Original Article

Wild bumblebees use both absolute and relative evaluation when foraging

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Foraging theory assumes that animals assess value based on objective payoffs; however, animals often evaluate rewards comparatively, forming expectations based on recent experience. This form of evaluation may be particularly relevant for nectar foragers such as bumblebees, where individuals can visit thousands of flowers daily that vary in nectar quality. While many animals, including bees, demonstrate reference-based evaluation in experimental contexts, it is unclear whether this occurs in the wild. Here, we asked how daily experience with wildflower nectar influenced wild bumblebees' reward evaluation. We measured the daily nectar concentration of bee-visited wildflowers (*Penstemon* spp.), before presenting foragers with conspecific flowers filled with a range of artificial nectar concentrations. We recorded bees' acceptance of artificial nectar, the probability of subsequent visits to flowers on the same plant, and residence time. While bees had a minimum threshold of nectar acceptability that was unaffected by experience, when there was higher-concentration environmental nectar, they were less likely to accept lower-quality rewards on manipulated plants. Bees also visited more flowers and stayed longer on plants with higher-concentration nectar. This study shows evidence for both absolute and reference-based evaluation in wild bees and points towards differences between bees' behavior in lab- and wild-foraging contexts.

Key words: ***Bombus*, floral rewards, nectar, pollinators, reference-based evaluation, value perception.**

INTRODUCTION

Understanding how individuals evaluate food rewards is central to explaining how foraging decisions are made (Stephen and Krebs 1986; Real 1987). In nature, prey or food items can vary in their quality or quantity ("reward value"), and foragers assess these metrics to make foraging decisions. Most foraging models of decision-making consider a highly simplified environment in which the availability of different options is known to the forager and does not change over time (Evans et al. 2013; Fawcett et al. 2014). Assuming other factors (i.e., predation risk, time investment, etc.) are equal, foragers are expected to maximize some measure of energetic intake (Emlen 1966; MacArthur and Pianka 1966; Schoener 1971). This suggests that individuals assess resource values according to their absolute payoffs and should exhibit a threshold of acceptability (Charnov 1976; McNamara 1982). Once reward values fall below this threshold, foragers should move to another patch or explore novel food types (e.g., Latty and Beekman 2010; Thiel 2011; Townsend-Mehler et al. 2011; Townsend-Mehler and Dyer 2012).

In most natural environments, however, both the availability and quality of options fluctuate in time and space. As such, the ability to evaluate options relative to each other and continually update reward acceptability based on recent experience may be necessary to maximize energy intake (Krebs and Inman 1994; Fawcett et al. 2014; Dunlap and Stephens 2016). This type of reference-based evaluation appears to be taxonomically widespread and may play a large role in determining how foragers choose between options that differ in their payoffs (Bitterman 1976; Flaherty 1982; Couvillon and Bitterman 1984; Mustaca et al. 2000; Daniel et al. 2008; Oberhauser and Czaczkes 2018; Wendt and Czaczkes 2020). One consequence of this form of evaluation is that a given reward will either be accepted or rejected depending on an individual's recent foraging experience. For instance, if a forager encounters a series of highly rewarding options, they may perceive a medium-quality option as low quality, but if that same option was encountered after a series of low-quality options, it would be perceived as high quality (Fawcett et al. 2014).

Reference-based evaluation should be adaptive in environments where conditions change rapidly and unpredictably in a number of ways, making past experience important in informing expectations about the future (McNamara et al. 2013). This may be particularly relevant for generalist foragers, such as bumblebees (*Bombus* spp.) and honeybees (*Apis mellifera*). Each day, an individual bee can visit

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thousands of flowers of multiple species that vary in their floral signals, handling times, and reward payoffs (Heinrich 1976, 1979; Chittka et al. 1997). Even within a single plant species, bees must choose between plants (Wilson and Price 1977; Waser and Price 1981; Ishii 2006) and individual flowers on a plant (Pyke 1979; Waddington and Heinrich 1979; Thomson et al. 1982; Harder 1988). Honeybees and bumblebees also show floral constancy, where individuals restrict visits primarily to one plant species within or across foraging bouts (Heinrich 1979; Thomson 1981; Chittka et al. 1999). In doing so, foraging bees sample many flowers of the same species and develop flower- or stimulus-specific expectations about reward properties across visits (Boisvert et al. 2007; Hemingway and Muth 2022).

Evidence from laboratory studies on reward perception suggests that bees judge rewards subjectively, that is, in a reference-based manner. Bees show negative incentive contrast effects, or exaggerated responses of aversion or rejection, when there is a discrepancy between reward expectation and perceived value (Bitterman 1976; Couvillon and Bitterman 1984; Cibula and Zimmerman 1987; Wiegmann et al. 2003; Waldron et al. 2005; Gil et al. 2007; Biernaskie et al. 2009; Wiegmann and Smith 2009; Townsend-Mehler et al. 2011; Hemingway and Muth 2022; Solvi et al. 2022). Individuals may be particularly sensitive to inter-specific differences in reward quality: foraging bees that experienced a down-shift in reward quality rejected this "nectar" for longer when it was on a color previously paired with the higher-quality reward, than on a novel flower color (Hemingway and Muth 2022). While lab studies indicate that incentive contrast effects are reasonably robust, bees are typically tested under much simpler conditions than would be encountered in a field setting. As such, it is unclear whether wild-foraging bees form similar types of reward expectations when foraging in natural floral communities. By tracking daily changes in nectar quality in their environment and adjusting the acceptability of rewards accordingly, wild-foraging bees may be able to maximize their nutritional intake. Reference-based evaluation may also have consequences for plant fitness, since it may affect the number of flowers bees visit per plant and the time they spend on plants; variables that affect pollen transfer (Thomson 1986; Ostevik et al. 2010).

Here, we asked how recent experience with wildflower nectar influenced wild bumblebees' (*Bombus* spp.) acceptance of nectar rewards. To do this, we measured the daily nectar concentration of bee-visited wildflowers (*Penstemon* spp.) as a proxy for the quality of nectar foraging bees encountered on a given test day. We then presented individual foragers with manipulated wildflowers filled with known concentrations of artificial nectar. We recorded whether bees accepted (consumed) the reward on the plant. If bees have an absolute threshold for acceptable nectar concentrations, foraging responses should be independent of environmental nectar quality. In contrast, if pollinators evaluate resources in relation to their experience as has been shown in lab-based work, we expected that bees would increase their acceptability threshold when nectar in the environment was of higher quality. In addition, we also measured other foraging behaviors relevant to plant fitness: the number of flowers bees visited on a given plant and the time spent on the plant (Thomson 1986; Ostevik et al. 2010). Based on previous work, we expected that bees would spend more time on higher-quality plants (Thomson 1986). However, if this effect was reference-based, then the effect would be stronger when there was lower-quality nectar available in the environment.

