Oecologia
https://doi.org/10.1007/s00442-021-04979-8

METHODS

Measuring foraging preferences in bumble bees: a comparison of popular laboratory methods and a test for sucrose preferences following neonicotinoid exposure

Sarah K. Richman[1] · Felicity Muth[2] · Anne S. Leonard[1]

Received: 26 August 2020 / Accepted: 23 June 2021

Abstract
Animals develop food preferences based on taste, nutritional quality and to avoid environmental toxins. Yet, measuring preferences in an experimental setting can be challenging since ecologically realistic assays can be time consuming, while simplified assays may not capture natural sampling behavior. Field realism is a particular challenge when studying behavioral responses to environmental toxins in lab-based assays, given that toxins can themselves impact sampling behavior, masking our ability to detect preferences. We address these challenges by comparing different experimental methods for measuring sucrose concentration preference in bumble bees (*Bombus impatiens*), evaluating the utility of two preference chamber-based methods (ad libitum versus a novel restricted-sampling assay) in replicating bees' preferences when they fly freely between artificial flowers in a foraging arena. We find that the restricted-sampling method matched a free-flying scenario more closely than the ad libitum protocol, and we advocate for expanded use of this approach, given its ease of implementation. We then performed a second experiment using the new protocol to ask whether consuming the neonicotinoid pesticide imidacloprid, known to suppress feeding motivation, interfered with the expression of sucrose preferences. After consuming imidacloprid, bees were less likely to choose the higher-quality sucrose even as they gained experience with both options. Thus, we provide evidence that pesticides interfere with bees' ability to discriminate between floral rewards that differ in value. This work highlights a simple protocol for assessing realistic foraging preferences in bees and provides an efficient way for researchers to measure the impacts of anthropogenic factors on preference expression.

Keywords Behavioral assays · *Bombus impatiens* · Imidacloprid · Nectar · Preference

Introduction

Animal fitness hinges upon an individual's ability to exploit resources through foraging. Understanding the role of dietary preferences in foraging decisions has therefore become a fixture in the field of ecology (Chesson 1983; Perry and Pianka 1997; Simpson et al. 2004). Bumble bees are a tractable system long used as a model to understand the relationship between foraging preference and energetic gain (e.g., Heinrich 1976; Real and Caraco 1986; Chittka et al. 1997), because the decisions they make about flowers are critical for their metabolic demands (Zimmerman 1981; Pyke 1984; Pleasants 1989). This work shows that bumble bees generally show preferences for flowers providing nectar rewards that are rich in carbohydrates (Cnaani et al. 2006 and references therein). However, physiological state also affects the expression of their dietary preferences. For example, bumble bee workers (*Bombus impatiens* and *B. vagans*) infected with the gut parasite *Crithidia bombii* preferentially foraged on *Chelone glabra* flowers with artificially increased levels of nectar iridoid glycosides, which reduce *C. bombii* infections (Richardson et al. 2015, 2016). Behavioral experiments to measure pollinators' floral preferences are thus critical for disentangling state-dependent and environmental factors driving observed preferences. Given that nectar availability can limit colony growth in bumble bees (Rotheray et al. 2017), better understanding of their nectar assessment can

Communicated by Diethart Matthies.

✉ Sarah K. Richman
sarahkrichman@gmail.com

[1] Department of Biology, University of Nevada, Reno, Reno, NV 89557, USA

[2] Department of Integrative Biology, University of Texas at Austin, Austin, TX 78712, USA

Published online: 11 July 2021

help inform conservation (Woodard and Jha 2017), including research into the effects of pesticides on bees' ability to obtain high-quality resources from flowers (Mogren and Lundgren 2016).

To that end, researchers have developed several methods for assessing nectar preferences and their mediating factors in bumble bees, summarized in Table 1. One popular approach is to use high-throughput assays that involve observing individual bees foraging inside small, clear tubes for artificial nectar (as used in Muth et al. 2018). This approach offers several advantages compared to free-flying experiments in lab or field settings, including the ease of manipulating test subjects and increased sample size per unit of experimenter effort. Researchers often have limited time and resources available to conduct field-realistic preference assays and would therefore benefit from simple methods that can adequately capture the dynamics of a more complex system. Yet, the extent to which different assays (e.g. restricted feeding vs. ad libitum, free-flying vs. preference tube-based) return similar results has never, to our knowledge, been explored. Although these lab-based methods aim to reveal what ecologists may interpret as inherent foraging preferences in bees, it would be worth knowing whether differences in experimental protocol drive differences in observed choices that could lead to incongruent conclusions about preference.

Here, we address these issues by asking (1) whether bumble bees' foraging preferences for sucrose solutions of different concentrations are replicable under different protocols and (2) whether experimental approaches can be adapted to be more efficient without sacrificing the reliability of the results. We investigated these two questions by comparing different laboratory protocols used to quantify nectar sugar concentration preferences in the bumble bee *Bombus impatiens*: one assay using free-flying bees inside a foraging arena and two assays using preference tubes as in Muth et al. (2018). This allowed us to ask: how accurately do the results of preference tube assays reproduce the results of free-flying assays in the lab? Given the broad appeal of testing bumble bees' dietary preferences in a wide range of ecological contexts (Table 1), and given that the technique of free-flying assays can be difficult and time consuming to develop, our aim was to identify experimental approaches that yield similar results.

After identifying a new high-throughput approach [restricted volume preference assay (RVPA)] that best approximated a free-flying scenario, we used this method to ask whether consumption of a neonicotinoid insecticide known to impair foraging motivation (Lämsä et al. 2018), impacted the expression of bumble bees' nectar preferences. This application of our method was inspired by the recent interest in the effects of neonicotinoid pesticides on bees' behavior and cognition (reviewed in Blacquière et al. 2012). Neonicotinoids have strong negative effects on multiple aspects of bee performance and play a role in pollinator declines. Therefore, understanding the sublethal effects of these pesticides on bee foraging and nutrition is widely recognized as a critical research effort (Goulson 2013). In these studies, researchers seek methods that balance ecological realism vs. throughput sufficient to enable large sample sizes and to expand the taxonomic breadth of test subjects (Lundin et al. 2015). Our work here presents a new method which

Table 1 Summary of the scope of approaches to studying food preferences in bumblebees, including studies that examine how preference changes with mediating factors

Bee condition	Flowers and setting	Type of preference tested	Bumble bee species	References
Free flying	Real flowers, natural setting	Male vs. female flowers	*B. vagans*	Bell et al. (1984)
	Artificial flowers, flight cage	Flowers with and without nectar yeasts	*B. impatiens*	Schaeffer et al. (2016)
	Artificial flowers, lab	Sucrose concentration + flower color, with and without pesticide exposure	*B. impatiens*	Phelps et al. (2018)
	Ad libitum feeders, lab	Sucrose with and without neonicotinoids	*B. terrestris*	Arce et al. (2018)
Preference chambers	Choice between two feeders ad libitum,lab	Sucrose with and without neonicotinoids	*B. terrestris*	Kessler et al. (2015)
	Choice between two feeder tubes, lab	Sucrose with and without phytochemical	*B. impatiens*	Palmer-Young et al. (2018)
Harnessed bees	Proboscis extension response, lab	Sucrose concentration, sucrose type	*B. terrestris*	Mommaerts et al. (2013)
Walking	Maze-based protocol, lab	Contaminated vs. uncontaminated chambers	*B. impatiens*	Sprayberry et al. (2013)

The studies listed here are not meant to be exhaustive, but are rather meant to represent a diversity of experimental designs for measuring preference

offers a combination of such features that may be useful in these and other contexts.

Materials and methods

Colony maintenance and general methods

Bumblebee colonies (*Bombus impatiens,* 50–70 individuals/colony with natal queen) were purchased from Koppert Biological Systems (MI, USA) and connected to foraging arenas. For the free-flying experiment (Experiment 1, Protocol A), colonies were connected to a 0.60 × 1.23 × 0.60 m (h, w, d) foraging arena (True-Lite: F32T8-TL, Interlectric Corp., Warren, PA, USA) by a gated passageway. In all other experiments, colonies were connected to a smaller arena (0.5 m^2) containing a feeder, which bees were collected from before being tested for preferences in tubes. Bees in all experiments were provided with 15% (w/w) sucrose solution ad libitum via a white-wicked feeder in each arena, and colonies were given ~ 0.5 g/day of honeybee-collected pollen (Koppert Biological Systems). We used workers from two colonies for each experiment, aiming for 20 bees/treatment represented equally across both colonies; see exact sample sizes below. In all protocols, subjects were offered a choice between 15% and either 30% or 45% sucrose in order to determine whether different protocols could capture different levels of discrimination. To assess whether there were differences in experimenter effort across protocols, we calculated the amount of time required to complete each experiment (see Table S1).

