



Bumble bees' food preferences are jointly shaped by rapid evaluation of nectar sugar concentration and viscosity

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Animals are often assumed to follow a strategy of energy maximization, and therefore should evaluate feeding options based on energy intake rates. However, at the proximal level, a direct estimate of energy intake rates, if that is possible at all, might require postabsorptive senses with relatively longer processing times, whereas an indirect estimate of energy intake through proxies like pre-absorptive senses of different sensory food properties might support rapid foraging decisions. Here, we show that nectar sugar concentration (sweetness) and nectar viscosity (resistance) drive preferences of bumble bees, *Bombus terrestris*, classical models for economic and foraging decision making. Using a tasteless/odourless biopolymer (Tylose), we created feeding options that differed in sweetness and resistance, properties that affect energy intake rate and can be immediately sensed. When energy intake rates were similar, bumble bees developed preferences based on sweetness and resistance. When energy intake rates were different, but sweetness and resistance were balanced against each other, bees developed no preferences. Decision dynamics during training indicated that bumble bees simultaneously evaluated sweetness and resistance to make decisions quickly (in seconds). These results suggest that bumble bees' food preferences are jointly affected by the immediate sensation of nectar sweetness and resistance as positively and negatively reinforcing properties, respectively, indicating a pre-absorptive proximate mechanism for rapid energy-sensitive decisions in bumble bees.

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Foraging animals make decisions and develop preferences for which foods to approach and which to ignore based on the various properties of the different options (Bateson et al., 2002; Baumont et al., 2000; Heil, 2011; Hemingway et al., 2021; Latty & Beekman, 2010; Latty & Trueblood, 2020; Pyke, 2016). A widely adopted framework, namely value-based decision making (Rangel et al., 2008; Sanfey et al., 2006), states that animals assign a subjective value to each option and then prefer the option with the largest value. It is thus critical to understand on which properties animals are placing values.

Energy gain per unit time, or energy intake rate, has been proposed to be the 'currency' that is maximized by humans and many

other animals (King & Marshall, 2022; MacArthur & Pianka, 1966; Pyke, 1984). This energy maximization strategy is commonly assumed to be the major driving force for foraging decisions and learned preferences for many pollinating animals. In particular, for bumble bees, *Bombus terrestris*, a model species for economic decision making, it has been suggested that bees' choices in multi-floral patches are in accordance with maximizing energy intake rates, for example when choosing between flowers with different nectar volumes or moving between different flower patches (Harder & Real, 1987; Heinrich, 1983; Hodges, 1981; Pyke, 1978, 1980; Waddington & Heinrich, 1979).

However, energy intake rate perception by bees, if this is possible at all, should take at least many seconds or even minutes to achieve, as it would likely require feedback from the postabsorptive senses for caloric content (Dus et al., 2011, 2015; Stafford et al., 2012). In

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contrast, empirical evidence suggests that pre-absorptive sensory nectar properties such as sugar concentration and viscosity can be almost instantly perceived by bumble bees (Lechantre et al., 2019; Miriyala et al., 2018). In fact, nectar sugar concentration (sweetness) determines the energy content, and nectar viscosity (resistance) affects the nectar collection time, due to bumble bees' tongue-dipping technique that involves periodic protraction and retraction of a tongue covered by elongated papillae (Harder, 1986; Kim et al., 2011; Lechantre et al., 2021; Pattrick et al., 2020). It is thus possible that instead of relying on relatively slower postabsorptive preferences to estimate energy intake rate directly, bumble bees could use those sensory nectar properties for an indirect, rough, but rapid sensing of energy, allowing them to make timely decisions in the everchanging floral supermarket.

