Ethology

Flower Choice and Learning in Foraging Bumblebees: Effects of Variation in Nectar Volume and Concentration

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Abstract

Bees collect food from flowers that differ in morphology, color, and scent. Nectar-seeking foragers can rapidly associate a flower's cues with its profitability, measured as caloric value or 'net energy gain,' and generally develop preferences for more profitable species. If two flower types are equally easy to discover and feed from, differences in profitability will arise from differences in the volume or the sugar concentration of their nectar crops. Although there has been much study of how bees respond to one or the other of these two kinds of nectar variation, few studies have considered both at once. We presented free-foraging bumblebees with two different types of equally rewarding artificial flowers. After a period of familiarization, we made one type more rewarding than the other by increasing its nectar concentration, volume, or both. Bees responded more rapidly to a change in the reward's sugar concentration than to a change in its volume, even if the profitability differences were approximately equal. Sucrose concentration differences (40% vs. 13%) caused bees to virtually abandon the more dilute flower type, whether both types offered the same volume (2 μl) or the less concentrated reward offered higher volume (7 μl vs. 0.85 μl). When the two types of flower differed only in nectar volume (7 μl vs. 0.85 μl), the less rewarding type continued to receive 22% of the visits. We propose three different hypotheses to explain the stronger response of the bees to changes in sugar concentration: (i) their response threshold to sucrose concentration might change; (ii) less time is needed to assess the concentration of a reward than its volume; and (iii) a smaller sample size may be needed for reliable estimation of profitability when flowers differ in concentration.

Introduction

Many flower visitors, including bumblebees, interact with a diversity of host plants. Proficiency at handling several floral types requires considerable learning ability (reviews by Papaj & Lewis 1993; Menzel 2001). These bees rapidly learn which of several flower species is more profitable and specialize on the more profitable ones. They learn to associate a reward with a flower's color, pattern, shape, odor, or microtexture. Honeybees memorize the location of a profitable food source and the time of day when the reward is available. Honeybees and bumblebees also possess an impressive capacity to learn how to find and extract rewards from flowers of radically different morphologies (Laverty 1994).

External factors involved in associative learning can be categorized by the properties of the cues associated with the reward (i.e., the conditioned stimuli) and the properties of the reward (i.e., the unconditioned stimuli). Research on bee learning has focused more on cues than on rewards (Greggers & Mauelshagen 1997; Scheiner et al. 1999). Given that the nectar and pollen rewards form the basis for the angiosperm–pollinator mutualism, and nectar/pollen economics directly affects the reproductive success of both interactants, this lack is surprising.

The two properties of the nectar reward that are usually addressed in relation to bee learning and floral choice are nectar concentration and nectar volume. Bees prefer to forage on floral types associated with more concentrated nectar (Bitterman 1976; Whitham 1977; Hodges & Wolf 1981; Loo & Bitterman 1992; Hill et al. 2001). Scheiner et al. (1999, 2001) showed that a honeybee's response to sugar concentration in nectar rewards is linked to learning and memory. The honeybee's sensitivity to sugar (which is assayed by a 'response threshold' equal to the lowest sugar concentration that evokes proboscis extension), has a striking effect on both the acquisition and extinction of conditioned tactile stimuli. Bees with lower thresholds (i.e., higher sensitivity), acquire a conditioned response faster; when the experience is terminated, they are slower to extinguish the response. Recent experience affects perception of sugar concentration (Pankiw et al. 2001).

Bees can also prefer flowers that offer higher nectar volumes when flower-handling cost is controlled (Waddington & Gottlieb 1990), but contradictory results are sometimes obtained. For example, Menzel & Erber (1972) reported that appetitive conditioning was independent of the volume of reward. In contrast, others have found significant effects of nectar volume on learning. Depending on the training protocol and the cues used, greater nectar volumes were associated either with faster acquisition (i.e., greater 'associative strength') or similar acquisition but greater asymptotic response (i.e., a learning curve with a higher asymptote) (Buchanan & Bitterman 1988; Lee & Bitterman 1990; Couvillon et al. 1991; Couvillon & Bitterman 1993).

In the field, when bees choose between two food sources, both will usually be rewarding, but the expected value of the reward will typically differ [either in caloric value or expected net energy gain (NEG)]. Through sampling, bees assess the reward and associate this value with some cue that identifies the plant. Harder & Real (1987) showed that bumblebees reacted to variation in nectar volume in ways that yielded higher NEG, for which those authors derived an equation. Nectar reward value is a function of both concentration and volume, but surprisingly, we were able to find only one research program that systematically altered the concentration and volume of a reward in a common setting. Bateson et al. (2002, 2003) used such a design to study decision-making in hummingbirds, but their experiments were designed to test whether the birds assign an absolute or relative value to the alternative options, rather than to understand the learning process.

