral species (Lewis 1986; Waser 1986; Dukas & Real, in press, unpublished data).

Although bees learned more slowly when there was variance in nectar than when there was no variance, learning rates did not significantly decrease with an increase in nectar variance. When foraging from flowers with higher nectar variances, bees must process information by one of the following alterna-

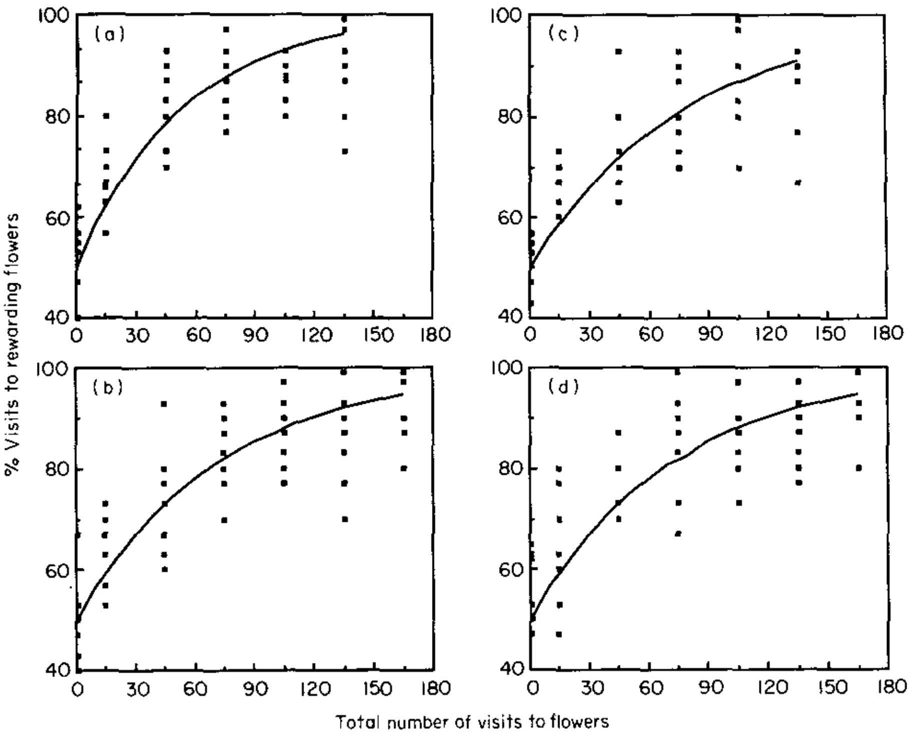

Figure 1. Changes in the proportions of visits to rewarding flowers by bumble bees with experience. (a) Nectar variance = 0 μl², (b) variance = 1 μl², (c) variance = 2 μl², and (d) variance = 4 μl². Each data point presents the proportion of bee visits to rewarding flowers in a trial of 30 visits; some overlapping data points are hidden.

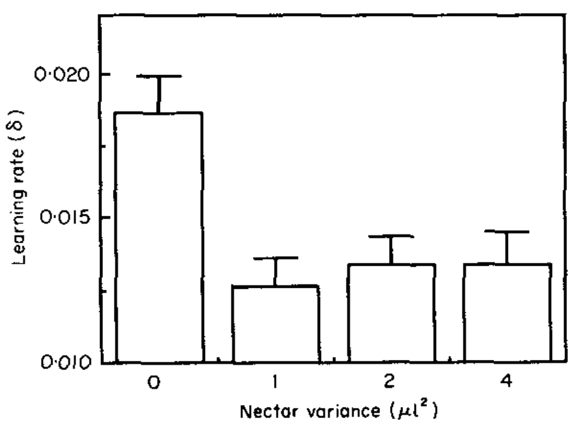

Figure 2. Comparison of mean (±SE) learning rates of bumble bees in the experiments with four nectar variances. Learning rates are δ-values from the model $N = 30 - 15 \times e^{-\delta v}$. The learning rate δ was significantly greater in the zero variance compared with the non-zero variance experiments ($F = 15{\cdot}2$, $df = 1, 259$, $P < 0{\cdot}001$).

tives, or a combination of both: (1) integrate information about nectar volumes from a larger number of flowers, or (2) sample a certain maximum number of flowers before choosing between alternative floral types. While longer sampling period is associated with lower learning rate, sampling flowers up to a certain limit may be associated with making the wrong floral choice, i.e. bees may choose the floral type that offers less mean reward, as a result of the limited and highly variable information obtained. Bumble bees probably have an upper limit to the amount of information they can remember and integrate successfully (Real et al. 1990; Real 1991). Therefore, increasing nectar variance may be associated with an increase in wrong floral choices instead of reduced learning rate. Such mistaken floral choices may occur under natural settings, but did not occur in our experiment when one of the two floral types was always non-rewarding. It seems therefore, that in order to further explore and quantify the effects of increasing nectar variance on learning performance, one must present bees with two or more variable alternatives, while carefully separating behaviour related to learning from behaviour resulting from risk sensitivity.