In natural settings, while sweetness increases linearly with nectar energy density, it increases exponentially with nectar resistance (Pattrick et al., 2020), which could result in a longer collection time (Harder, 1986; Josens et al., 1998; Nardone et al., 2013). Therefore, to make energy-sensitive decisions, bees need to consider both nectar sweetness and resistance. Indeed, empirical evidence suggests that sweetness alone might not be able to account for bumble bees' feeding preferences: bumble bees show no preference between 55% and 68% sugar concentration (Bailes et al., 2018), even though physical models predict that 55% provides a higher (optimal) energy intake rate (Kim et al., 2011; Lechantre et al., 2021; Pattrick et al., 2020). Note that bumble bees can discriminate between sugar concentrations with an absolute difference of 2%, which is a 0.1 relative difference (20% versus 22%; Nachev et al., 2013; Whitney et al., 2008). Therefore, they should have little difficulty discriminating between sugar concentrations of 55% and 68% (a 0.24 relative difference). The reported lack of preference between 55% and 68% by bumble bees may thus be a result of combined effects of resistance and sweetness in these options.

As no study has investigated the impact of the combination of sugar concentration and viscosity on foraging preferences of bumble bees, here we set out to provide such direct investigations. Using Tylose, a cellulose that can increase the viscosity of liquid without changing its energy content (Nicolson et al., 2013), we manipulated the sugar concentration and viscosity of sucrose solutions, and investigated how instantaneous sensation of these sensory properties may jointly shape bumble bees' food preferences.

GENERAL METHODS

Animals and Set-up

Three colonies of bumble bees, *Bombus terrestris*, were obtained from Biobest's Chinese branch (Biobest Belgium N.V., Westerlo, Belgium) and housed in wooden nestboxes (28 × 16 cm and 11 cm high). Each nestbox was connected to a flight arena (40 × 60 cm and 40 cm high; Fig. 1a) covered by a UV-transparent acrylic ceiling, via a transparent acrylic tunnel (4 × 4 cm and 30 cm long) with three sliding doors, which allowed us to control individual access to the arena. Illumination was provided by two simulated daylight LED tubes (TruD65TM, 3nh, Shenzhen, China). Bees were fed daily with 15% (w/w) sucrose solution and pollen ad libitum every day outside the experiments. Individual bees were identified by a number tag (Opalithplättchen, Warnholz & Bienenvoigt, Ellerau, Germany) attached to their thorax.

Energy Intake Rate Prediction

Following recent physical modelling of bumble bee nectar collection (Pattrick et al., 2020), we modelled the volumetric transfer rates ($Q, \mu\text{l s}^{-1}$) of sucrose solution for drinking as $Q = 10^{0.236} \times \mu^{-0.180}$,

where μ is the viscosity in mPa·s. At a given temperature T (°C), the viscosity of a sucrose solution can be reliably approximated by $\log_{10} \mu = -0.114 + 22.46 \times x + (30 - T) / (91 + T) \times (1.1 + 43.1 \times x^n)$, where x is the mole fraction of sucrose at concentration c (w/w), and is given by $x = (c / 342.3) / ((100 - c) / 18.02 + c / 342.3)$. The concentration-specific density ρ_c (g/ml) can be calculated as $\rho_c = 0.9988603 + 0.0037291c + 0.0000178c^2$. The energy intake rate \dot{E} (J/s) can then be computed as $\dot{E} = \sigma \times Q \times \rho_c \times c$ (Lechantre et al., 2021), where σ is the energy content per unit mass of sucrose, a constant provided by sugar manufacturers (16.98 kJ/g). To determine the effect of varying sugar concentration and viscosity on nectar energy intake rate, we first estimated energy intake rate for a range of fixed concentrations and separately for a range of fixed viscosities, thereby showing the effect of each food quality in theoretical isolation. To illustrate the interplay between sweetness, resistance and energy intake rate, we utilized an established physical model of bee drinking (Pattrick et al., 2020) and estimated the theoretical energy intake rates for a range of nectar sugar concentrations while holding nectar viscosity constant, and the energy intake rates for a range of viscosities while holding sugar concentration constant (Fig. 2: blue and red lines). We were then able to adjust and set viscosity and sugar concentration for different options as described in the following section.