METHODS
Study site and species

We collected data in June 2021 at two sites in montane meadow and mixed conifer forests. The first site was in the Humboldt-Toiyabe National Forest (Dog Valley Meadow, California), which was at a lower elevation and bloomed earlier. The second site was in Tahoe National Forest (Van Norden, California). These sites are referred to hereafter as Dog Valley and Van Norden. Both sites are considered high-altitude desert, characterized by infrequent rain, including in the 2-week period leading up to this study. The average daily temperatures during data collection (i.e., active bee foraging) ranged from 21.1 to 27 °C at Dog Valley and 10.1 to 21 °C at Van Norden (NOAA). Data were collected for 6 days in Dog Valley and 4 days in Van Norden (Supplementary Figure S1).

Experiments were conducted using the four most abundant species of bumblebee ($n = 180$): *Bombus vosnesenskii* ($n = 88$), *B. vandykei* ($n = 16$), *B. centralis* ($n = 52$), and *B. vancouverensis nearcticus* ($n = 5$). Of these 180 bees, 19 were not measured or identified to species due to experimenter error, although these bees belonged to either *B. vosnesenkii* or *B. vandykei*. For each behavioral response, we pooled data across all bee species, although there was variation in representation between our two field sites (Supplementary Table S1). We focused on two flower species within the genus *Penstemon: P. rydbergii* at Dog Valley and *P. heterodoxus* at Van Norden. We chose these species because they were the most abundant flowering species at each site and visited most frequently by nectar-foraging bumblebees. Bees collected nectar from these flowers at both field sites but were never observed collecting pollen from these flowers (Hemingway, personal observation). These two species are visually very similar to each other and did not differ in their average daily nectar volume or concentration (see Supplementary Figure S2 and Supplementary Table S2). Experimental plants of both species had an average of 52 flowers per plant with a range of 10–102 flowers.

Study meadow

Both sampling sites were generally similar in their environmental conditions; however, the two sites varied slightly in size, determined by the natural boundaries of *Penstemon* wildflowers blooming within the meadows at the time of testing (Dog Valley = 12 m × 35 m; Van Norden = 35 m × 20 m). To estimate how the two sites varied in their overall availability/quality of foraging resources, we measured flowering plant abundance and diversity at each site. To do this, we sampled 5% of the area by randomly throwing a 0.5 m × 0.5 m quadrat and measuring the number of species, the number of plants, and the number of *Penstemon* species within the quadrat. This required 84 measurements for Dog Valley and 140 measurements for Van Norden. At Dog Valley, the average (±SD) number of species per quadrat was 1.6 ± 0.92; the average number of plants was 7.36 ± 7.48 and of *Penstemon* plants was 2.49 ± 3.66. At Van Norden, the average (±SD) number of species per quadrat was 1.09 ± 0.56; the average number of plants was 12.56 ± 13.76; and *Penstemon* plants was 12.07 ± 13.71.

Experimental design

Each testing day, we calculated the average concentration of nectar in *Penstemon* in the study meadow, before offering foraging bumblebees *Penstemon* plants containing artificial nectar that varied across a range of concentrations. This allowed us to determine whether bees' acceptance of nectar rewards varied based on their short-term foraging experience (Figure 1).

To calculate wildflower nectar concentration, we collected nectar each day from 15 to 30 plants using 1 μL microcapillary tubes (Drummond Scientific). These plants were bagged 24 h earlier (when unopened) using white mesh organza drawstring bags (10 × 15 cm) to prevent visits from other insects prior to nectar extraction (Cibula and Zimmerman 1987), as in Sun et al. (2017). We selected plants via haphazard sampling to bag across the entire study meadow. Because mesh does not significantly change humidity or temperature, this approach allowed us to obtain nectar production estimates that reflected natural conditions (Wyatt et al. 1992). We extracted nectar from approximately 5–10 flowers per hour over a 4-h period (0800–1200 h); bees started foraging as early as 0700 at both sites. This allowed us to infer the nectar concentration bees experienced across time, which we expected would change with increasing temperatures throughout the day. Nectar samples were pooled across plants for each hour of collection (i.e., 2–4 h/15–30 samples total) to reach a volume necessary to measure the sugar concentration of nectar samples using a refractometer (Aichose Brix Refractometer 0–80%). Pooling samples across multiple plants is a common technique for measuring sugar composition when volumes are insufficient for analysis (Nicolson 2022). Once nectar was extracted from all flowers on a plant, we cut plants at the base of the stem for use in behavioral experiments, using a different plant for each bee.

Following nectar extraction, we filled all previously emptied flowers on each plant with 1 μL of a known sucrose solution of one of six concentrations (w/w): 0%, 1%, 5%, 10%, 25%, and 50% (hereafter "artificial nectar"). This volume was within the range of that observed in the flowers bagged overnight (see Supplementary Table S3). Although we could not use real nectar

for our experimental treatments due to the small amount produced by these flowers, sucrose solution is a common proxy in behavioral experiments in the field (Taneyhill 2010; Burdon et al. 2020). The plant was then placed in an empty 15 mL conical falcon tube through a hole in the lid and attached to a 1 m wooden pole using tape (Figure 1). This setup was a modified version of the "interview stick" used in (Thomson 1981; Heiling et al. 2021). After following a focal individual bumblebee that foraged for nectar on *Penstemon* for at least five successive visits, we presented it with the manipulated plant by holding the flower in the direct path of the bee and waiting for it to land on it. Focal bees were haphazardly selected within the study meadow. Approximately 90% of bees landed on and sampled a manipulated plant offered on the interview stick. We only tested foragers (i.e., no foraging queens or males), with approximately 30 bees per artificial nectar treatment (sample sizes: 0% = 29; 1% = 30; 5% = 31; 10% = 30; 25% = 30; 50% = 30). Treatments were randomized across both sampling times and days. All bees tested exclusively visited *Penstemon* plants prior to testing. Focal bees were only tested once.