ACKNOWLEDGMENTS

We thank J. R. Walters, S. Ellner, L. Harder, E. D. Seaman and an anonymous referee for comments on the manuscript. This study was supported in part by Sigma Xi grant to R.D. and NSF grants BNS 8719292 and BNS 8900292 to L.A.R.

REFERENCES

Cartar, R. V. 1991. A test of risk-sensitive foraging in wild bumble bees. *Ecology*, **72**, 888–895.

Couvillon, P. A. & Bitterman, M. E. 1980. Some phenomena of associative learning in honeybees. *J. comp. physiol. Psychol.*, **94**, 878–885.

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.

Dukas, R. & Real, L. A. 1991. Learning foraging tasks by bees: a comparison between solitary and social species. *Anim. Behav.*, **42**, 269–276.

Dukas, R. & Real, A. L. In press. Cognition in bees: from stimulus reception to behavioral change. In: *Insect Learning: Ecological and Evolutionary Perspectives* (Ed. by D. Papaj & A. Lewis) London: Chapman & Hall.

Gould, J. L. 1987. The role of learning in honey bee foraging. In *Foraging Behaviour* (Ed. by A. C. Kamil, J. R. Krebs & H. R. Pulliam), pp. 479–496. New York: Plenum Press.

Heinrich, B. 1976. Foraging specialization of individual bumblebees. *Ecol. Monogr.*, **46**, 105–128.

Heinrich, B. 1979. Majoring and Minoring by foraging bumblebees; *Bombus vagance*: an experimental study. *Ecology*, **60**, 245–255.

Heinrich, B., Mudge, P. R. & Deringis, P. G. 1977. Laboratory analysis of flower constancy in foraging bumblebees: *Bombus ternarius* and *Bombus terricola*. *Behav. Ecol. Sociobiol.*, **2**, 247–265.

Lewis, C. A. 1986. Memory constraints and flower choice in *Pieris rapae*. *Science*, **232**, 863–865.

Menzel, R., Erber, J. & Masuhr, T. 1974. Learning and memory in the honeybee. In: *Experimental Analysis of Insect Behaviour* (Ed. by L. Barton-Browne), pp. 195–217. Berlin: Springer-Verlag.

Pleasants, J. M. & Zimmerman, M. 1983. The distribution of standing crop of nectar: what does it really tell us? *Oecologia (Berl.)*, **57**, 412–414.

Real, L. 1981. Uncertainty and pollinator-plant interactions: The foraging behavior of bees and wasps on artificial flowers. *Ecology*, **62**, 20–26.

Real, L. 1991. Animal choice behavior and the evolution of cognitive architecture. *Science*, **253**, 980–986.

Real, L. & Caraco, T. 1986. Risk and foraging in stochastic environments. *A. Rev. Ecol. Syst.*, **17**, 371–390.

Real, L., Ellner, S. & Harder, L. D. 1990. Short-term energy maximization and risk-aversion in bumble bees: a reply to Possingham et al. *Ecology*, **71**, 1625–1628.

Real, L. & Rathcke, B. J. 1988. Patterns of individual variability in floral resources. *Ecology*, **69**, 728–735.

SAS Institute. 1985. *SAS User Guide*. Cary, North Carolina: SAS Institute, Inc.

Southwick, A. K. & Southwick, E. E. 1983. Aging effect on nectar production in two clones of common milkweed, *Asclepias syriaca*. *Oecologia (Berl.)*, **56**, 121–125.

Stephens, D. W. & Krebs, J. R. 1986. *Foraging Theory*. Princeton, New Jersey: Princeton University Press.

Teuber, L. R. & Barnes, D. K. 1979. Environmental and genetic influences on quantity and quality of alfalfa nectar. *Crop Sci.*, **19**, 874–878.

Waser, N. M. 1986. Flower constancy: definition, cause, and measurement. *Am. Nat.*, **127**, 593–603.

Waser, N. M. & Mitchell, R. J. 1990. Nectar standing crops in *Delphinium nelsonii* flowers: spatial autocorrelation among plants? *Ecology*, **71**, 116–123.

Zimmerman, M. 1981. Patchiness in the dispersion of nectar resources: probable causes. *Oecologia (Berl.)*, **49**, 154–157.

Zimmerman, M. 1983. Plant reproduction and optimal foraging: environmental nectar manipulation in *Delphinium nelsonii*. *Oikos*, **41**, 57–63.