We sought to characterize the ability of bees to differentiate among flower types that differed in nectar concentration or volume. Specifically, we presented foraging bumblebees with two types of equally rewarding flowers. After an initial familiarization period, we changed the characteristics of the two types by making one flower type better than the other, either by changing sugar concentration, or nectar volume, or both. Bees typically shifted their visits to the more profitable type; we quantified the speed and extent of the shift by fitting a sigmoid response curve. Although our principal interest was in the speed with which bees reacted to different sorts of variation, our experiments also address whether Harder & Real's (1987) findings about variation in nectar volume can be extended to variation in nectar concentration. When both aspects of nectar quality vary, are bees' choices still determined by NEG?

Materials and Methods

Bees

A colony of *Bombus impatiens* (Cresson) containing about 30 workers (BioBest Canada Ltd, Leamington, ON, Canada) was maintained in the laboratory in its original nest box (29 × 21 × 14 cm) at room temperature. It was supplied with pollen collected from honeybee colonies. A tube connected the colony to a flight cage (2 m × 3 m × 2 m) where the experiments were conducted. To maintain a consistent need for nectar foraging, we kept the colony in a sugar-limited state by supplying sucrose solution from two feeders, similar in their shape to the electronic feeders described below, located in the flight cage. These feeders each contained 12 ml of 20% nectar (g sucrose/g water), and were made available after each day's experiments were completed. If all honey-pots in the hive were empty the next morning, more nectar was added to the honey-pots using a syringe. On the contrary, if many honey-pots in the hive were full, no nectar was provided in the cage.

The Flowers

We constructed automatically dispensing artificial flowers, following Keasar et al. (1996) with some modifications (Fig. 1). Each flower comprised an acrylic plastic disk (landing surface) with a hole drilled in its center, covering a cylinder that held approx. 10 ml of nectar (sugar water). A cork with a magnetic collar floated on the nectar. The bee had access only to a small cup mounted on top of the cork. This cup was replenished by activating an electromagnet, which pulled the cup down below the surface of the nectar. After the electromagnet was deactivated, the nectar-filled cup returned to its original position, a process that took 3 s to complete. During the experiments, cups that could hold three different volumes of nectar were used (0.85, 2, and 7 μl). The artificial flowers were controlled by a computer running two different software programs. One program recorded the time of probing and the specific flower that was visited. The second program specified the interval between refilling (IR) of each individual flower. At the specified IR, the magnet of each flower was activated. Flowers that had been emptied refilled themselves, whereas those that were full remained full. Therefore, refilling was unrelated to whether visitation had occurred.

Sixteen flowers were organized in a 4 × 4 square grid, 25 cm apart, on a white table. All flowers had identical yellow disks. They were divided into two groups of eight, marked by different scents to facilitate discrimination by the bee. The scent was applied to the flowers' disks using a glass microcapillary. Flowers of the first type (rows 1 and 3) were marked by 2 μl of geranium oil (G) diluted 1:200 in pentane (after Kunze & Gumbert 2001). Flowers of the other type (rows 2 and 4) were marked by the same dilution of rose oil (R).

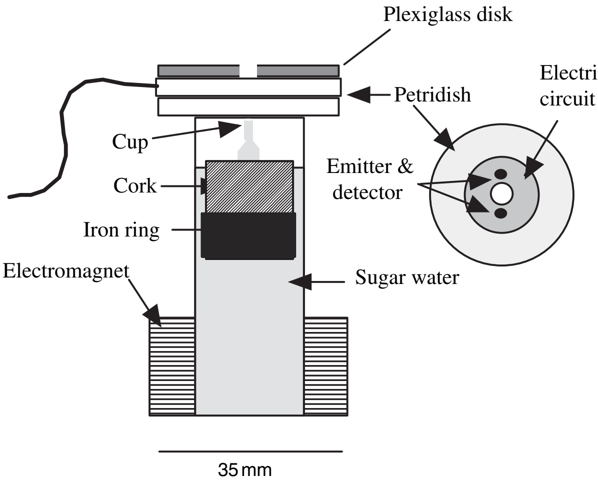

Fig. 1: Schematic drawing of the feeder used in the experiment. Left: side view; right: inner view of the Petri dish