In Experiment 1, Protocol A (free-flying), naïve subjects gained experience foraging from artificial flowers during a pretest "shaping" period. In Experiment 1, Protocols B (RVPA) and C (ad libitum) and Experiment 2, bees were collected from sucrose feeders upon landing using an insect aspirator (Bioquip Products, CA, USA). Bees were then cold anesthetized and transferred to a preference tube. In all experiments, we used spatial location (e.g. left vs. right) to indicate sucrose concentration (reward quality); artificial flowers (or preference tubes) offering different solutions were visually identical. We switched the position (right or left) of the sucrose solutions between subjects. In all experiments, preferences were assessed individually to reduce social influences, such as copying other individuals' choices (Leadbeater and Chittka 2007; Worden and Papaj 2005). In all cases, preference tubes and artificial flowers were wiped down with 70% ethanol between subjects.

Experiment 1, Protocol A: Sucrose preferences in a free-flying context in a foraging arena

To establish free-flying bumblebee sucrose preferences, we observed individuals foraging on artificial flowers in an arena over the course of a single foraging trip. The ceiling and three walls of the arena were made from metal mesh; the floor and the wall farthest from the colony connector tube were green-painted wood ("Ivy Topiary", Behr Ultra, Santa Ana CA). Artificial flowers consisted of a 25 mm diameter circle made of gray craft foam (Creatology, Irving, TX, USA) with a 1.5 mL Eppendorf tube at the center. We drilled two holes in the Eppendorf tube, one at the tip and one in the center (Fig. 1b). Before experimental trials, shaping took place as follows: we inserted three artificial flowers horizontally, in the center of the wooden wall, with the Eppendorf center hole facing upward. We filled the tip of each Eppendorf tube with 40 µL of 15% sucrose and allowed bees free access to the flowers. After a bee consumed sucrose from the flower, we refilled it using a repeater pipette (Eppendorf M4, Hauppauge, NY, USA). When an individual bee successfully collected sucrose from 10 flowers, we marked its thorax with a paint pen (Posca, Tokyo, Japan) by inserting the tip of the pen into the Eppendorf center hole while the bee was feeding. After this shaping period, we let all bees return to the nest box and set up the arena for the experimental trials.

During an experimental trial, a marked bee was allowed to freely forage on an array with five artificial flowers each on the left and right side of the back wall in visually identical groupings (Fig. 1a). Flowers on one side were each filled with 4 µL of 15% sucrose and the other side each with 4 µL of either 30% or 45% sucrose ($n = 21$ and 20 bees for the 15% vs 30% and 45% treatments, respectively). For each visit, we recorded the sucrose concentration of the flower, as well as whether a bee consumed the sucrose solution (a 'drinking visit'), probed the solution with its proboscis without collecting it, or landed on the flower without probing. After a bee collected all the sucrose from a flower, which could be visually confirmed by an observer, the observer refilled it once the bee flew away. The design of the arena ensured that this hidden observer did not disturb the forager. Bees were allowed to forage until they attempted to return to the colony by entering the connector tube. If 20 min had elapsed since the last floral visit, and the minimum-visit threshold (10) was not met, the bee was removed from the experiment. In our analysis, we only included bees that made at least ten visits total after consuming both sucrose types. All bouts were recorded using a digital video camera (Canon QuickShot, 32 FPS). After completion of a bout (at least 10 visits, with no more than 20 min between visits), bees were removed from the arena using an insect aspirator and euthanized by freezing.

Experiment 1, Protocol B: Sucrose preferences under sequential offerings in a preference tube assay—"restricted volume preference assay (RVPA)" protocol

To test whether bees confined to individual preference tubes exhibited sucrose concentration preferences similar

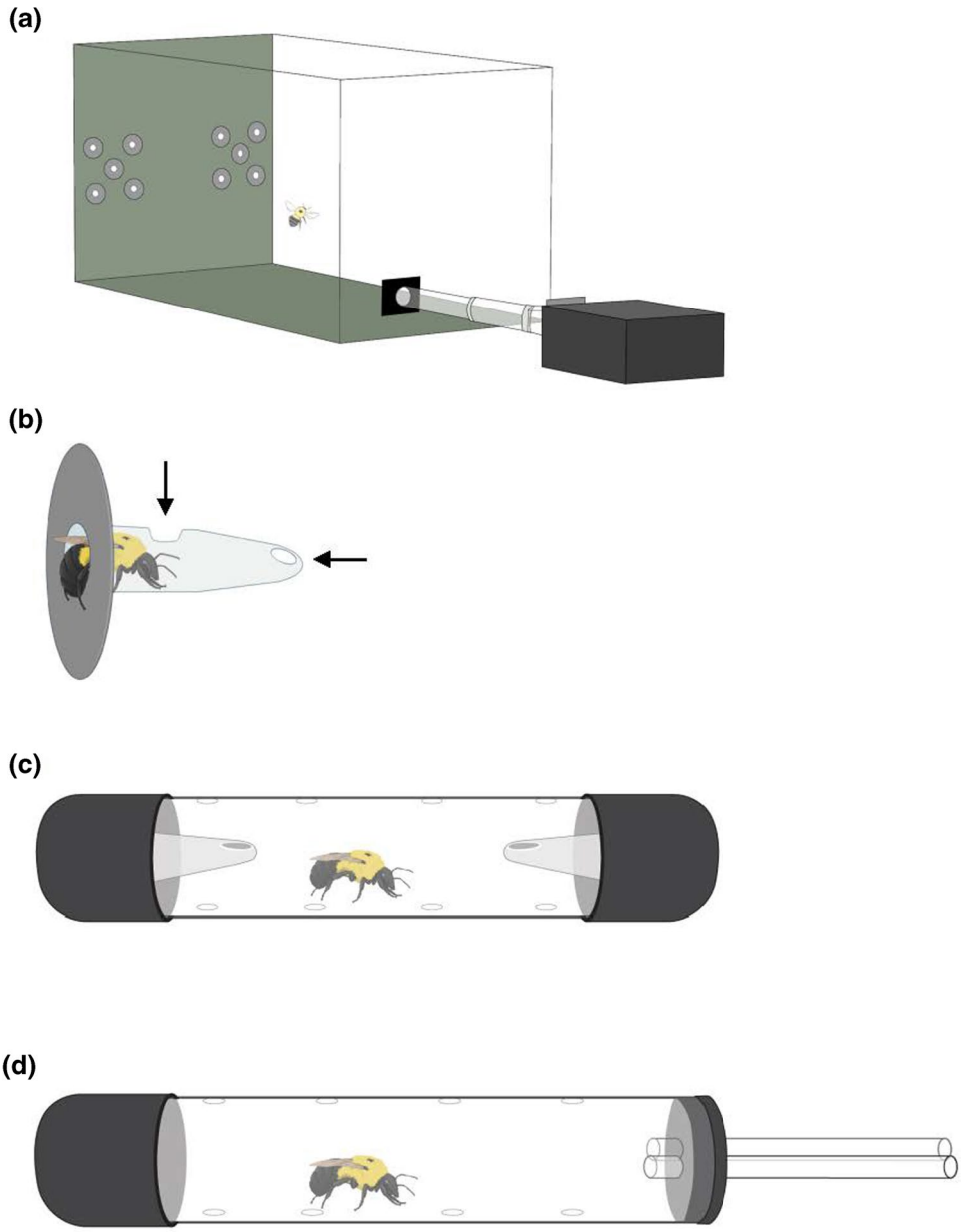

Fig. 1 Diagrams of experimental protocols. **a** Foraging arena equipped with two groups of five artificial flowers. Flower groups contained either the high-quality (30% or 45% (w/w)) or low-quality (15% w/w) sucrose type. A connector tube connected the colony box to the foraging arena, allowing individual bees to make foraging trips. **b** Artificial flowers used for shaping in testing in Experiment 1, Protocol A (free-flying assay). Arrows point to holes drilled in the Eppendorf tube where sucrose is injected into the artificial flower (horizontal arrow) and where bees are paint-marked on their thorax (vertical arrow). **c** Preference tubes used in the sequential reward offering assays (Experiments 1, Protocol B [restricted volume preference assay (RVPA)] and Experiment 2). Four µL of sucrose solution is injected into each Eppendorf tube, and replaced after a bee consumes all of the sucrose. **d** Preference tubes used in the ad libitum assay (Experiment 1, Protocol C) Two capillary tubes of either the high- or low-quality sucrose solution were inserted into the end of the preference tube. Illustrations by Ann Sanderson