Feeding Option Preparation

Each type of feeding option consisted of a 20 μl droplet of artificial nectar (sucrose solution; hereafter nectar). Each experiment offered two different nectars each with a specific sugar concentration and viscosity. Sugar concentration was set using granulated sucrose. Viscosity was adjusted (when required) using Tylose (HS 30000 YP2, Shin-Etsu, Tokyo, Japan). Tylose is a nontoxic, odourless and (according to the literature) tasteless hydroxyethyl cellulose (Gloor et al., 1950) that has been used as a food thickener to investigate the effect of viscosity for a variety of nectivorous animals, for example sunbirds (Köhler et al., 2010), beetles (Yang et al., 2023), honey bees (Nicolson et al., 2013), hawk moths (Josens & Farina, 2001) and ants (Lois-Milevicich et al., 2021). To ensure that the sucrose concentrations and viscosities were correctly adjusted, after adding Tylose, the viscosities of all prepared solutions were measured in a rotational rheometer (NDJ-9S, Lichen, Shanghai, China) at ambient temperature (ca. 25 °C), and the sugar concentrations were checked with a pocket refractometer (SN-DR-8201, range 0–82% w/w, Lichen, Shanghai, China).

Experimental Procedures

Bees were first subjected to a pretraining phase, during which they were individually trained to collect a full crop of 15% sucrose solution from eight artificial flowers (hereafter flowers) and return to the nest, for three consecutive bouts. Each flower consisted of a transparent acrylic square (25 × 25 mm and 5 mm thick) placed on top of a glass vial, and it provided 20 μl of sucrose solution in its centre. Eight flowers were placed pseudorandomly in the arena. Following pretraining, the training phase consisted of 100 trials (voluntarily landing on and drinking the nectar of a flower) or 10 training blocks (each block included 10 consecutive landings), whereby the bees acquired associations between nectar type and flower colour (Dyer & Chittka, 2004). The number of bouts used in experiments 1–4 ranged from 7 to 13 (mean = 9.8), 5 to 16 (mean = 9.7), 8 to 13 (mean = 10.7) and 11 to 21 (mean = 15.4) respectively.

In experiment 1, the bumble bees were trained with two different nectars: one had 20% sucrose (w/w) and a viscosity of 2.78 mPa·s (0.04% w/w Tylose added; equivalent to that of a 30% sucrose solution), and another had 30% sucrose and a viscosity of 44.45