The Paradigm and Data Analysis

Our two-phase experiments followed a paradigm described by Cnaani et al. (2003). During phase 1 (training), a single naive bee was allowed to make 150–190 individual flower visits (three to five foraging bouts) to flowers that were equally rewarding. After training was completed, the reward in each flower type was changed and the same bee allowed another 420 visits (phase 2 – the testing). The behavioral response of each bee was analyzed individually by breaking the sequence of choices into 14 blocks of 30 consecutive visits, and calculating the proportion of visits to each flower type within each block. The testing phase was long enough for bees to switch from their initial proportion (first asymptote) and equilibrate at a different proportion (second asymptote). Changes in these proportions over time were summarized by fitting a four-parameter nonlinear sigmoid regression (Eqn 1, Fig. 2),

$$Y = Y_0 + \frac{a}{1 + e^{-(X - X_0/b)}} \tag{1}$$

where 'a' is the amplitude (the distance between the two asymptotes), 'b' the rate of transition between the two asymptotes, X_0 the inflection point, where 50% of the transitions between the two asymptotes have occurred and Y_0 the second asymptote. Before curve-fitting, we set the value of the first asymptote equal to the average proportion of visits to geranium-scented flowers during the training phase. This step avoids the effects of small changes in the proportion of visits during the training phase on X_0 and b parameters.

Experimental Procedure

Between experiments, the 16 artificial flowers were hidden by a paper cover, and two feeders (similar in their shape to the artificial flower but offering full access to the nectar) were available. Each morning, all bees found in the flight cage were returned to the hive, the 16 artificial flowers uncovered, and the two feeding flowers removed. We equipped all flowers with 2-μl cups, loaded them with 20% sucrose solution, and allowed bees to enter. When one or more had begun to forage, and the first forager

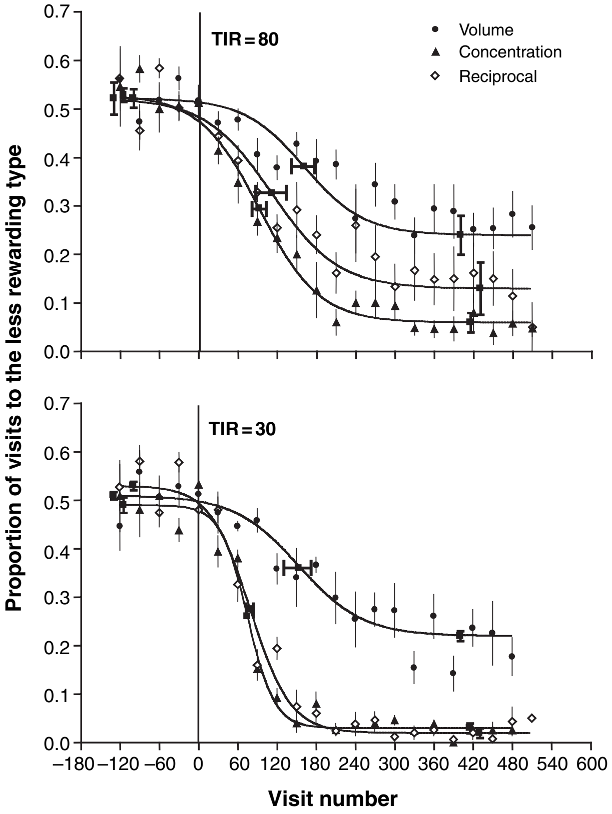

Fig. 2: Change in the proportion of visits to the geranium-scented flowers over 570 visits. In the first 150 visits (number − 150 to 0), the two types of flowers were equally rewarding, with a IR of 160 s. At the second phase of the experiment, IR was changed to 80 s (upper chart) or 30 s (lower chart). To build these graphs, the proportion of visits of each bee to the geranium flowers was calculated for each set of 30 visits, and was analyzed by a four-parameter sigmoid regression. We than calculated the average (±SE) value for each of the parameters in each treatment (type of change) and IR. These average values of the parameters were used to construct the curves. The mean value of each parameter and its standard error are presented by a black square with error bars (Y_0 on the right, a–Y_0 on the left, X_0, the inflection point in the middle). The mean (±SE) proportion of visits to the less rewarding type in each set of 30 visits is also shown

returned to the hive, we returned all workers in the flight cage to the hive. The flowers were then scent-marked G or R. The IR was set to 160 s for both types of flowers. Because the average time between two successive flower visits was about 7 s, an IR of 160 s meant that bees would find a reward in about 70% of random flower visits. Phase 1 of the experiment began when the test bee left the hive. The bee was allowed to perform at least 150 visits, which required three to five foraging bouts. After its last foraging bout in phase 1, while the bee was inside the hive, one of three possible changes in the reward was made (see also Table 1):