to those expressed in a free-flying scenario, we offered bees the same choices as in Protocol A (15% vs. 30% or 15% vs. 45%). Subjects were contained in transparent plastic cylindrical preference tubes with ventilation holes (TAP plastics, USA; L x D 13 × 2.5 cm, wall thickness: 1.6 mm, Fig. 1c). Each preference tube was sealed at both ends with rubber caps fitted with modified 1.5 mL Eppendorf tubes. We drilled a hole in the tip of each Eppendorf tube and removed its cap, then fitted it inside of the rubber cap so that the tip faced into the preference tube (Fig. 1c). Bees were removed from the white-wicked feeders in the arena and briefly cold anesthetized (10 min at 4 °C) before being placed individually into preference tubes. Bees were given 90 min to acclimatize to the preference tube environment before a trial. When a trial began, we filled the tip of one Eppendorf feeder with 4 µL of 15% sucrose and filled the other feeder tip with 4 µL of either 30% or 45% sucrose using a repeater pipette ($n = 20$ and 19 bees for the 15% vs 30% and 45% treatments, respectively). We switched the position of the high and low sucrose treatments (right or left side) on each observation day. We observed bees for 30 min, recording each time a bee consumed sucrose from a feeder tip. After consumption, we immediately refilled the Eppendorf feeder. Analogously to Protocol A, in order for a trial to be included in analysis, the bee was required to consume sucrose from each feeder tip at least once, followed by at least ten additional consumption events at either tip within the 30 min observation period. Bees were

videotaped during the trial and euthanized following it as in free-flying protocol.

Experiment 1, Protocol C: Sucrose preferences under ad libitum feeding in a preference tube assay

To test whether sucrose preferences among bees in preference chambers offered unrestricted volumes of solution align with free-flying and RVPA methods, we assayed bees' ($n = 19$ bees/treatment) responses to the same sucrose concentration differentials as in Protocols A and B. Bees were tested in the transparent plastic cylindrical preference tubes used in Protocol B. Each preference tube was sealed at one end with a rubber cap and fitted with two feeding tubes at the other end (capillary tubes ID × L: 3.4 × 150 mm, World Precision Instruments, USA, Fig. 1d). One feeding tube was filled with 400 µL of 15% sucrose and the other was filled with 400 µL of either 30% or 45% sucrose. We removed bees from the white-wicked feeders and cold anesthetized them (10 min at 4 °C), before placing them into the preference tubes. After 90 min of acclimatization to the preference tubes, we provided bees with the feeding tubes and allowed them to feed ad libitum for 180 min. At the beginning of a trial, we used permanent marker to indicate the position of the meniscus of the sucrose in the feeding tubes, then recorded consumption at standardized time intervals by measuring the distance from the mark to the sucrose meniscus. We took measurements at 15, 30, 60, 90, 120, and 180 min. We ran separate evaporative controls ($n = 20$/treatment) following the same protocol, without a bee inside the preference tube. The data revealed no significant difference in evaporation rate among the different sucrose types (ANOVA $F_{2,57} = 0.617$, $p = 0.54$). All trials were recorded using a digital video camera as in Protocols A and B. After trials were completed, we analyzed behavior by watching the videos at 4 × speed (Figure S1). We calculated the number of times feeding on each sucrose type occurred, which we used to perform analyses analogous to Protocols A and B (see "Statistical Methods").

Experiment 2: Using the RVPA protocol to assess sucrose preferences following an acute dose of imidacloprid

After establishing that the RVPA protocol was a reliable proxy for a free-flying assay (Table 2), we used this approach to ask whether an acute dose of the neonicotinoid imidacloprid altered bees' preferences for 15% versus 30% sucrose. We dissolved 93.00 mg of analytical standard PEDESTAL® imidacloprid powder in 93 ml of acetone, resulting in a 1:1 stock solution. An aliquot of 20 µL of this solution was then added to 1 L of 15% sucrose solution. For the control solution containing no pesticide, the same volume of acetone was added to 1 L of the same concentration of sucrose. Solutions were stored in amber bottles in a refrigerator (at 4 ° C) and were always fed to bees immediately after being poured from these bottles (solutions were then immediately returned to the refrigerator). After 45 min, we used a pipette to dispense a 20 µL droplet of either the imidacloprid ($n = 22$ bees) or the control solution ($n = 18$ bees) and observed bees drink the entire droplet. Bees that did not drink the entire droplet were removed from the experiment. We allowed another 45 min to elapse (to maximize pesticide absorbance (Samuelson et al. 2016)) before commencing behavioral trials using the RVPA described in Experiment 1.

Statistical methods

All analyses were performed using R version 3.5.0 (R Core Team 2018). We evaluated sucrose preferences using three different measures, since even within a particular type of protocol, preference is often statistically analyzed in a variety of ways. The use of diverse approaches also allows for evaluation of different aspects of bee performance. For example, measuring the relative consumption of high- and low-quality sucrose solutions provides information on overall energy intake, while measuring sucrose choice over time provides information on how experience shapes preference. We performed identical analyses for Experiment 1, Protocols A, B, and C. Doing so allowed us to qualitatively compare the results of each protocol and assess the extent to which each of the tube-based protocols could replicate results of the free-flying protocol. If results are largely replicable, researchers may feel more confident in designing preference tube experiments without worrying about sacrificing ecological realism. We performed similar analyses for Experiment 2; however, we used a different explanatory variable, which we note below.

First, we made a general assessment of preference by asking whether bees made more drinking visits overall to the high-quality versus low-quality sucrose using separate paired *t* tests for the 15% versus 30% treatment and the 15% versus 45% treatment. In the Experiment 2 analysis, we tested imidacloprid-exposed versus non-exposed bees. Second, we asked whether a greater difference in sucrose concentration caused bees to make relatively more visits to the high-quality reward (15% versus 30% or 45%). For this test, we performed a generalized linear mixed model (binomial error distribution) using the proportion of drinking visits to the high-quality sucrose as the response variable and sucrose treatment (15% versus 30% or 45%) as the explanatory variable (lme4 package; Bates et al. 2015). In the Experiment 2 analysis, imidacloprid exposure (exposed/not exposed) was used as the explanatory variable. Because the range of visits made before bees consumed both sucrose types ranged from 2 to > 15, the number of drinking visits a bee made until it consumed both sucrose types

Table 2 Summary of Experiment 1 protocols and their outcomes

Question	Protocol	Assay	Test	Result	Replicate results of free-flying assay?
Do bees make more visits to the higher concentration than the lower concentration?	A	Free flying	Paired *t* test	Yes for bees in both treatment groups	n/a
	B	RVPA		Yes for bees in both treatment groups	Yes
	C	Ad libitum		Yes for bees in both treatment groups	Yes
Does a greater difference in sucrose concentration lead to more visits to the high-quality sucrose?	A	Free flying	Binomial GLM	Yes, bees in the 15% vs 45% treatment group made a higher proportion of visits to the high-quality sucrose than bees in the 15% vs 30% treatment	
	B	RVPA		Yes, bees in the 15% vs 45% treatment group made a higher proportion of visits to the high-quality sucrose than bees in the 15% vs 30% treatment	Yes
	C	Ad libitum		No, there was no between-treatment difference in proportion of visits to the high-quality sucrose	No
Are (a) bees more likely over time to choose the high-quality sucrose type, and (b) does the likelihood differ between treatments?	A	Free flying	Binomial GLM	No, bees were not more likely to choose the high-quality sucrose over time; however, bees in the 15% vs 45% treatment were more likely to do so overall	
	B	RVPA		Yes, bees were more likely to choose the high-quality sucrose over time, with a marginal between-treatment difference	No to (a), yes to (b)
	C	Ad libitum		No, bees were not more likely to choose the high-quality sucrose over time, nor was there a between-treatment difference	Yes to (a), no to (b)

Protocols B (Restricted Volume Preference Assay) and C (ad libitum preference assay) were conducted to determine whether sucrose preferences observed in Protocol A (free-flying assay) were replicable using a protocol that confined bees to a preference tube. Generally, tube-based approaches can serve as a proxy for free-flying assays, with the sequential offering approach more closely matching free-flying results than the ad libitum approach

was included as a covariate. Bee identity was included as an observation-level random effect to account for overdispersion. We originally included colony as a separate random effect; however, colony-level variance was estimated at zero, so it was removed from the final model. Finally, we asked whether bees were more likely to choose the higher-quality sucrose over time, i.e., as they gained experience during a single foraging bout. This was determined using a generalized linear mixed model (binomial error distribution) with a choice of the high-quality sucrose as the response variable (0/1), and visit number and treatment as crossed explanatory variables, spatial location of the high-quality sucrose as a fixed factor, and bee ID as a random effect (lme4 package; Bates et al. 2015). In the Experiment 2 analysis, imidacloprid exposure replaced treatment as an explanatory variable. We originally included colony as a separate random effect; however, colony-level variance was estimated at zero, so it was removed from the final model. We tested for overall treatment effects of categorical variables using likelihood ratio tests. We also assessed sampling behavior; methods and results for these analyses are reported in Online Supplement 1.