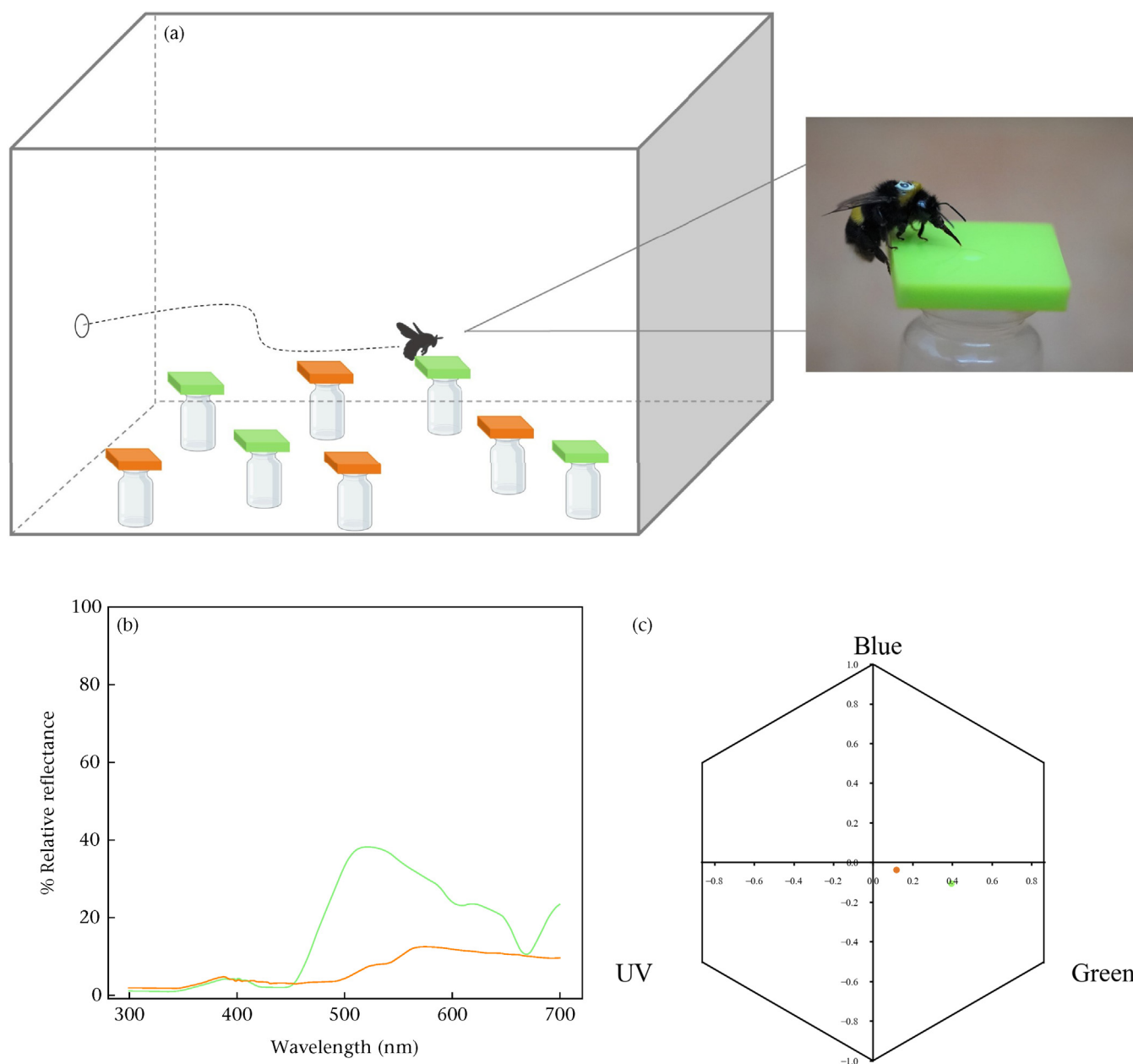


Figure 1. Experimental apparatus and the colours used in the behavioural experiments. (a) In each experiment, coloured acrylic artificial flowers (orange and lime) were presented horizontally in the flight arena. (b) Spectral reflectance of the two colours used in the experiments. (c) Loci of colours in the hexagonal bee colour space, determined by the bees' UV, blue and green photoreceptors (Chittka, 1992). Dots indicate each of the chip colours used in the experiments and are shown with the colours they appeared to human observers.

mPa·s (0.27% w/w Tylose added; equivalent to that of a 60% sucrose solution). In experiment 2, the two nectars both had 20% sucrose, but one had a viscosity of 2.78 mPa·s (0.04% w/w Tylose added) and the other had a viscosity of 44.45 mPa·s (0.34% w/w Tylose added). In experiment 3, one nectar had 20% sucrose and a viscosity of 2.78 mPa·s (0.04% w/w Tylose added), and the other nectar had 22% sucrose and a viscosity of 44.45 mPa·s (0.33% w/w Tylose added). The nectars used in experiment 4 had 20% (with a natural viscosity of 1.71 mPa·s) and 22% (with a natural viscosity of 1.86 mPa·s) sucrose, respectively, and did not contain any Tylose. Across all experiments, flowers were coloured orange or lime (human perception), and the reflectance of the two colours was measured with a wavelength range of 300–700 nm with 1 nm increments (Fig. 1b), using a spectrophotometer (Ocean Optics USB 2000+,