Once the bee landed on the manipulated plant, we verbally recorded several behavioral responses using iPhones. Namely, we stated the time that a bee landed on the plant, how many flowers she visited (typically via walking between flowers, but in one case, the bee flew off the plant and then returned to another flower), whether she accepted or rejected the sucrose solution in each flower, and the time that she departed from the plant, upon which we stopped timing. In almost every case, bees flew directly to a neighboring plant, where we caught them for measurements and identification. We recorded the species of this plant; all but one bee visited *Penstemon*. While one experimenter (S.P.) measured standing
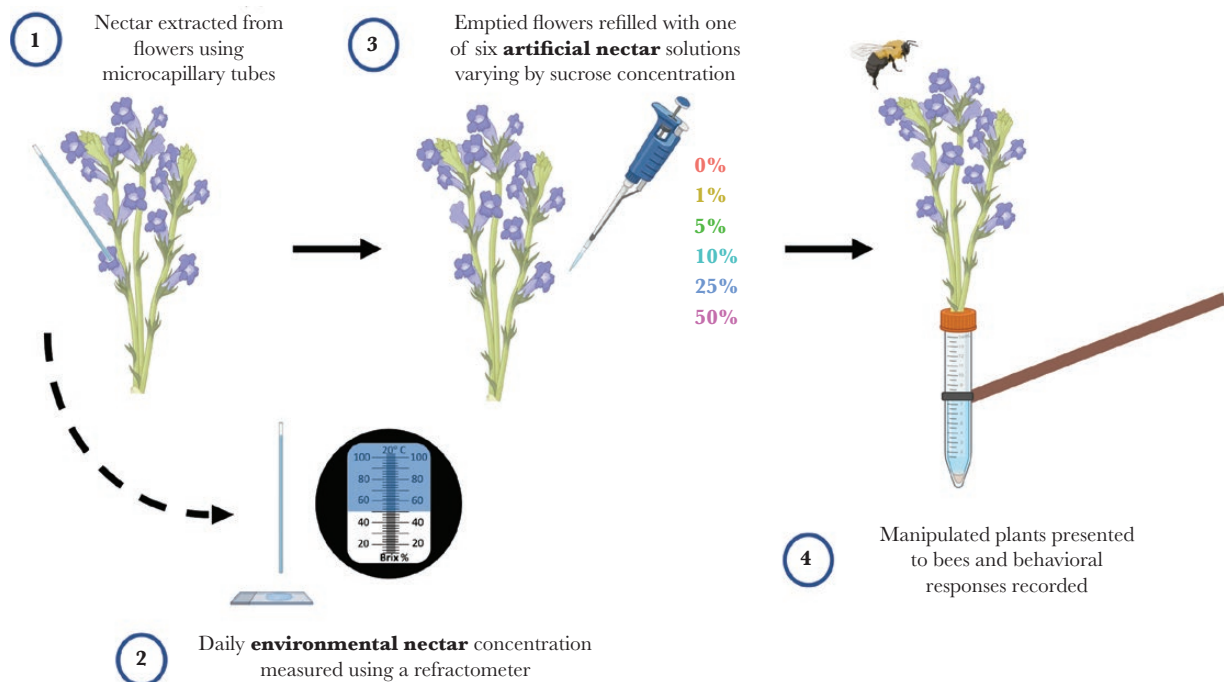

Figure 1
Experimental methods. Step 1: Nectar was extracted daily from all flowers on each plant using 1 μL microcapillary tubes as an estimate of environmental nectar concentration. Step 2: The sugar concentration of extracted environmental nectar was then measured using a brix refractometer. Step 3: Emptied flowers were refilled with 1 μL of artificial nectar that was one of six known sucrose concentrations (colors correspond to nectar treatments in Figures 2 and 3). Step 4: Plants containing artificial nectar were presented to bees and behavioral responses were verbally recorded.

nectar in bagged plants, the other (C.T.H.) pipetted sucrose solutions into flowers on the manipulated plant and immediately presented them to bees. Because of this, the observer was not blind to the experimental treatment. However, a single observer (C.T.H.) conducted all plant presentations to reduce variation in observer behavior.

Following testing, bees were captured using an insect net and transferred to falcon tubes, which were chilled for ~10 min on ice to allow for bee identification and marking. We recorded the species identification using "Bumblebees of the Western United States" (Koch et al. 2011). We also photographed the head, thorax, and abdomen, and measured body size of each individual using intertegular distance as a proxy (Hagen and Dupont 2013). Finally, we paint-marked bees' thoraces using non-toxic, water-based paint markers (POSCA USA) to avoid re-testing the same individual and released them at the initial capture location (Briggs et al. 2022). Bees are not covered under the US Institutional Care and Use Committee; however, care was taken while testing and handling animals.

Behavioral responses

Nectar acceptance was characterized by bees drinking the sucrose solution in the flower (taking ≥1 s), while rejection was characterized by a bee probing the solution with their proboscis and moving on to the next flower or plant without drinking (<1 s). We also addressed how nectar concentration affected foraging behavior by measuring the number of flowers visited and probed by the bee. This measure encompassed all flowers sampled, whether the nectar was accepted or rejected, and was standardized by plant size by accounting for the total number of flowers per plant. Finally, we measured the residence time on the plant, which can correlate with pollination success, and since bees have been shown to spend more time on plants that have higher nectar concentrations (Thomson 1986; Wolff et al. 2006; Thomson et al. 2012).

Statistical analyses

All data were analyzed in R 4.2.3 (R Core Team 2020). Data and analyses are publicly available (Hemingway et al. 2023). In general, we ran models including all experimental variables, and interaction terms of interest, to ask specific questions. For covariates that were not an aspect of the experiment but may have affected results (e.g., time of day), we included these variables and relevant interaction terms but removed them in a step-wise fashion if nonsignificant; more details are given below for each analysis.

To determine the effects of daily standing nectar and nectar manipulations on acceptance behavior, we used a GLMM with a binomial distribution and logit link using the lme4 package (Bates et al. 2015). In our model, acceptance was entered as a binary response (accept = 1, reject = 0) for each flower visited. Experimental nectar treatment (continuous variable: 0%, 1%, 5% 10%, 25%, or 50% (w/w) sucrose), daily environmental nectar concentration (continuous variable), and location (Dog Valley or Van Norden) were included as predictor variables and bee ID was included as a random factor. Because we were also interested in whether the relationship between bees' acceptance behavior and daily nectar quality differed between the experimental treatments, we tested for an interaction between nectar treatment and daily environmental nectar concentration. Because the effects of nectar treatment or daily nectar concentration may have varied by location, we tested for interactions between treatment and location and between daily nectar and location. We

also tested for the main effects of two covariates that were not part of the experimental question being asked, but that may have influenced results: the time of day that bee foraging occurred and bee species. These terms could not be included in the maximal model initially because of singularity issues. To help summarize model fit, we also calculated pseudo-R^2 values using the MuMIn package for our best-fitting model for acceptance behavior. While pseudo-R^2 values to help summarize the models fit, they are not generally recommended for model selection (Nakagawa and Schielzeth 2013).

To determine whether daily nectar and/or experimental nectar treatment affected the proportion of flowers bees visited, we ran a GLMM with a binomial distribution and logit link with the visitation of nectar-filled flowers on each plant as a binary response (visited = 1, not visited = 0). We again included experimental nectar treatment, daily environmental nectar concentration, and location as predictor variables and bee as a random factor. We tested for potential interactions between the three predictor variables. We also tested for the main effects of time of day and bee species in our final model. We again calculated pseudo-R^2 values for our best-fitting.

Finally, we addressed how daily nectar and nectar treatment affected bees' residence times on plants. Residence times were continuous and had a positive right skew, therefore we log-transformed these data. For this analysis, we only had one observation per bee (i.e., no repeated measures), and the response variable fitted a Gaussian distribution; we therefore carried out linear models using the lm() function. We again included daily nectar, experimental treatment, location, and interactions as predictor variables. Time of day and bee species were included as potential covariates in the final model.