We performed separate analyses for Experiment 1, Protocol C in addition to those described above. Measuring consumption volume over time is a popular method for determining sucrose preferences in bees (Nicolson et al. 2013; Kessler et al. 2015). While not all researchers record ad libitum preference trials (some simply measure consumption at a given timepoint), we opted to also videotape these trials to better understand behavioral differences that might manifest across protocols. For example, the order in which bees encountered different sucrose solutions or how much experience they had with one solution before sampling another could shape their preferences. For the consumption analyses, we calculated a preference index as (volume high-quality sucrose consumed—volume low-quality sucrose consumed)/(volume high-quality sucrose consumed + volume low-quality sucrose consumed) (Nicolson et al. 2013; Kessler et al. 2015). Positive values indicate a preference for the high-quality sucrose type and negative values indicate a preference for the low-quality sucrose type (Asparch et al. 2016). We separately assessed cumulative and non-cumulative differences in consumption volume over time. In each analysis, we performed a linear model with the difference in consumption volume as the response variable and sucrose treatment (15% versus 30% or 15% versus 45%) and the measurement time as categorical explanatory variables. We included bee ID as a random effect to account for repeated measures.

Results

Experiment 1, Protocol A: Sucrose preferences in a free-flying context in a foraging arena

As expected, our free-flying protocol captured a preference for higher concentration sucrose in both choice contexts (Table 3). This effect was stronger when there was a greater difference between the sucrose concentrations. Bees in the 15% versus 45% sucrose treatment were more likely to drink the more rewarding sucrose type than bees in the 15% versus 30% sucrose treatment (model estimate $\pm$ SE = 1.367 $\pm$ 0.384, $z = 3.563$, $p < 0.001$, Fig. 2a). Bees' preferences were not biased by their initial choices, since their probability of drinking the high-quality sucrose was not explained by the number of visits until both sucrose types were sampled, nor by whether the bee sampled the high-quality sucrose first. Bees were not more likely to choose the high-quality sucrose over time on a per-visit basis when considering both treatments together (visit number; model estimate $\pm$ SE = 0.011 $\pm$ 0.018, $z = 0.600$, $p = 0.55$; Fig. 2d); however, the significant interaction between visit number and treatment ($\chi^2_2 = 34.943$, $p < 0.001$) indicates that bees in the 15% versus 45% treatment were faster to consistently choose the high-quality sucrose. Bees in the 15% versus 45% treatment were also more likely to choose the high-quality sucrose overall ($\chi^2_2 = 4.007$, $p = 0.04$).

Experiment 1, Protocol B: Sucrose preferences in a restricted volume preference assay (RVPA)

Bees in the RVPA protocol generally exhibited the same behavioral patterns and preferences as bees in the free-flying assay. In both the small-difference and large-difference treatment groups, bees collected more high-quality sucrose (Table 3), but the magnitude of this preference was dependent on treatment. Bees in the 15% versus 45% sucrose treatment were more likely to consume the higher concentration sucrose solution than bees in the 15% versus 30% sucrose treatment (all visit types: model estimate $\pm$ SE = 0.911 $\pm$ 0.262, $z = 3.480$, $p < 0.001$; drinking visits only: model estimate $\pm$ SE = 1.499 $\pm$ 0.403, $z = 3.719$, $p < 0.001$; Fig. 2b). Again, these preferences were not influenced by the first choices bees made: the proportion of visits to the high-quality sucrose was not explained by the number of visits until both sucrose types were sampled, nor by whether the bee sampled the high-quality sucrose first. While bees followed the same behavioral trend as free-flying bees on a per-visit basis, there were some quantitative differences between free-flying and RVPA

Table 3 Results of paired *t* tests for all experiments, asking if bees preferred the high-quality sucrose over the low-quality sucrose, based upon behavior displayed during laboratory assays

Experiment	Treatment group	Treatment level	Mean ± SD	*t* value	*df*	*p* value
1 (Free flying)	15% vs 30%	High quality	9.55 ± 3.24	2.81	19	0.01
		Low quality	6.45 ± 3.25			
1 (Free flying)	15% vs 45%	High quality	12.76 ± 4.04	7.41	20	< 0.001
		Low quality	2.90 ± 2.76			
1 (RVPA)	15% vs 30%	High quality	14.10 ± 5.99	3.92	19	< 0.001
		Low quality	5.25 ± 6.23			
1 (RVPA)	15% vs 45%	High quality	17.21 ± 5.94	11.22	18	< 0.001
		Low quality	1.58 ± 1.07			
1 (ad libitum)	15% vs 30%	High quality	112.47 ± 41.93	5.38	18	< 0.001
		Low quality	98.95 ± 35.72			
1 (ad libitum)	15% vs 45%	High quality	121.47 ± 52.58	6.65	18	< 0.001
		Low quality	102.84 ± 48.16			
2	Imidacloprid	High quality	10.50 ± 5.91	3.65	21	0.002
		Low quality	4.45 ± 3.83			
2	Control	High quality	12.83 ± 5.14	6.35	17	< 0.001
		Low quality	3.06 ± 2.90			

In Experiment 1, the high-quality sucrose is indicated by "treatment group", with the percentages indicating sucrose concentration. A higher concentration is considered higher quality. In Experiment 2, the high- and low-quality sucrose concentrations are always 30% and 15%, respectively. Mean and SD values represent the number of times a bee chose a given sucrose concentration

protocols (Fig. 2e). In the RVPA, bees were more likely to choose the high-quality sucrose over time (model estimate ± SE = 0.127 ± 0.017, $z = 7.337$, $p < 0.001$; Fig. 2e), and this effect was stronger in the 15% versus 45% treatment group (interaction between visit number and treatment: $\chi^2_2 = 7.519$, $p < 0.001$). Additionally, while there was evidence for bees in the 15% versus 45% treatment being more likely to choose the high-quality sucrose overall (model estimate ± SE = 0.816 ± 0.48), the difference was not statistically significant ($\chi^2_2 = 2.850$, $p = 0.09$).

Experiment 1, Protocol C: Sucrose preferences under ad libitum feeding in a preference tube assay

In contrast to the RVPA protocols, preferences measured in the ad libitum protocol did not always represent those of free-flying bees. While bees in each treatment group made significantly more visits to the high-quality reward (similar to free-flying and RVPA; Table 3), the proportion of drinking visits to the high-quality sucrose type did not significantly differ between sucrose treatments (Fig. 2c). Also in contrast to free-flying and RVPA protocols (where the ordering of encounters with different solution types did not matter), bees that encountered the high-quality sucrose type first made fewer subsequent visits to it (model covariate, model estimate ± SE = − 1.816 ± 0.606, $z = -2.995$, $p = 0.003$). When we examined bee sucrose concentration preferences over the course of the observation period, we again found marked differences between the results of the ad libitum protocol and those of the free-flying and RVPA methods: there was no significant difference between the proportion of visits to the low- versus high-quality sucrose (model estimate ± SE = − 0.01 ± 0.02, $z = -1.322$, $p = 0.19$, Fig. 2c), nor was there a between-treatment significant difference in whether bees were more likely overall to choose the high-quality sucrose ($\chi^2_2 = 0.018$, $p = 0.89$, Fig. 2f). There was, however, a significant interaction between visit number and treatment ($\chi^2_2 = 6.731$, $p = 0.009$) that was qualitatively reversed from the patterns observed in the free-flying and RVPA methods. Bees in the 15% versus 30% treatment took fewer visits to begin consistently drinking the high-quality sucrose than bees in the 15% versus 45% treatment. Using consumption data (preference index), we were generally unable to determine a preference for high-quality sucrose (cumulative difference: $\chi^2_1 = 1.98$, $p = 0.16$; non-cumulative: difference: $\chi^2_2 = 2.35$, $p = 0.13$; Fig. 3).