Orlando, FL, U.S.A.) with a deuterium/halogen light source. The calculated distance between these two colours was 0.29 hexagon units (Chittka, 1992; Fig. 1c), and thus bumble bees could easily differentiate between them (Dyer & Chittka, 2004). After the 100th trial, bees were gently captured with a cup and transported back to the hive. The flowers were washed with 75% ethanol in water to remove any potential scent marks left by bees and were moved to new positions to prevent bees from potentially associating nectar type with location in the arena. The colours associated with nectar type were counterbalanced across bees. After training, bees underwent a 2 min unrewarded test in which each flower provided 20 μ l of distilled water. All bees were trained and tested individually.

During the training phase, an observable pattern emerged across all experiments where bumble bees, at times, opted not to

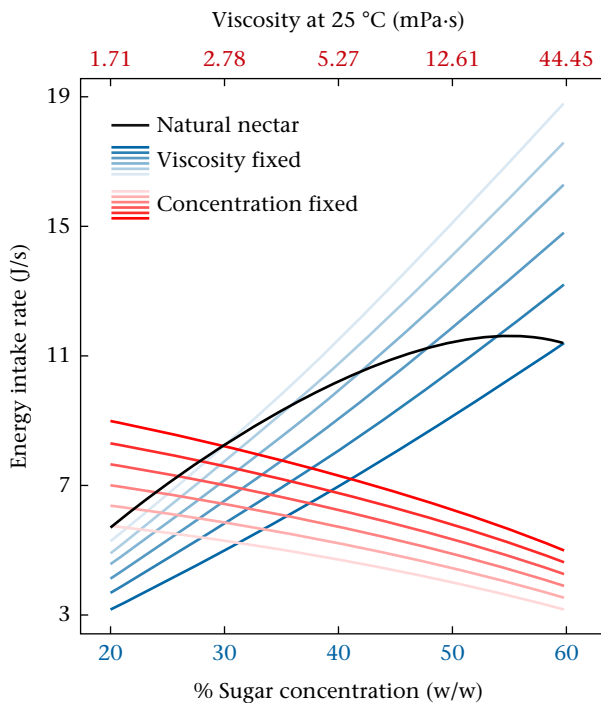


Figure 2. Visualization of theoretical energy intake rates of nectar by bumblebees as a result of sugar concentration alone, viscosity alone, or sugar concentration and viscosity together. Blue lines represent the predicted energy intake rate changes as a result of sugar concentration increases (from 20% to 60% w/w; corresponding to the bottom X-axis coloured in blue) if viscosity is fixed (different shades of blue represent six different viscosity fixation levels, from 2.78 mPa·s to 44.45 mPa·s). Note how an increase in sugar concentration increases energy intake rate. Red lines represent the predicted energy intake rate changes as a result of viscosity increases (from 1.71 mPa·s to 44.45 mPa·s; corresponding to the top X-axis coloured in red) if sugar concentration is fixed (different shades of red represent six different concentration fixation levels, from 20% w/w to 30% w/w). Importantly, an increase in viscosity reduces energy intake rate. The black line represents energy intake rate changes of nectar (over naturally observed sugar concentrations and viscosities) as a natural result of viscosity increasing with sugar concentration (Patrick et al., 2020). Note how the effect of increased sugar concentration overpowers the negative effect of viscosity on energy intake rate until approximately 55% sugar concentration and 22.72 mPa·s viscosity at which point energy intake rate begins to drop due to the significant impact of viscosity. The rates were calculated following Patrick et al. (2020).

fully consume the entire sucrose droplet (20 μ l) provided on a flower during one visit and instead left the unfinished feeder to make a new choice. To capture this behaviour quantitatively, we recorded not only the numbers of landings, which served as training trial numbers, but also the instances of incomplete consumption (when a bee departed from a feeder leaving behind a visibly noticeable amount of the sucrose droplet). The proportions of these disrupted consummatory behaviours were then visualized and compared across all experiments.