RESULTS

1.Acceptance of experimental nectar

Bees were more likely to accept artificial nectar in flowers when standing nectar in the environment was less concentrated (Figure 2; daily nectar: $z = -2.505$, $P = 0.012$), indicating that nectar acceptability was at least partially evaluated according to recent experience. This effect was driven by lower experimental nectar concentrations: bees were less likely to accept water (0%), 1%, or 5% sucrose when environmental nectar was of higher quality, while nectar of 10% and higher was nearly always accepted, regardless of daily nectar concentration (Figure 2; treatment × daily nectar: $z = -2.047$, $P = 0.041$). Surprisingly, acceptance overall was very high, with bees accepting 5% sucrose most of the time, and 1% sucrose around half the time (Figure 2, Supplementary Figure S3). As expected, bees were more likely to consume higher concentrations of artificial nectar from flowers overall (Figure 2, Supplementary Figure S3; treatment: $z = 4.187$, $P < 0.001$). There was no effect of location (Supplementary Figure S4; location: $z = -0.144$, $P = 0.886$), time of day ($\chi^2 = 0.525$, df = 1, $P = 0.469$), or bee species ID ($\chi^2 = 0.528$, df = 3, $P = 0.913$) on acceptance behavior. Parameter estimates and 95% bootstrapped confidence intervals for final model terms are included in Supplementary Table S4. Overall, our model had strong explanatory power (conditional pseudo-$R^2 = 0.990$, delta < 0.001).

2.Flower visitation on plant

Visitation behavior was also influenced by daily nectar concentration, with bees visiting more flowers per plant when the nectar concentration in the environment was higher (Figure 3; daily nectar: z
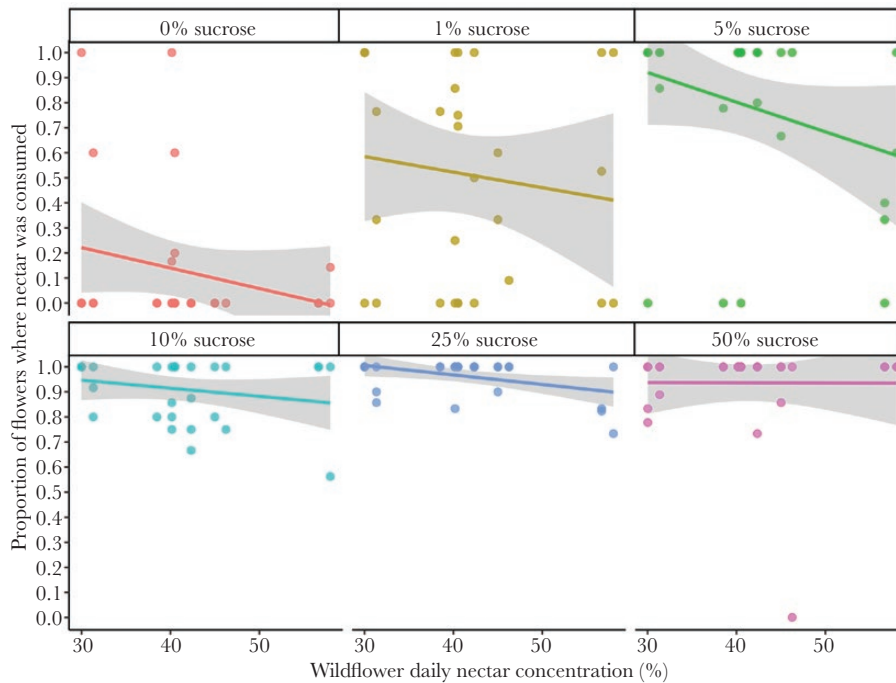
Figure 2

The proportion of flowers that bees accepted (consumed nectar from) out of the total number of flowers sampled on a given plant. Acceptance is shown according to treatment (0%, 1%, 5%, 10%, 25%, and 50% artificial nectar solution offered) and across a range of concentrations found naturally that day in *Penstemon* wildflowers.

=2.077, P = 0.038). As expected, we found that bees visited more flowers per plant when they contained higher artificial nectar concentrations (Figure 3, Supplementary Figure S5; treatment: z = 2.091, P = 0.036), although this effect was weaker than for acceptance behavior (Supplementary Table S4). There was not a significant interaction between these two variables (treatment × daily nectar: z = 0.716, P = 0.474), indicating that bees' responses to daily nectar did not vary across the different experimental treatments, so this term was removed from the final model. We found an effect of location, with bees visiting more flowers per plant at Dog Valley relative to Van Norden (Supplementary Figure S6; location: z = −3.158, P = 0.002). This may be explained, in part, by the higher density of flowering plants, including the focal species at Van Norden (see "Study meadow" section in Methods). Neither time of day (χ^2 = 0.153, P = 0.696), nor bee species ID (χ^2 = 2.712, P = 0.438) affected the proportion of flowers that bees visited. Parameter estimates and 95% bootstrapped confidence intervals for final model terms are included in Supplementary Table S4. For our visitation model, the fit was still quite high (pseudo-R^2 = 0.432, delta = 0.384).

3. Residence time on plants

Bees stayed longer on plants when encountering higher artificial nectar concentrations (treatment: t = 2.969, P = 0.003) and when there was more concentrated nectar in their environment, although this effect was not significant (Supplementary Figure S7; daily nectar: t = 1.732, P = 0.085). Bees spent more time on plants at Dog Valley (the lower floral density location) relative to Van Norden (Supplementary Figure S8; location: t = −4.105, P < 0.001). *B. vancouverensis nearcticus* spent less time on plants relative to other bee species (Supplementary Figure S9; bee species ID:

F = 3.171, P = 0.026). Residence time was not affected by time of day (f = 1.216, P = 0.272).

DISCUSSION

Although most models of decision making assume that foragers evaluate food in absolute terms, many animals evaluate rewards according to recent experience (Crespi 1942; Flaherty 1982; Papini et al. 1988). While comparative evaluation appears taxonomically widespread (Daniel 2020), it has rarely been investigated in wild animals. Here, we asked if bees' acceptance of nectar was influenced by recent experience. We found that bees used a combination of both absolute and reference-based evaluation. Specifically, bees almost always accepted nectar above a certain threshold (10% sucrose concentration). However, bees appear to rely on reference-based evaluation when deciding whether to accept lower-quality (0–5%) sucrose.