Experiment 2: Sucrose preferences following an acute dose of imidacloprid in a restricted volume preference assay

Bees that consumed imidacloprid prior to being tested were slower to develop a preference for the high-quality sucrose than control bees; when we examined whether bees were more likely to choose the high-quality sucrose on a per-visit basis, we found that control bees were more likely to do so ($\chi^2_2 = 3.973$, $p = 0.04$, Fig. 4b). While both treatment and control bees developed a preference for the higher-quality sucrose over trials (model estimate ± SE = 0.222 ± 0.027, $z = 8.099$, $p < 0.001$, Fig. 4b),

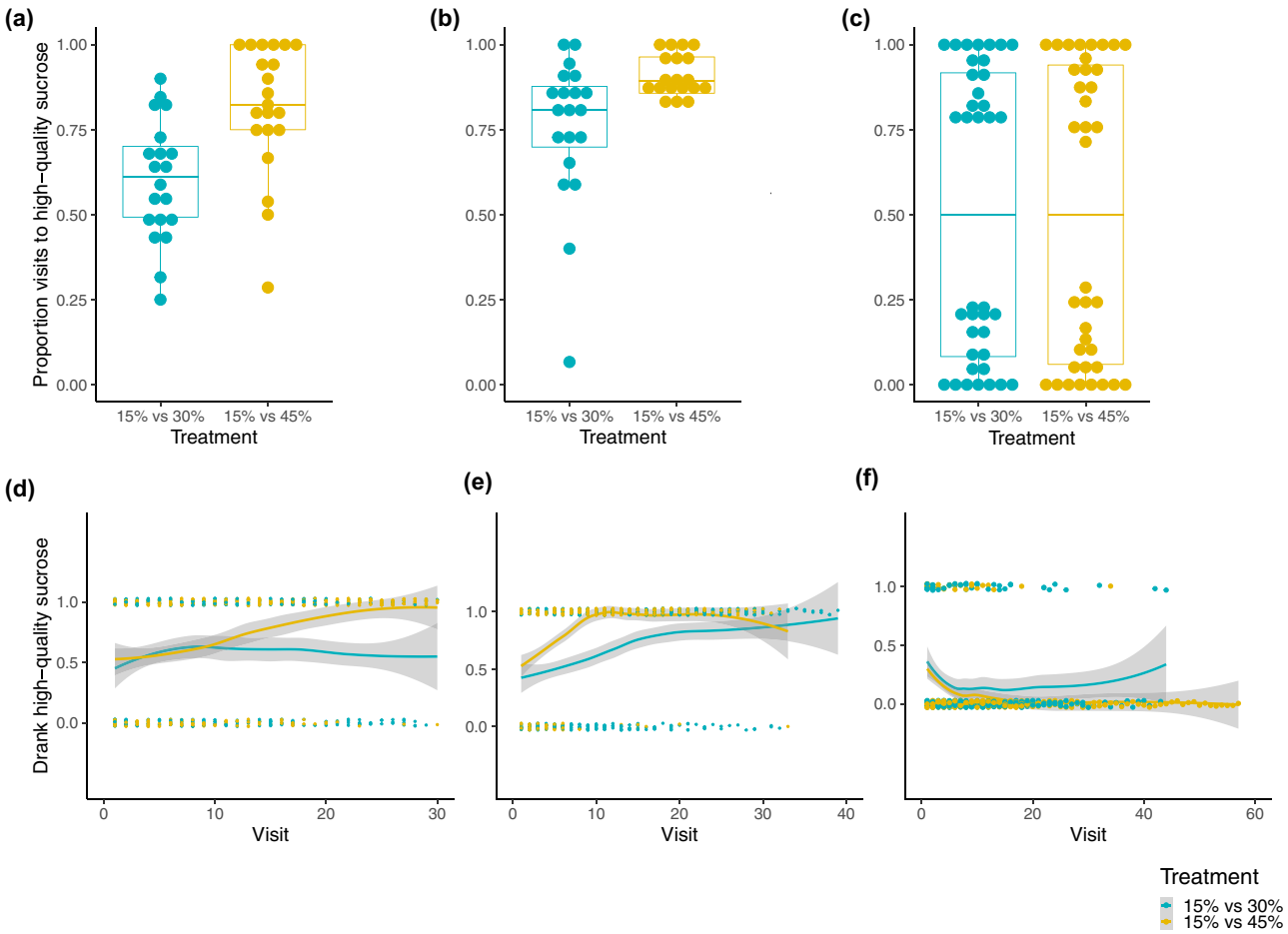

Fig. 2 Results of Experiment 1. **a**–**c** Proportion of visits made to different sucrose types. Boxplot represents median and interquartile range, and each dot represents a single data point (bee). Boxplot on the right shows data for the 15% vs. 30% treatment; boxplot on the left shows data for the 15% vs 45% treatment. Each panel represents a different protocol used in Experiment 1: **a** free flying, **b** restricted volume preference assay, **c** ad libitum. **d**–**f** Probability that a bee consumed the high-quality sucrose for each drinking visit. Blue points represent a smaller difference in sucrose concentrations (15% vs. 30%) and yellow points represent a larger difference in sucrose concentrations (15% vs. 45%). Each point represents a visit; curves are fit using Loess smoothing and shaded region represents SE. Each panel represents a different protocol used in experiment 1: **d** free flying, **e** restricted volume preference assay, **f** ad libitum. Note differences in *x*-axis scale

neonicotinoid-exposed bees were slower to develop this preference (significant visit number × treatment interaction: $\chi^2_2 = 12.370$, $p < 0.001$), suggesting that bees given an acute dose of imidacloprid discriminate less strongly against the lower-quality sucrose. When considering preference independent of time, bees in the two treatments did not statistically significantly differ in their proportion of visits to the high-quality sucrose, although the model estimate and standard error indicate a trend toward lower preference in IMD-dosed bees (model estimate ± SE = − 0.808 ± 0.477, $z = -1.696$ $p = 0.07$; Fig. 4a). We found no effects of the other variables we addressed on preference, including the number of visits until both sucrose types were sampled and whether the bee sampled the high-quality sucrose first.

Discussion

Accurately measuring bee foraging preferences is critical in the face of global declines, where nutritional factors are increasingly recognized as prime drivers of performance in the face of land use-associated changes in floral resources and exposure to systemic pesticides (Goulson et al. 2015). Yet, studies attempting to quantify bee nectar preferences use a variety of different methods that vary in their practical difficulty, throughput, and ecological realism. Here we compared two broad categories of popular approaches (free-flying on artificial flowers vs. confined to preference tubes) and found that they returned important differences in the foraging choices made by individual

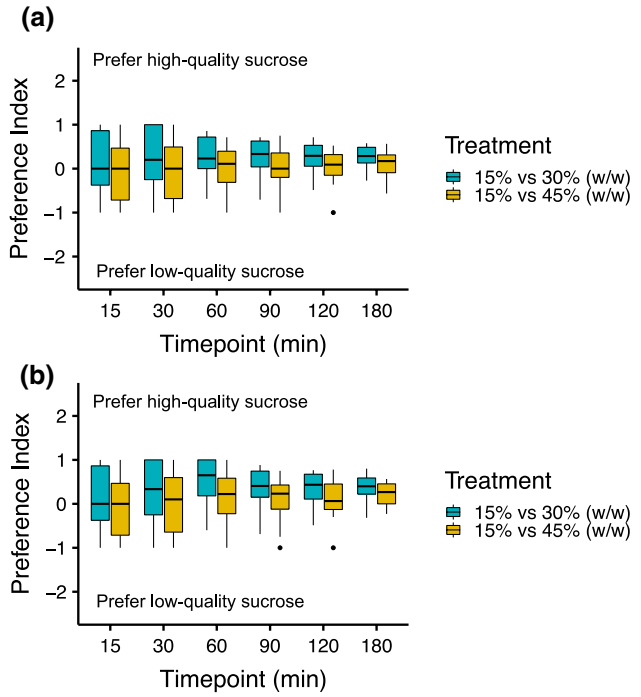

Fig. 3 Preference index data from Experiment 1, Protocol C (ad libitum) based on cumulative consumption differences (**a**) and non-cumulative consumption differences (**b**). Box-and-whisker plots represent bee preference for the high-quality sucrose in the 15% vs. 30% (w/w) treatment (blue bars) and the 15% vs. 45% (w/w) treatment (yellow bars) at each timepoint. Positive values indicate a preference for the high-quality sucrose type; negative values indicate a preference for the low-quality sucrose type