Table 1: Summary of reward properties in each flower type

Group	Scent	Volume (μl)	Concentration (%)	Energy content (J)	Expected NEG (J/s)
Phase I					
A	Rose	2	20	6.67	0.99
B	Geranium	2	20	6.67	0.99
Phase II					
Volume change					
A	Rose	0.85	20	2.83	0.54
B	Geranium	7	20	23.34	2.55
Concentration change					
A	Rose	2	13	4.21	0.61
B	Geranium	2	40	14.51	2.2
Reciprocal change					
A	Rose	7	13	14.75	1.6
B	Geranium	0.85	40	6.17	1.2

A summary of reward properties for each flower type (scented with rose or geranium) and each phase of the experiment. 'Energy content' presents the energetic value of the sugar available in a flower with specific nectar volume and concentration. The corresponding expected net energy gain (NEG) for a bee that will concentrate on this type of flower only, is presented in the right most column. NEG was calculated according to Harder & Real (1987). Values of body mass, flight time and handling time (needed to calculate the expected NEG) were taken from this study. All other data were taken from Harder & Real (1987).

1 Volume change: Replacing the 2-μl cup with a 7-μl cup (rose) or a 0.85-μl cup (geranium).
2 Concentration change: Replacing the 20% nectar by 40% (rose) and 13% (geranium).
3 Reciprocal change: Setting the 'rose' flower to offer 0.85 μl of 40% nectar, and the 'geranium' flowers to offer 7 μl of 13% nectar.

The IR was also changed, and set to be either 80 s (in early trials) or 30 s (in later trials). The decrease in IR was meant to 'compensate' a bee that specialized on the more rewarding type, by still allowing it to encounter reward at roughly the same rate as in the training phase, even though it was visiting half as many flowers. Obviously, any such compensation would be approximate, because the realized encounter rate depends on the bee's behavior. The change from 80 to 30 s was made to provide better compensation (see below).

To calculate the NEG for flowers with different volume and concentration combinations (Table 1), we used the equation of Harder & Real (1987). Our specific values of concentration and volume were chosen because they are in the range of nectar volumes found in the field (Petanidou & Smets 1995); they are within the range over which NEG for the bee is approximately a linear function of nectar volume (Harder & Real 1987); and the concentrations are low enough to not affect ingestion rate (Harder 1986). The specific values in phase 2 of the experiment were originally designed so that the net value of the augmented flowers would be doubled, compared with the flowers in phase 1, and the value of the diminished flowers would be halved. If this had worked as planned, the high-concentration flowers and the high-volume flowers would have been equal in value, as would the low-volume and low-concentration flowers. This would have permitted direct comparisons across treatments. However, it became apparent to us that augmented volumes slowed foraging rates because longer handling times were required to consume the nectar. The slower pace of these bees meant that most of the flowers had refilled between visits, so these bees seldom encountered empties. In contrast, augmented concentrations allowed speedier foraging, and the bees in those treatments continued to encounter empty flowers in 30–50% of visits.

These differences complicate comparisons of learning rates across treatments. For simplicity, suppose that learning the difference between two differently rewarding types of flowers requires a bee to experience 'n' rewarding visits to each type. If all visits are rewarding, as in the volume change experiments, the bee will need about '2n' visits to learn the difference between the two types. If only half of the visits are rewarding, as in the 'concentration change' experiments, it will take closer to '4n' visits. These likely differences in learning rate result from different rates of encountering reward and not from differences in reward properties. Setting IR to 30 s, which was about the shortest obtainable with the apparatus, was aimed at minimizing this problem; it allowed bees that visited flowers with small volumes of nectar (in the 'concentration' and 'reciprocal' experiments) to encounter rewards at almost every visit. In practice, changing the IR from 80 to 30 s had only a marginal (technically non-significant) effect on learning parameters (see Results).

Our estimates of NEG for the volume and concentration experiments (Table 1) show that both of these manipulations did produce roughly similar contrasts in NEG during phase 2, although the ratio of the better to the worse flower type was greater in the volume treatment (2.55/0.54 = 4.7) than in the concentration treatment (2.20/0.61 = 3.6). In the Reciprocal treatment, which was intended to produce equal NEGs by trading off volume and concentration, the high-volume/low-concentration flowers yielded a 1.33-fold greater NEG than the low-volume/high-concentration ones did.