Our findings indicate a key difference between bees' reference-based evaluation in the field and what has typically been observed in the lab. In our study, bees encountered 30–50% nectar in *Penstemon* wildflowers, yet still readily accepted 10% sucrose. Previous lab-based studies have found that bees foraging on 40–50% sucrose will reject 20–30% (Bitterman 1976; Waldron et al. 2005; Hemingway and Muth 2022). Thus, while wild-foraging bees still show evidence of reference-based evaluation, these effects are smaller than those detected in lab studies. Even without immediate prior experience with higher-quality nectar, the ranges of accepted sucrose here are typically rejected in a laboratory setting (Page Jr et al. 1998; Mujagic and Erber 2009; Mommaerts et al. 2013). This difference may be explained by wild-foraging bees encountering lower-volume and/or less-predictable nectar and having higher energetic
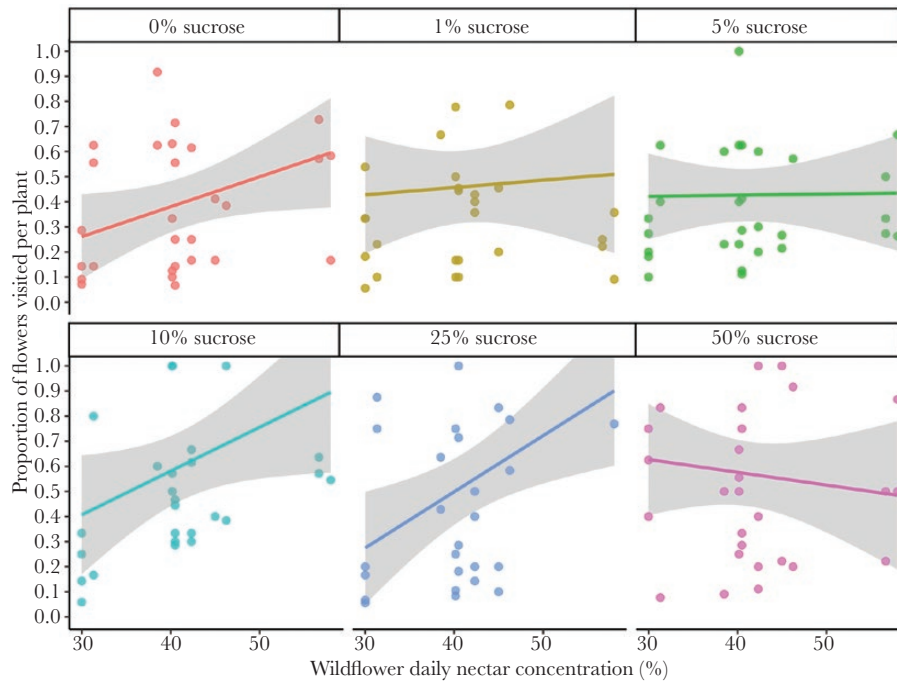
Figure 3
Proportion of flowers visited (consumed or rejected after sampling) by foraging bees out of the total number of flowers available per plant. Visitation is shown according to artificial nectar treatment (0%, 1%, 5%, 10%, 25%, and 50%) and across a range of concentrations found naturally that day in *Penstemon* wildflowers.

needs than captive bees. Wild-foraging individuals may also have a greater need for water (Ferry and Corbet 1996; Nicolson 2022).

The concentrations of artificial nectar evaluated in a reference-dependent manner (0–5% sucrose) were lower than any nectar concentrations measured in *Penstemon* at either site. As such, it is unclear whether reference-based evaluation drives nectar acceptance of this plant species. That the *Penstemon* nectar was always found to be in the 30–60% range might reflect that lower concentrations would be less likely to be accepted by bumblebees. However, in a co-blooming species, *Wyethia mollis*, we recorded lower average concentrations of nectar (i.e., 5–20%), and bumblebees were frequently observed foraging for nectar on this species (Hemingway, personal communication). This raises the question of whether bees might have different acceptance thresholds for different species, as has been previously suggested (Townsend-Mehler et al. 2011; Hemingway and Muth 2022).

Reference-based evaluation may be underpinned by "contrast effects," where responses depend on whether recently encountered options were better or worse (Fawcett et al. 2014). In uncertain environments, animals should invest more time and energy exploring alternative options when currently available options deteriorate (McNamara et al. 2013). These effects should be strongest in animals adapted to rapidly changing environments, such as generalist bees. Many lab-based studies have found evidence for contrast effects in bees (Bitterman 1976; Couvillon and Bitterman 1984; Wiegmann et al. 2003, 2003; Waldron et al. 2005; Townsend-Mehler et al. 2011; Hemingway and Muth 2022), showing that they evaluate rewards relative to expectations based on prior experience with both nectar (Bitterman 1976; Wiegmann and Smith 2009) and associated floral stimuli (Couvillon and Bitterman 1984; Hemingway and Muth 2022). Our finding that wild-foraging bumblebees did not reject all concentrations lower than what they

had likely encountered differs from these lab-based studies. In addition to the variables mentioned above that may explain this discrepancy (i.e., nectar reliability and variability), another important difference is that while lab-based studies always use sucrose solution as a proxy for nectar, here the bees' prior experience was with real nectar. Nectar is not only composed of several other types of sugars, including glucose and fructose, but also contains amino acids, fatty acids, salts, and secondary metabolites (Nicolson 2022), which may influence how bees perceive and evaluate nectar quality.

While our acceptance behavior results can be explained at an individual cognitive level in terms of incentive contrast effects, they may also be explained by changes in the nutritional state of colonies between days (Hendriksma et al. 2019). For example, when environmental nectar is higher in concentration, colony energetic reserves might also be higher, meaning that bees should be more selective in the quality of nectar that they collect. This explanation is not mutually exclusive with the sensory/cognitive explanation of incentive contrast effects; however, given the timeframes over which we tested bees, it seems unlikely that colony state would have changed substantially, and thus, the sensory explanation is more likely. Resource demands also change dynamically across the lifetime of the colony. Although this study was conducted fairly early in the season, we had no way of assessing colony states, and there may have been variation between colonies. Experiments conducted later in the season could help determine how reference-based evaluation changes depending on a colony's energetic reserves.

We also found that bees tended to stay longer and visit more flowers per plant when encountering higher-quality artificial nectar, and, to a lesser extent, with increasing daily nectar concentration. This agrees with previous work showing that bees remained longer and sampled more flowers per plant when the patch was more rewarding than their previous experience (Biernaskie and Gegear

2007). Similarly, many field-based studies using nectar volume as the measure of reward quality have shown that bees track daily changes in nectar quality in their environment and adjust expectations accordingly (Thomson et al. 1982; Hodges 1985; Pleasants 1989; Kadmon and Shmida 1992; Biernaskie et al. 2002). For example, bumblebees that encountered a series of enriched plants (flowers bagged for 40–50 h nectar production) initially increased the average number of flowers visited per plant (Cibula and Zimmerman 1987).

That bees visited more flowers per plant and trended towards staying longer on plants with increasing daily nectar concentration is somewhat surprising, since we expected that bees' higher rejection of low-quality rewards would translate to shorter residence times. These results may indicate that bees that find nectar on a plant, even if it is lower quality than expected, may stay longer searching on this plant when high-quality nectar is present in their environment. This could be because the presence of nectar, even if lower quality, might indicate nectar rewards "missed" by competing pollinators. However, bees regularly abandoned a given plant before sampling every flower, visiting only around 35–65% of available flowers. This was true even at the highest concentration of artificial nectar. This may reflect a general departure rule, and for pollinators visiting mass-flowering plants, it is common for individuals to sample only a portion of flowers available (e.g., Harder et al. 2004), which may reduce the probability of revisiting previously emptied flowers (Pleasants 1989; Kadmon and Shmida 1992; Ohashi and Yahara 2002). It is worth noting that unlike normal plants, all flowers in manipulated plants for our study were filled with sucrose solution, which may also have influenced departure behavior.