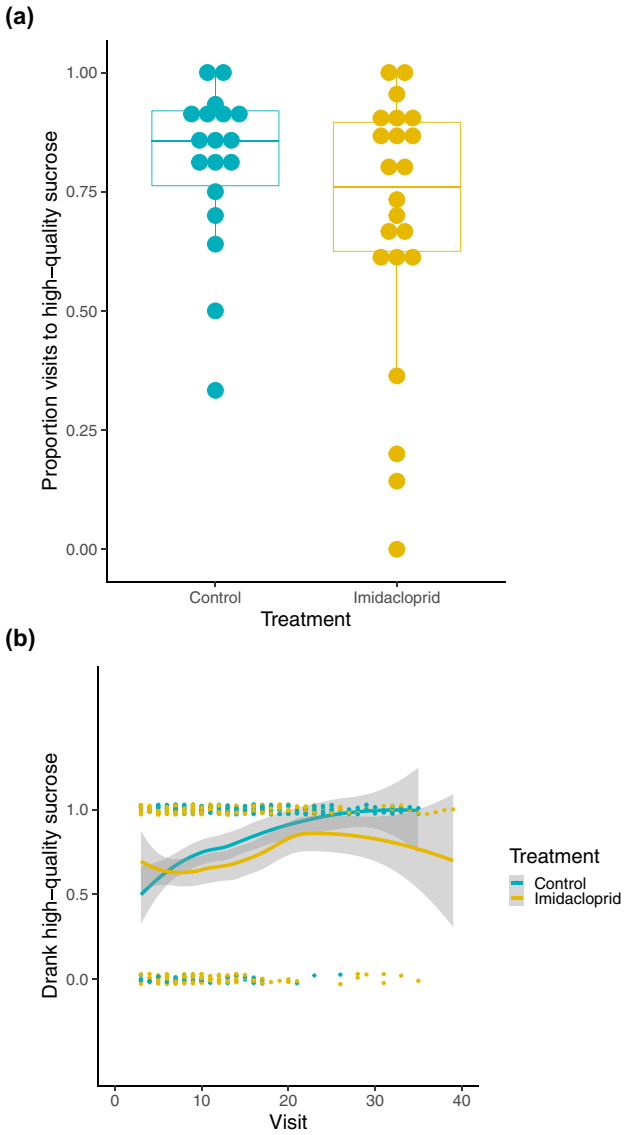

Fig. 4 Results of Experiment 2, which tests the effect of acute imidacloprid exposure on bees' ability to discriminate between high- and low-quality sucrose. **a** Proportion of visits made to different sucrose types (15%. vs 30%). Boxplots represent proportion of visits made to the high-quality sucrose type by bees that did not ingest imidacloprid (left side) and those that did (right side). Each point represents an individual bee. **b** Probability that a bee consumed the high-quality sucrose for each drinking visit. Blue points represent control bees (no ingestion of imidacloprid) and yellow points represent bees that did ingest imidacloprid. Each point represents a visit; curves are fit using Loess smoothing and shaded region represents SE

bumble bee workers. We developed a novel methodology (RVPA) which we argue may serve as a better proxy to a free-flying scenario than the ad libitum feeding assays in common use. Further, RVPA is time-efficient, yielding replicable data of free-flying assays, but following a simpler and more executable protocol. We demonstrated the utility of the RVPA approach for research into the sublethal effects of pesticides on bee foraging behavior by characterizing the sugar concentration preferences of bees exposed to an acute dose of imidacloprid. We found that bees exposed to this common neonicotinoid are less likely to choose high-quality rewards over time. RVPA offers a high-throughput approach that can easily be adopted in the laboratory to address questions about foraging behavior in a number of ecological contexts including understanding how pesticides or other nectar constituents might alter foraging decision-making in bees.

Sucrose preferences in a free-flying context

In Experiment 1, Protocol A (free-flying scenario), bees clearly preferred 30% and 45% sucrose over 15% sucrose (Table 3). These results are broadly consistent with previous work reporting bumble bee preferences for higher sugar concentrations in nectar (Cnaani et al. 2006). Furthermore, they were more likely to prefer the high-quality sucrose when the difference in concentration between the low- and high-quality options was greater (Fig. 2a). When the concentration in the high-quality option was twice that of the

low-quality option, bees tended to sample both options more frequently, switching back and forth between the two types (Online Supplement 1). These results suggest a threshold level of difference between sugar concentrations of different flowers, above which bees will abandon one option in favor of another. This finding is consistent with studies reporting nonlinear increases in visits to high-concentration sucrose relative to the difference in concentrations (*e.g.*, distance effect; Waddington 2001; Nachev et al. 2013). In nature, nectar sugar concentration can vary within and between plant species (Lanza et al. 1995; Herrera et al. 2006). To cope with the variability of nectar reward quality, bees may continue to sample different flower types so long as the difference in quality between them is negligible. A recent laboratory study testing *B. impatiens* workers' ability to track high-sucrose rewards when reward quality was highly variable suggests bees made more discrimination errors (and therefore sampled more widely) when the difference in concentration was low (Dunlap et al. 2017). When the difference in quality between flower types becomes great enough, bees may make the decision to stay constant to the high-quality option. For example, *Bombus fervidus* workers presented with artificial flowers containing either 10%, 20%, or 30% sucrose solution did not display preferences for any one type; conversely, *B. impatiens* workers presented with artificial flowers containing either 13% or 40% sucrose solution highly preferred the high-concentration sucrose (Waddington 1995; Cnaani et al. 2006).

Sucrose preferences using preference tube assays

One of the goals of this study was to evaluate experimental approaches that use preference tubes in terms of their utility as an analog to free-flying laboratory assays. A major benefit of preference tube approaches is the ability to collect data in a relatively short amount of time (see Table S1 for our estimates of experimenter effort/data point). In free-flying methodologies, bees generally need to be trained to visit artificial flowers before undergoing lengthy testing protocols. In tube-based methodologies, the training and testing process is greatly simplified. Furthermore, because preference tubes are small and contained compared to large flight arenas, researchers are able to collect data from multiple subjects simultaneously. Despite the clear benefits of tube-based approaches, the extent to which they replicate a free-flying scenario has been an open question.

We found that the structure of reward presentation plays a large role in whether bees behave similarly in preference tubes and in flight arenas. Specifically, offering sequential rewards (small amounts given out over a period of time following the RVPA protocol) yielded results that were almost completely replicable to a free-flying scenario, *i.e.* bees expressed the same sucrose preferences as in Protocol A (Table 3, Fig. 2a–b, d–e). When rewards were provided in large quantities for bees to feed ad libitum (Protocol C), we were less able to discern the same sucrose preferences as bees in the free-flying assay (Figs. 2a, c, d, f, 3). Additionally, bees feeding ad libitum that encountered the high-quality sucrose type first made fewer subsequent visits to it. This behavior may be attributed to the fact that bees become satiated by consuming a large amount of sucrose in a single visit. If so, following an ad libitum protocol may not adequately capture natural sampling behavior, especially considering that most flowers visited by bumble bees naturally produce relatively small nectar volumes (Willmer 2011). In contrast, our results clearly show that preference tube assays providing sequential rewards are a highly effective method for conducting experiments that yield similar results to a free-flying scenario. By offering bees small amounts of sucrose sequentially, we were able to simulate natural flower sampling, *e.g.* forcing bees to continue seeking out floral rewards. This approach ensures that bees are not able to satiate themselves on one single food source and therefore should allow experimenters to more easily discern preference based on observed foraging choices. Therefore, the RVPA approach may work better than an ad libitum approach in keeping bees motivated to sample. It is not known whether restricting bees to a tube, where they must crawl rather than fly, affects foraging motivation generally.