To minimize effects of past experience or motivational status, we used only those bees that started to forage spontaneously, and we used each bee only once. The acrylic disks on top of the artificial flowers were replaced by clean disks at the beginning of each experiment, to eliminate any scent marks left by the previous bee.

Learning parameters were estimated by the nonlinear fitting routine in the Origin graphics package (OriginLab Corp. 2002). We assessed the effects of nectar treatment (volume, concentration, or reciprocal) and interval between refilling (80 or 30 s) on the fitted learning parameters in a factorial analysis of variance computed by SAS PROC GLM, using type III sums of squares, followed by Tukey's Studentized range (HSD) tests for pairwise comparisons of treatments (SAS Institute, Inc. 1999). For phase 1, preferences for geranium or rose were made by ordinary ANOVA on the proportions of visits to each type.

Results

During phase 1, bees did not discriminate between the two scents. The mean proportions of visits to the geranium-scented flowers were 0.52 ± 0.04, 0.51 ± 0.03, and 0.52 ± 0.03 for the volume change, concentration change, and the reciprocal change experiments, respectively (ANOVA, $F_{2,28} = 0.22$, $p = 0.8$).

As expected, during phase 2, bees reacted to each of the three changes in the reward by reducing the proportion of visits they made to the less-rewarding flower type (Fig. 2). The rates of learning varied with treatment, however (Table 2). Taking the four parameters in turn, there was no effect of nectar treatment or IR on 'b', the transition rate. Both the strength of the learning response (second asymptote), and the speed of response (infection point, X_0) were affected by the nectar treatment, however. None of the interactions involving nectar treatment and refilling rate were significant. The refilling rate IR by itself had no effect on X_0, but a marginal effect on Y_0 ($p = 0.0625$). [If the model is re-computed after dropping the insignificant interaction terms, the effect of IR on Y_0 increases to the cusp of conventional statistical significance ($p = 0.051$). We will not dwell further on this possible effect of variation in IR because we did not set out to study it and because the pattern is too weak to support speculation, but we note it as an effect to be considered in further work with flowers of this design.]

Returning to the clearly significant effects of nectar treatment on the strength (Y_0) and speed (X_0) of bees' responses, the Tukey's tests showed that bees responded significantly faster and more strongly to the concentration and the reciprocal treatment than they did to the volume treatment, but that the concentration and reciprocal treatments did not differ from each other. Therefore, bees reacted more definitively to treatments that included concentration differences.

We do not present a separate analysis of the amplitude 'a' between the first and second asymptotes; because the first asymptote was the same (about 0.5, above) for all treatments, the amplitude is highly correlated with the second asymptote Y_0 and adds no further information.

Discussion

When a foraging bumblebee experiences a novel change in the concentrations of nectar in two flower types, it shifts to the better type faster (smaller inflection point, X_0) and more completely (higher preference asymptote, Y_0) than does a bee that experiences a change in nectar volume. The stronger response to concentration is particularly noteworthy because, in our experiments, the contrasts in NEG would suggest that bees ought to have been more responsive to volume, not less. Following a reciprocal change in which sugar concentration was higher in one type of flower while nectar volume was higher in the other type, foraging bees clearly preferred the flower type that has more concentrated nectar, even though this type was less profitable. Therefore, bees reacted more strongly to nectar concentration increases than to volume increases, even if energetic considerations suggest that the opposite should have been true.

The equation for NEG was derived by Harder & Real (1987) to explain why two flower types offering the same mean volume of nectar could differ in terms of the mean rate of net energy uptake by the bee. Harder and Real argued that an observed preference for flowers with smaller variance in nectar volume (but with the same mean value) arose because it provided a higher rate of NEG. The power of their model and one of its important advantages is that it introduced several more 'dimensions' into the decision making system of the bee than had previously been considered. They showed that the foraging bee not only considers the average caloric value of the reward, but also considers its time budget, body mass, and ingestion rate. In our study, the Harder and Real equation failed to predict the bumblebee choice. Harder and Real developed their model and tested it on bees that had to choose between flower types having different variance of nectar volume; they did not examine cases where nectar volume and nectar concentration both varied. Although some costs associated with different nectar concentration were incorporated into the model, it is possible that other costs were missing. An example could be the cost associated with getting rid of the excess water in the low-concentration nectar. Still another explanation for the failure of the model could relate to its assumption that bees are making objective and rational decisions. Alternatively, a bee's choice could be affected by its subjective perception of the reward, which is not formulated into the function (see Waddington & Gottlieb 1990; Bate-