One variable we did not account for in the current study was the variability between flowers in nectar concentration within a day, since we pooled samples from different plants during concentration measurements due to the small amount of nectar present in each flower. Variability can be caused by daily patterns of reward depletion and renewal (e.g., Pleasants and Zimmerman 1979; Thomson 1988), genetic differences between plants (Parachnowitsch et al. 2019), and differences in environmental variables like sun exposure (Pleasants 1983), temperature (Jakobsen and Kritjánsson 1994), and availability of water (Carroll et al. 2001; Phillips et al. 2018). Bees can assess variability between plants (e.g., Shafir 2000; Biernaskie et al. 2002, 2009; Biernaskie and Cartar 2004), and generally avoid variable rewards in favor of constant ones (Real 1981; Waddington et al. 1981; Dunlap et al. 2017). As such, nectar variability may influence bees' tendency to use reference-based vs. absolute comparisons; exploring this may be a worthwhile avenue for future research.

We found some site-specific behaviors: bees visited more flowers per plant and resided on plants for longer in Dog Valley relative to Van Norden, while our findings related to nectar acceptance did not differ. One variable that differed between the two sites was plant species: *P. rydbergii* was only found at Dog Valley and *P. heterodoxus* was only found at Van Norden. However, species differences are unlikely to explain our results, as there were no differences in their nectar concentration and, at least from a human perspective, the flowers were indistinguishable. Instead, a more likely explanation for the site-specific differences is that Dog Valley had a much lower floral density than Van Norden. As such, foraging theory would predict that bees should stay longer on a given plant (Charnov 1976). It is also possible that the higher temperatures at Dog Valley relative to Van Norden drove these effects, since flight endurance

and the likelihood of flight decreases above 25 °C (Kenna et al. 2021). We also observed a slight difference in residence time between *B. vancouverensis nearcticus* and the other three species. This may be due to species differences in behavior, although it seems more likely that this is driven by random variation, due to the small sample size for this species.

Our results may have implications for floral reward strategies since pollinator behavior on the flower can affect plant fitness. We found that bees probed more flowers, but drank less nectar, as the concentration of environmental nectar increased. These results indicate that it is possible that plants offering lower-quality nectar when standing nectar levels are on average higher may benefit from more visits, and potentially pollen transfer, while suffering lower costs of nectar depletion by foraging bees, at least in the short term. In the longer term, we would expect bees to spend less time on these flowers or reject them altogether. Here, we considered visitation and residence time as a proxy for fitness, but future studies should measure seed set to more directly link behavior to plant fitness.

In the present study, we examined foraging behavior during a brief period where bees foraged for nectar primarily on a single, highly rewarding species. In the wild, bumblebees forage for nectar rewards from multiple floral species (Heinrich 1979). Additionally, nectar rewards vary in several other aspects of reward quality, such as volume and variance, and bees likely have to integrate information about these different reward dimensions when making foraging decisions (Latty and Trueblood, 2020). Future work could incorporate some of the additional natural complexity, including nectar volume and variability, as well as traits of other competing plants. By examining the mechanisms of reward perception of pollinators, especially in natural floral communities, we can develop more predictive models of pollinator visitation and foraging behavior.

SUPPLEMENTARY MATERIAL

FUNDING

AUTHOR CONTRIBUTIONS

C.T.H. and F.M. conceived the ideas and designed methodology; C.T.H. and S.P. collected the data; C.T.H. analyzed the data; C.T.H. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

CONFLICT OF INTEREST

We declare no competing interests.

DATA AVAILABILITY

Analyses reported in this article can be reproduced using the data provided by Hemingway et al. (2023).

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REFERENCES

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J Stat Softw 67(1):1–48. doi:10.18637/jss.v067.i01

Biernaskie JM, Cartar RV. 2004. Variation in rate of nectar production depends on floral display size: a pollinator manipulation hypothesis. Funct Ecol. 18(1):125–129. doi:10.1111/j.1365-2435.2004.00815.x

Biernaskie JM, Cartar RV, Hurly TA. 2002. Risk-averse inflorescence departure in hummingbirds and bumble bees: could plants benefit from variable nectar volumes? Oikos 98(1):98–104. doi:10.1034/j.1600-0706.2002.980110.x

Biernaskie JM, Gegear RJ. 2007. Habitat assessment ability of bumble-bees implies frequency-dependent selection on floral rewards and display size. Proc Biol Sci. 274(1625):2595–2601. doi:10.1098/rspb.2007.0705

Biernaskie JM, Walker SC, Gegear RJ. 2009. Bumblebees learn to forage like bayesians. Am Nat. 174(3):413–423. doi:10.1086/603629

Bitterman M. 1976. Incentive contrast in honey bees. Science. 192(4237):380–382. doi:10.1126/science.1257773

Boisvert MJ, Veal AJ, Sherry DF. 2007. Floral reward production is timed by an insect pollinator. Proc Biol Sci. 274(1620):1831–1837. doi:10.1098/rspb.2007.0445

Briggs EL, Baranski C, Münzer SO, Garrison G, Collazo JA, Youngsteadt E. 2022. Estimating bee abundance: can mark-recapture methods validate common sampling protocols? Apidologie. 53(10):10.

Burdon RCF, Raguso RA, Gegear RJ, Pierce EC, Kessler A, Parachnowitsch AL. 2020. Scented nectar and the challenge of measuring honest signals in pollination. J Ecol. 108(5):2132–2144. doi:10.1111/1365-2745.13432

Carroll AB, Pallardy SG, Galen C. 2001. Dought stress, plant water status, and floral trait expression in fireweed, Epilobium angustifolium (Onagraceae). Am J Bot. 88(3):438–446.

Charnov EL. 1976. Optimal foraging, the marginal value theorem. Theor Popul Biol. 9(2):129–136. doi:10.1016/0040-5809(76)90040-X

Chittka L, Gumbert A, Kunze J. 1997. Foraging dynamics of bumble bees: correlates of movements within and between plant species. Behav Ecol. 8(3):239–249.

Chittka L, Thomson JD, Waser NM. 1999. Flower constancy, insect psychology, and plant evolution. Naturwissenschaften. 86(8):361–377. doi:10.1007/s001140050636

Cibula DA, Zimmerman M. 1987. Bumblebee foraging behavior: changes in departure decisions as a function of experimental nectar manipulations. Am Midl Nat. 117(2):386. doi:10.2307/2425981

Couvillon PA, Bitterman ME. 1984. The overlearning-extinction effect and successive negative contrast in honeybees (Apis mellifera). J Comp Psychol. 98(1):100–109.

Crespi L. 1942. Quantitative variation of incentive and performance in the white rat. Am J Psychol. 55(4):467–517.