It is important to note that experimenter effort required may also be highly variable, especially in a free-flying scenario. Individual colonies may produce workers that vary in their foraging motivation, which can lead to differences in the length of time it takes to collect data. The amount of experimenter effort required for ad libitum protocols also depends on whether or not experimenters videotape subjects, which is not always necessary to answer questions about consumption volume; however, it is worth noting that the behavioral data we gleaned from videos of the ad libitum trials returned results matching those of free-flying protocol much better than consumption alone (Figure S1). Use of tracking software may allow researchers to reduce video analysis time and therefore make longer-term assays such as the ad libitum protocol more feasible. However, given the reduced accuracy of the ad libitum protocol in capturing behaviors seen in the free-flying assay and potential for satiation that affects sampling, we advocate for the RVPA protocol in experiments meant to capture ecologically realistic scenarios on the timescale of bee foraging bouts. Because we ran the ad libitum protocol for a maximum of 180 min, the extent to which this protocol might better match bee free-flying foraging preferences over longer timescales (e.g. 24 h or across multiple days) remains an open question. To our knowledge, the RVPA protocol is a novel approach, or at least widely under-used given its utility. Given the complexity of animal behavior, particularly when conducting studies

incorporating multiple behavioral drivers (for example, in this study, pesticide exposure and differences in nectar sugar concentration), researchers seek methods that best capture the dynamics of a natural setting, and the RVPA holds this potential.

Sucrose preferences following imidacloprid exposure

When we tested for preference on a per-visit basis, we found strong evidence that ingestion of imidacloprid reduces the probability that bees choose the high-quality sucrose option (Fig. 4b). As noted above and shown in Experiment 1 free-flying and RVPA assays, bees generally display a keen ability to discriminate between sucrose concentrations. However, we found that consuming an acute dose of a neonicotinoid appears to interfere with that process, slowing down the development of a preference for high-quality sucrose. To our knowledge, this result is novel among studies examining the effects of neonicotinoids on bees' preference expression. A suppressed ability to distinguish high-concentration from low-concentration floral nectar may have negative repercussions for individual *Bombus* workers and for entire colonies. These effects may be sensory as well as metabolic. For instance, reduced functioning of the gustatory system, driven by neonicotinoid exposure, may impair workers' ability to discriminate between food sources in honey- and bumble bees (Lambin et al. 2001; Alkassab and Kirchner 2017). Reduced sensory function may go on to impair metabolic performance, as workers rely on floral nectar to meet their caloric and carbohydrate needs (Plowright and Silverman 2000). The inability to preferentially forage on nectar high in sugar may lead to inefficient metabolism, which can decrease worker longevity and decrease their ability to regulate processes within the colony (Bowers 1986; Plowright and Silverman 2000). However, bees may be able to make up for the loss in sugar intake. In honey bees (*Apis mellifera*), exposure to the neonicotinoid thiamethoxam caused a reduced sensitivity (proboscis-extension response) to rising sucrose levels, albeit on a longer timescale (14 days) than was measured in this study (Démares et al. 2016). If bees were given the option to balance their diet with adequate protein, however, survival did not suffer (Démares et al. 2016). It is possible that bees are less discriminating between high and low sucrose concentration following exposure to imidacloprid because their metabolism is already suppressed and they do not require as much sugar as they would without having been exposed. Previous work has shown that neonicotinoids greatly reduce feeding motivation in bees (Lämsä et al. 2018), and it may be that individual workers can afford to be less discriminating of floral rewards. It is important to note that reduced feeding motivation can also cause workers to neglect colony provisioning and nursing behavior (Crall et al. 2018), which can have negative fitness consequences at the colony level.

While we did find some evidence that consumption of imidacloprid reduced the proportion of visits to the high-quality sucrose type (by evaluating the effect size and its standard error), the result was not statistically significant. One reason we did not see a stronger effect of imidacloprid on bee behavior could be due to the concentration of imidacloprid used in the sucrose solution. We chose a concentration of 20 ppb, based upon previous studies reporting concentrations of neonicotinoids found in natural floral nectar (Goulson 2013). However, in studies that report dose-dependent effects of neonicotinoids on bumble bee foraging or sucrose sampling behavior, generally bees perform worse under exposure to higher pesticide concentrations (Stanley et al. 2016; Phelps et al. 2018). Considering a range of imidacloprid concentrations may yield further insight into the effect of neonicotinoid exposure on bees' ability to discriminate between different floral rewards.

Conclusions

Bumble bees develop preferences for floral rewards based on energetic needs, nutritional context, and physiological state. Quantifying bumble bee preferences is critical for understanding how these preferences may be shaped by ecological factors, as well as how preferences scale up to affect plant-pollinator interactions. We provide researchers with an easy-to-implement laboratory preference assay (RVPA) that can be used as an analog for more complicated free-flying approaches. We advocate for expanded use of this protocol to ask more nuanced questions; for example, how drivers of global change such as exposure to pesticides affect preference expression. We provide an example of how the protocol could be used to test whether exposure to neonicotinoids affects the expression of sucrose concentration preference and find that the pesticide imidacloprid may inhibit bees' ability to distinguish between low- and highly rewarding sucrose types. Altogether, the results will be useful for future studies that aim to understand how foraging preferences contribute to pollinator fitness.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00442-021-04979-8.

Acknowledgements The authors thank Koppert Biological Systems for bumble bee colonies, and T. Catlett, R. Dhami, J. Kailey, and S. Miller for assistance with experiments. We thank two anonymous reviewers for insightful comments on a previous version of the manuscript. Funding was provided by the United States Department of Agriculture (NIFA 2018-67014-27543) to ASL and US Department of Agriculture NIFA postdoctoral fellowship and a L'Oréal For Women in Science postdoctoral fellowship to FM.

Author contribution statement All authors conceived of the study and designed methodology. SR collected data. SR and FM analyzed data. SR led the writing of the manuscript. All authors contributed critically to drafts and gave final approval for publication.

References

Alkassab AT, Kirchner WH (2017) Sublethal exposure to neonicotinoids and related side effects on insect pollinators: honeybees, bumblebees, and solitary bees. J Plant Dis Prot 124:1–30. https://doi.org/10.1007/s41348-016-0041-0

Arce AN, Ramos Rodrigues A, Yu J et al (2018) Foraging bumblebees acquire a preference for neonicotinoid-treated food with prolonged exposure. Proc R Soc B Biol Sci 285:20180655. https://doi.org/10.1098/rspb.2018.0655

Asparch Y, Pontes G, Masagué S et al (2016) Kissing bugs can generalize and discriminate between different bitter compounds. J Physiol Paris 110:99–106. https://doi.org/10.1016/j.jphysparis.2016.11.006

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

Bell G, Lefebvre L, Giraldeau L-A, Weary D (1984) Partial preference of insects for the male flowers of an annual herb. Oecologia 64(3):287–294. https://doi.org/10.1007/BF00379123

Blacquière T, Smagghe G, van Gestel CAM, Mommaerts V (2012) Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. Ecotoxicology 21:973–992. https://doi.org/10.1007/s10646-012-0863-x

Bowers MA (1986) Resource availability and timing of reproduction in bumble bee colonies (Hymenoptera: Apidae). Environ Entomol 15:750–755. https://doi.org/10.1093/ee/15.3.750

Chesson J (1983) The estimation and analysis of preference and its relationship to foraging models. Ecology 64:1297–1304. https://doi.org/10.2307/1937838

Chittka L, Gumbert A, Kunze J (1997) Foraging dynamics of bumble bees: correlates of movements within and between plant species. Behav Ecol 8:239–249. https://doi.org/10.1093/beheco/8.3.239

Cnaani J, Thomson JD, Papaj DR (2006) Flower choice and learning in foraging bumblebees: effects of variation in nectar volume and concentration. Ethology 112:278–285. https://doi.org/10.1111/j.1439-0310.2006.01174.x

Crall JD, Switzer CM, Oppenheimer RL et al (2018) Neonicotinoid exposure disrupts bumblebee nest behavior, social networks, and thermoregulation. Science 362:683. https://doi.org/10.1126/science.aat1598

Démares FJ, Crous KL, Pirk CWW et al (2016) Sucrose sensitivity of honey bees is differently affected by dietary protein and a neonicotinoid pesticide. PLoS ONE 11:e0156584. https://doi.org/10.1371/journal.pone.0156584

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

Goulson D (2013) An overview of the environmental risks posed by neonicotinoid insecticides. J Appl Ecol 50:977–987. https://doi.org/10.1111/1365-2664.12111