Table 2: Learning parameters for the different nectar-property treatments

	Y_0 – second asymptote	b – transition rate	X_0 – inflection point
Mean ± SE			
Concentration 80 (n = 5)	0.058 ± 0.017	−45.66 ± 5.27	92.59 ± 10.05
Concentration 30 (n = 5)	0.033 ± 0.005	−20.59 ± 4.02	74.70 ± 1.26
Volume 80 (n = 4)	0.238 ± 0.038	−45.94 ± 26.62	160.95 ± 16.85
Volume 30 (n = 5)	0.225 ± 0.011	−49.11 ± 10.16	153.02 ± 25.51
Reciprocal 80 (n = 6)	0.135 ± 0.055	−50.68 ± 15.16	111.56 ± 26.41
Reciprocal 30 (n = 5)	0.023 ± 0.006	−30.68 ± 6.08	78.79 ± 8.55
Significance of effect (from GLM)			
Type of change (C/V/R)	$p < 0.0001$	$p = 0.548$	$p = 0.0013$
IR (30 or 80 s)	$p = 0.0625$	$p = 0.190$	$p = 0.200$
Interaction	$p = 0.241$	$p = 0.159$	$p = 0.167$

Mean (±SE) of the sigmoid regression parameters for each of the three different types of changes in nectar treatment and the two different IRs. The results of the two-factor analysis of variance (GLM) for type of change (volume, concentration or reciprocal) and IR (80/30) effect on each of the parameters are presented. For both of the significant treatment effects (bold), Tukey's HSD tests indicate that responses to volume were weaker than to concentration or reciprocal, but that concentration and reciprocal did not differ from each other.

son et al. 2002, 2003; Shafir et al. 2002; and further discussion below).

We propose two possible mechanistic explanations that may account for the different behavioral responses to concentration and volume changes. The first explanation proposes that 'actual' and 'perceived' differences in profitability are not the same. It is possible that after experiencing 40% in some of the flowers in the second phase, the bees perceive the much lower concentration of 13% as akin to plain water. If bees do not usually experience such dilute rewards in nature, selection may not have had an opportunity to enhance sensory discrimination at low concentrations (Waddington & Gottlieb 1990). It is possible, therefore, that the bees were underestimating the lower concentration of sugar despite the high volume of 7 μl. These differences in perception may explain the high preference for concentrated nectar in the reciprocal change experiment.

Another explanation concerns the 'timing' of information about nectar properties. Sugar concentration is assessed by chemoreceptors on the bee's glossa (Whitehead & Larsen 1976), so the sugar concentration is probably perceivable as soon as the glossa touches the nectar. Little is known about the mechanism by which bees assess nectar volume, however. Nectar is typically not visible (both in our experimental system and in many real flowers), so the bee will acquire information about nectar volume only after consuming the whole amount. Processing this information presumably requires an integration of sensory information (from either mechanoreceptors or chemoreceptors) and drinking duration (time). The slow acquisition of volume data may cause the bee to leave a flower with dilute nectar before consuming the whole amount of nectar and without having assessed the volume. Incomplete assessment of nectar volume may therefore explain why bees are less responsive to volume change. Moreover, in cases where volume and concentration both vary, a bee can immediately learn that a flower of type A has a higher sugar concentration than one of type B, but will learn that the amount of nectar in type B is higher only if it is 'patient' enough to drink all the nectar.

Stronger behavioral responses to concentration than to volume might also arise ultimately from differences in the statistical reliability of information about the relative profitability of flower types. In the field, individual bumblebee foragers may restrict their visits to one species of flower and occasionally sample others (termed 'major' and 'minors', respectively; Heinrich 1976; Chittka et al. 1999). Switching to a more rewarding species after such sampling can be beneficial, but sampling entails a risk of making an incorrect estimate. Therefore, bees may have been selected to pay more attention to more reliable cues. Nectar standing crops will be highly variable because of exploitation, so a good estimate of mean volume will require a large number of visits. In contrast, nectar concentration will be less affected by exploitation, and will typically be more stable (Shafir et al. 2003). Concentration may therefore be a more reliable indication of an important aspect of a flower's value. Field studies of concentration, volume, and foraging preferences of the bees are required to better understand the process of switching between flower species.

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