Daniel AM. 2020. Scaling relative incentive value in honey bees, Apis mellifera. Learn Motiv. 69:101614. doi:10.1016/j.lmot.2020.101614

Daniel AM, Wood M, Pellegrini S, Norris JN, Papini MR. 2008. Can contextual cues control consummatory successive negative contrast? Learn Motiv. 39(2):146–162. doi:10.1016/j.lmot.2007.11.001

Dunlap AS, Papaj DR, Dornhaus A. 2017. Sampling and tracking a changing environment: persistence and reward in the foraging decisions of bumblebees. Interface Focus. 7(3):20160149. doi:10.1098/rsfs.2016.0149

Dunlap AS, Stephens DW. 2016. Reliability, uncertainty, and costs in the evolution of animal learning. Curr Opin Behav Sci. 12:73–79. doi:10.1016/j.cobeha.2016.09.010

Emlen JM. 1966. The role of time and energy in food preference. Am Nat. 100(916):611–617. doi:10.1086/282455

Evans MR, Grimm V, Johst K, Knuuttila T, De Langhe R, Lessells CM, Merz M, O'Malley MA, Orzack SH, Weisberg M, et al. 2013. Do simple models lead to generality in ecology? Trends Ecol Evol. 28(10):578–583. doi:10.1016/j.tree.2013.05.022

Fawcett TW, Fallenstein B, Higginson AD, Houston AI, Mallpress DEW, Trimmer PC, McNamara JM. 2014. The evolution of decision rules in complex environments. Trends Cogn Sci. 18(3):153–161. doi:10.1016/j.tics.2013.12.012

Ferry C, Corbet SA. 1996. Water collection by bumble bees. J Api Res. 35(3):120–122.

Flaherty CF. 1982. Incentive contrast: a review of behavioral changes following shifts in reward. Anim Learn Behav. 10(4):409–440. doi:10.3758/BF03212282

Gil M, De Marco RJ, Menzel R. 2007. Learning reward expectations in honeybees. Learn Mem 14(7):491–496. doi:10.1101/lm.618907

Hagen M, Dupont YL. 2013. Inter-tegular span and head width as estimators of fresh and dry body mass in bumblebees (Bombus spp.). Insectes Soc. 60(2):251–257. doi:10.1007/s00040-013-0290-x

Harder LD. 1988. Choice of individual flowers by bumble bees: interaction of morphology, time and energy. Behaviour 104(1):60–76.

Harder LD, Jordan CY, Gross WE, Routley MB. 2004. Beyond floricentrism: the pollination function of inflorescences. Plant Species Biol. 19(3):137–148. doi:10.1111/j.1442-1984.2004.00110.x

Heiling JM, Bronstein JL, Irwin RE. 2021. Nectar addition changes pollinator behavior but not plant reproduction in pollen-rewarding Lupinus argenteus. Am J Bot. 108(3):402–410. doi:10.1002/ajb2.1613

Heinrich B. 1976. The foraging specializations of individual bumblebees. Ecol Monogr. 46(2):105–128.

Heinrich B. 1979. "Majoring" and "minoring" by foraging bumblebees, Bombus vagans: an experimental analysis. Ecology. 60(2):245–255.

Hemingway C, Pimplikar S, Muth F. 2023. Dataset and R code from: "Wild bumblebees use both absolute and relative evaluation when foraging". Dryad Digital Repository. doi:10.5061/dryad.gb5mkkwwx

Hemingway CT, Muth F. 2022. Label-based expectations affect incentive contrast effects in bumblebees. Biol Lett. 18:20210549.

Hendriksma HP, Toth AL, Shafir S. 2019. Individual and colony level foraging decisions of bumble bees and honey bees in relation to balancing of nutrient needs. Front Ecol Evol. 7:177. doi:10.3389/fevo.2019.00177

Hodges CM. 1985. Bumble bee foraging: the threshold departure rule. Ecology. 66(1):179–187. doi:10.2307/1941318

Ishii H. 2006. Floral display size influences subsequent plant choice by bumble bees. Funct Ecol. 20(2):233–238.

Jakobsen H, Kritjánsson K. 1994. Influence of temperature and floret age on nectar secretion in Trifolium repens L. Ann Bot (Lond). 74(4):327–334. doi:10.1006/anbo.1994.1125

Kadmon R, Shmida A. 1992. Departure rules used by bees foraging for nectar: a field test. Evol Ecol. 6:142–151.

Kenna D, Pawar S, Gill RJ. 2021. Thermal flight performance reveals impact of warming on bumblebee foraging potential. Funct Ecol. 35(11):2508–2522. doi:10.1111/1365-2435.13887

Koch J, Strange J, Williams. (2011). Bumble bees of the Western United States. Publication No. FS-972.: U.S. Forest Service.

Krebs J, Inman A. (1994). Learning and foraging: individuals, groups, and populations. In: Real LA, editors. Behavioral mechanisms in evolutionary ecology. Chicago, IL: University of Chicago Press. p. 46–65.

Latty T, Beekman M. 2010. Food quality and the risk of light exposure affect patch-choice decisions in the slime mold Physarum polycephalum. Ecology. 91(1):22–27. doi:10.1890/09-0358.1

Latty T, Trueblood JS. 2020. How do insects choose flowers? A review of multi-attribute flower choice and decoy effects in flower-visiting insects. J Anim Ecol. 89(12):2750–2762.

MacArthur RH, Pianka ER. 1966. On optimal use of a patchy environment. Am Nat. 100(916):603–609. doi:10.1086/282454

McNamara JM. 1982. Optimal patch use in a stochastic enviornment. Theor Popul Biol. 21(2):269–288.

McNamara JM, Fawcett TW, Houston AI. 2013. An adaptive response to uncertainty generates positive and negative contrast effects. Science. 340(6136):1084–1086. doi:10.1126/science.1230599

Mommaerts V, Wackers F, Smagghe G. 2013. Assessment of gustatory responses to different sugars in harnessed and free-moving bumblebee workers (Bombus terrestris). Chem Senses. 38(5):399–407. doi:10.1093/chemse/bjt014

Mujagic S, Erber J. 2009. Sucrose acceptance, discrimination and proboscis responses of honey bees (Apis mellifera L.) in the field and the laboratory. J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 195(4):325–339. doi:10.1007/s00359-008-0409-0

Mustaca AE, Bentosela M, Papini MR. 2000. Consummatory Successive Negative Contrast in Mice. Learn Motiv. 31(3):272–282. doi:10.1006/lmot.2000.1055

Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods Ecol Evol. 4(2):133–142. doi:10.1111/j.2041-210x.2012.00261.x

Nicolson S. 2022. Sweet solutions: nectar chemistry and quality. Philos Trans Royal Soc B Lond: Biol Sci. 377(1853):20210163. doi:10.1098/rstb.2021.0163

Oberhauser FB, Czaczkes TJ. 2018. Tasting the unexpected: disconfirmation of expectations leads to lower perceived food value in an invertebrate. Biol Lett. 14(9):20180440. doi:10.1098/rsbl.2018.0440

Ohashi K, Yahara T. 2002. Visit larger displays but probe proportionately fewer flower: counterintuitive behaviour of nectar-collecting bumble bees achieves an ideal free distribution. Funct Ecol. 16(4):492–503.