Goulson D, Cruise JL, Sparrow KR et al (2007) Choosing rewarding flowers; perceptual limitations and innate preferences influence decision making in bumblebees and honeybees. Behav Ecol Sociobiol 61:1523–1529. https://doi.org/10.1007/s00265-007-0384-4

Goulson D, Nicholls E, Botías C, Rotheray EL (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347:1255957. https://doi.org/10.1126/science.1255957

Heinrich B (1976) The foraging specializations of individual bumblebees. Ecol Monogr 46:105–128. https://doi.org/10.2307/1942246

Herrera CM, Pérez R, Alonso C (2006) Extreme intraplant variation in nectar sugar composition in an insect-pollinated perennial herb. Am J Bot 93:575–581. https://doi.org/10.3732/ajb.93.4.575

Kessler SC, Tiedeken EJ, Simcock KL et al (2015) Bees prefer foods containing neonicotinoid pesticides. Nature 521:74–76. https://doi.org/10.1038/nature14414

Lambin M, Armengaud C, Raymond S, Gauthier M (2001) Imidacloprid-induced facilitation of the proboscis extension reflex habituation in the honeybee. Arch Insect Biochem Physiol 48:129–134. https://doi.org/10.1002/arch.1065

Lämsä J, Kuusela E, Tuomi J et al (2018) Low dose of neonicotinoid insecticide reduces foraging motivation of bumblebees. Proc R Soc B Biol Sci 285:20180506. https://doi.org/10.1098/rspb.2018.0506

Lanza J, Smith GC, Sack S, Cash A (1995) Variation in nectar volume and composition of Impatiens capensis at the individual, plant, and population levels. Oecologia 102:113–119

Leadbeater E, Chittka L (2007) Social learning in insects—from miniature brains to consensus building. Curr Biol 17:R703–R713. https://doi.org/10.1016/j.cub.2007.06.012

Levin DA (1972) Low frequency disadvantage in the exploitation of pollinators by corolla variants in *Phlox*. Am Nat 106:453–460

Lundin O, Rundlöf M, Smith HG, Fries I, Bommarco R (2015) Neonicotinoid insecticides and their impacts on bees: a systematic review of research approaches and identification of knowledge gaps. PLoS ONE 10(8):e0136928. https://doi.org/10.1371/journal.pone.0136928

Masek P, Scott K (2010) Limited taste discrimination in *Drosophila*. Proc Natl Acad Sci USA 107:14833. https://doi.org/10.1073/pnas.1009318107

Mogren CL, Lundgren JG (2016) Neonicotinoid-contaminated pollinator strips adjacent to cropland reduce honey bee nutritional status. Sci Rep 6:29608. https://doi.org/10.1038/srep29608

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

Muth F, Breslow PR, Masek P, Leonard AS (2018) A pollen fatty acid enhances learning and survival in bumblebees. Behav Ecol 29:1371–1379. https://doi.org/10.1093/beheco/ary111

Nachev V, Thomson JD, Winter Y (2013) The psychophysics of sugar concentration discrimination and contrast evaluation in bumblebees. Anim Cogn 16:417–427. https://doi.org/10.1007/s10071-012-0582-y

Nicolson SW, de Veer L, Köhler A, Pirk CWW (2013) Honeybees prefer warmer nectar and less viscous nectar, regardless of sugar concentration. Proc R Soc B Biol Sci 280:20131597. https://doi.org/10.1098/rspb.2013.1597

Palmer-Young EC, Calhoun AC, Mirzayeva A, Sadd BM (2018) Effects of the floral phytochemical eugenol on parasite evolution and bumble bee infection and preference. Sci Rep 8(1):1–11. https://doi.org/10.1038/s41598-018-20369-2

Perry G, Pianka ER (1997) Animal foraging: past, present and future. Trends Ecol Evol 12:360–364. https://doi.org/10.1016/S0169-5347(97)01097-5

Phelps JD, Strang CG, Gbylik-Sikorska M et al (2018) Imidacloprid slows the development of preference for rewarding food sources in bumblebees (*Bombus impatiens*). Ecotoxicology 27:175–187. https://doi.org/10.1007/s10646-017-1883-3

Pleasants JM (1989) Optimal foraging by nectarivores: a test of the marginal-value theorem. Am Nat 134:51–71. https://doi.org/10.1086/284965

Plowright CMS, Silverman A (2000) Nectar and pollen foraging by bumble bees (Hymenoptera: Apidae): choice and tradeoffs. Can Entomol 132:677–679. https://doi.org/10.4039/Ent132677-5

Pyke GH (1984) Optimal foraging theory: a critical review. Annu Rev Ecol Syst 15:523–575. https://doi.org/10.1146/annurev.es.15.110184.002515

R Core Team (2018) R: A language and environment for statistical computing. Version 3.5.0. R Foundation for Statistical Computing. Vienna. https://www.R-project.org/

Real L, Caraco T (1986) Risk and foraging in stochastic environments. Annu Rev Ecol Syst 17:371–390. https://doi.org/10.1146/annurev.es.17.110186.002103

Richardson LL, Adler LS, Leonard AS et al (2015) Secondary metabolites in floral nectar reduce parasite infections in bumblebees. Proc R Soc B Biol Sci 282:20142471. https://doi.org/10.1098/rspb.2014.2471

Richardson LL, Bowers MD, Irwin RE (2016) Nectar chemistry mediates the behavior of parasitized bees: consequences for plant fitness. Ecology 97:325–337. https://doi.org/10.1890/15-0263.1

Rotheray EL, Osborne JL, Goulson D (2017) Quantifying the food requirements and effects of food stress on bumble bee colony development. J Apic Res 56:288–299. https://doi.org/10.1080/00218839.2017.1307712

Samuelson EEW, Chen-Wishart ZP, Gill RJ, Leadbeater E (2016) Effect of acute pesticide exposure on bee spatial working memory using an analogue of the radial-arm maze. Sci Rep 6:38957. https://doi.org/10.1038/srep38957

Schaeffer RN, Mei YZ, Andicoechea J, Manson JS, Irwin RE (2017) Consequences of a nectar yeast for pollinator preference and performance. Funct Ecol 31(3):613-621. https://doi.org/10.1111/1365-2435.12762

Schiestl FP (2017) Innate receiver bias: its role in the ecology and evolution of plant–animal interactions. Annu Rev Ecol Evol Syst 48:585–603. https://doi.org/10.1146/annurev-ecolsys-110316-023039

Simpson SJ, Sibly RM, Lee KP et al (2004) Optimal foraging when regulating intake of multiple nutrients. Anim Behav 68:1299–1311. https://doi.org/10.1016/j.anbehav.2004.03.003

Smithson A, Macnair MR (1996) Frequency-dependent selection by pollinators: mechanisms and consequences with regard to behaviour of bumblebees *Bombus terrestris* (L.) (Hymenoptera: Apidae). J Evol Biol 9:571–588. https://doi.org/10.1046/j.1420-9101.1996.9050571.x

Sprayberry JDH, Ritter KA, Riffell JA (2013) The effect of olfactory exposure to non-insecticidal agrochemicals on bumblebee foraging behavior. PLoS ONE 8(10):e76273. https://doi.org/10.1371/journal.pone.0076273

Stanley DA, Russell AL, Morrison SJ et al (2016) Investigating the impacts of field-realistic exposure to a neonicotinoid pesticide on bumblebee foraging, homing ability and colony growth. J Appl Ecol 53:1440–1449. https://doi.org/10.1111/1365-2664.12689

Waddington KD (1995) Bumblebees do not respond to variance in nectar concentration. Ethology 101:33–38. https://doi.org/10.1111/j.1439-0310.1995.tb00342.x

Waddington KD (2001) Subjective evaluation and choice behavior by nectar-and pollen-collecting bees. Cognitive ecology of pollination. Cambridge University Press, Cambridge, pp 41–60

Willmer P (2011) Rewards 2: the biology of nectar. Pollination and floral ecology, Core Textbook. Princeton University Press, Princeton, pp 190–220

Woodard SH, Jha S (2017) Wild bee nutritional ecology: predicting pollinator population dynamics, movement, and services from floral resources. Curr Opin Insect Sci 21:83–90. https://doi.org/10.1016/j.cois.2017.05.011

Worden BD, Papaj DR (2005) Flower choice copying in bumblebees. Biol Lett 1(4):504–507. https://doi.org/10.1098/rsbl.2005.0368

Zimmerman M (1981) Optimal foraging, plant density and the marginal value theorem. Oecologia 49:148–153. https://doi.org/10.1007/BF00349181