Ostevik K, Manson J, Thomson J. 2010. Pollination potential of male bumble bees (*Bombus impatiens*): movement patterns and pollen-transfer efficiency. J Pollinat Ecol. 2(4):21–26. doi:10.26786/1920-7603(2010)3

Page RE Jr, Fondrk MK, Erber J. 1998. The effect of genotype on response thresholds to sucrose and foraging behavior of honey bees (*Apis mellifera L.*). J Comp Physiol A Sens Neural Behav Physiol. 182(4):489–500. doi:10.1007/s003590050196

Papini MR, Mustaca AE, Bitterman ME. 1988. Successive negative contrast in the consummatory responding of didelphid marsupials. Anim Learn Behav. 16(1):53–57. doi:10.3758/BF03209043

Parachnowitsch AL, Manson JS, Sletvold N. 2019. Evolutionary ecology of nectar. Ann Bot (Lond). 123(2):247–261. doi:10.1093/aob/mcy132

Phillips BB, Shaw RF, Holland MJ, Fry EL, Bardgett RD, Bullock JM, Osborne JL. 2018. Drought reduces floral resources for pollinators. Global Change Biol. 24(7):3226–3235. doi:10.1111/gcb.14130

Pleasants J. 1983. Nectar production patterns in *Ipomopsis aggregata (*Polemoniaceae). Am J Bot. 70(10):1468–1475.

Pleasants J. 1989. Optimal foraging in nectarivores: a test of the marginal value theorem. Am Naturalist. 134(1):51–71.

Pleasants JM, Zimmerman M. 1979. Patchiness in the dispersion of nectar resources: evidence for hot and cold spots. Oecologia. 41:283–288.

Pyke GH. 1979. Optimal foraging in bumblebees: rule of movement between flowers within inflorescences. Anim Behav. 27(4):1167–1181.

R Core Team. (2020). R: a language and environment for statistical computing (4.0.5) [Computer software]. R Foundation for Statistical Computing. https://www.R-project.org/

Real L. 1987. Objective benefit versus subjective perception in the theory of risk-sensitive foraging. Am Nat. 130(3):399–411.

Real LA. 1981. Uncertainty and pollinator-plant interactions: The foraging behavior of bees and wasps on artificial flowers. Ecology. 62(1):20–26.

Schoener TW. 1971. Theory of feeding strategies. Annu Rev Ecol Syst. 2(1):369–404.

Shafir S. 2000. Risk-sensitive foraging: the effect of relative variability. Oikos. 88(3):663–669. doi:10.1034/j.1600-0706.2000.880323.x

Solvi C, Zhou Y, Feng Y, Lu Y, Roper M, Sun L, Reid RJ, Chittka L, Barron AB, Peng F. 2022. Bumblebees retrieve only the ordinal ranking of foraging options when comparing memories obtained in distinct settings. eLife. 11:e78525. doi:10.7554/eLife.78525

Stephens D, Krebs J. 1986. Foraging theory. Princeton, NJ: Princeton University Press.

Sun S-G, Huang Z-H, Chen Z-B, Huang S-Q. 2017. Nectar properties and the role of sunbirds as pollinators of the golden-flowered tea (*Camellia petelotii*). Am J Bot. 104(3):468–476. doi:10.3732/ajb.1600428

Taneyhill DE. 2010. Patch departure behavior of bumble bees: Rules and mechanisms. Psyche. 2010:1–9. doi:10.1155/2010/872736

Thiel A. 2011. How to measure patch encounter rate: decision-making mechanisms in the parasitic wasp *Asobara tabida*. Anim Cogn. 14(1):73–82. doi:10.1007/s10071-010-0344-7

Thomson JD. 1981. Field measures of flower constancy in bumblebees. Am Mid Nat. 105(2):377–380.

Thomson JD. 1986. Pollen transport and deposition by bumble bees in Erythronium: Influences of floral nectar and bee grooming. J Ecol. 74(2):329–341.

Thomson JD. 1988. Effects of variation in inflorescence size and floral rewards on the visitation rates of traplining pollinators of *Aralia hispida*. Evol Ecol. 2:65–76.

Thomson JD, Maddison WP, Plowright RC. 1982. Behavior of bumble bee pollinators of *Aralia hispida Vent* (Araliaceae). Oecologia. 54:326–336.

Thomson JD, Ogilvie JE, Makino TT, Arisz A, Raju S, Rojas-Luengas V, Tan M. 2012. Estimating pollination success with novel artificial flowers: Effects of nectar concentration. J Pollinat Ecol. 9(14):108–114. doi:10.26786/1920-7603(2012)14

Townsend-Mehler JM, Dyer FC. 2012. An integrated look at decision-making in bees as they abandon a depleted food source. Behav Ecol Sociobiol. 66(2):275–286.

Townsend-Mehler JM, Dyer FC, Maida K. 2011. Deciding when to explore and when to persist: a comparison of honeybees and bumblebees in their response to downshifts in reward. Behav Ecol Sociobiol. 65(2):305–312. doi:10.1007/s00265-010-1047-4

Waddington K, Allen T, Heinrich B. 1981. Floral preferences of bumblebees (*Bombus edwardsii*) in relation to intermittent versus continuous rewards. Anim Behav. 29(3):779–784.

Waddington K, Heinrich B. 1979. The foraging movements of bumblebees on vertical inflorescences: an experimental analysis. J Comp Physiol A. 134(2):113–117.

Waldron FA, Wiegmann DD, Wiegmann DA. 2005. Negative incentive contrast induces economic choice behavior by bumble bees. Int J Comp Psychol 18(4):358–371.

Waser NM, Price M. 1981. Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*. Evolution 35(2):376–390.

Wendt S, Czaczkes TJ. 2020. Labeling effect in insects: cue associations influence perceived food value in ants (*Lasius niger*). J Comp Psychol 134(4):280–292.

Wiegmann D, Smith B. 2009. Incentive relativity and the specificity of reward expectations in honey bees. Int J Comp Psychol 22(3):141–152.

Wiegmann D, Wiegmann D, Waldron F. 2003. Effects of a reward downshift on the consummatory behavior and flower choices of bumblebee foragers. Physiology & Behavior. 79(4–5):561–566. doi:10.1016/S0031-9384(03)00122-7

Wilson M, Price M. 1977. The evolution of inflorescence size and Asclepias (Asclepiadaceae). Evolution. 31(3):495–511.

Wolff D, Witt T, Jürgens A, Gottsberger G. 2006. Nectar dynamics and reproductive success in *Saponaria officinalis* (Caryophyllaceae) in southern Germany. Flora - Morphol Distrib Funct Ecol Plants. 201(5):353–364. doi:10.1016/j.flora.2005.07.010

Wyatt R, Broyles SB, Derda GS. 1992. Environmental influences on nectar production in milkweeds (*Asclepias syriaca* and *A. exaltata*). Am J Bot. 79(6):636–642.