Statistical Analysis

The data were analysed in R (version 4.1.0, The R Foundation for Statistical Computing, Vienna, Austria, <http://www.r-project.org>) and visualized in Python (version 3.8, Python Software Foundation, Beaverton, OR, U.S.A., <http://www.python.org>; with matplotlib version 3.3.1 and seaborn version 0.10.1 packages). Performances during the training phase were analysed with generalized linear mixed models (GLMM; lme4 package in R). The proportion of choices over blocks of training was used as the dependent variable, and the GLMM models were fitted assuming a binomial distribution with a logit link function. The identity of individual bees and

colonies were set as random factors to account for repeated measurements, and the block number was included as a fixed factor. Flower colour (i.e. orange or lime colour represented the more viscous option) was excluded from the models because this did not significantly improve model fit in any experimental group (tested using likelihood ratio tests). Performances during the test or during a single training block (proportion of choices over one type of feeding option) were analysed with generalized linear models (GLM), assuming a binomial structure and a logit link function. The proportion of choices was used as the dependent variable, and the number of choices each bee performed was included as 'weight'.

Ethical Note

Although there are no current legal requirements regarding insect care and use in research, the bumble bees were cared for (e.g. see the guidelines by Fischer et al., 2023) daily by trained and competent staff, which included routine monitoring of welfare and provision of correct and adequate food during the experimental period. The behavioural tests were noninvasive, and the types of manipulations used (sucrose and water) are all experienced by bumble bees during their natural foraging life in the wild. Prior to the experiment, all bees were kept in their natal colony environment. The colonies were kept in a natural dark condition and were never short of food: about 5 g of pollen was provided directly to the colony by the experimenter each day under dark conditions, and sucrose solution was provided in the flight arena, allowing bees to forage freely. Only voluntarily engaged forager bees were trained and tested. All bee handling procedures were carefully done using plastic pots rather than forceps to reduce stress of handling.

EXPERIMENTAL METHODS AND RESULTS

Preferences and Energy Intake Rates

If bumble bees' choices were guided directly by energy gained per unit time, we should expect them to be indifferent to options offering similar energy intake rates. To test this idea, we trained a group of bees ($N = 10$) on a two-colour discrimination task using flowers containing nectar with similar predicted energy intake rates, but different levels of sweetness and resistance (experiment 1; Fig. 3a). In the first foraging block (10 trials; see General Methods), there was no preference for either option (GLM: $N = 10$, $Z = -0.400$, $P = 0.689$; Fig. 3b), but bees gradually developed a preference over training (GLMM: $N = 10$, $Z = 4.343$, $P = 1.41e-05$; Fig. 3b). Despite the energy intake rates of flowers being similar, bumble bees significantly preferred the type of flowers with higher sugar concentration and higher viscosity (Fig. 3a), both in the last training block (mean = 67%; GLM: $N = 10$, $Z = 3.330$, $P = 8.69e-04$; Fig. 3b) and in the subsequent unrewarded test (mean = 65.929%; GLM: $N = 10$, $Z = 2.610$, $P = 0.009$; Fig. 3). These results suggest that bumble bees' flower preferences are not simply determined by the theoretical energy intake rates of different feeding options.

Our interpretation of the results of experiment 1 relies on the assumption that the manipulated viscosity difference (41.67 mPa·s) between the two flower types can be perceived by bumble bees, otherwise the results of experiment 1 could be attributed to the difference in sugar concentration alone. To ensure this was true, in experiment 2, we trained another group of bees ($N = 10$) on two options with equal sugar concentration (20% w/w), but that differed in viscosity (2.78 versus 44.45 mPa·s). The effect of training block was not significant (GLMM: $N = 10$, $Z = 1.513$, $P = 0.130$; Fig. 3e), indicating that the viscosity difference of 41.67 mPa·s was perceptually small. However, bees showed a clear significant preference for the less viscous option in the last block of training

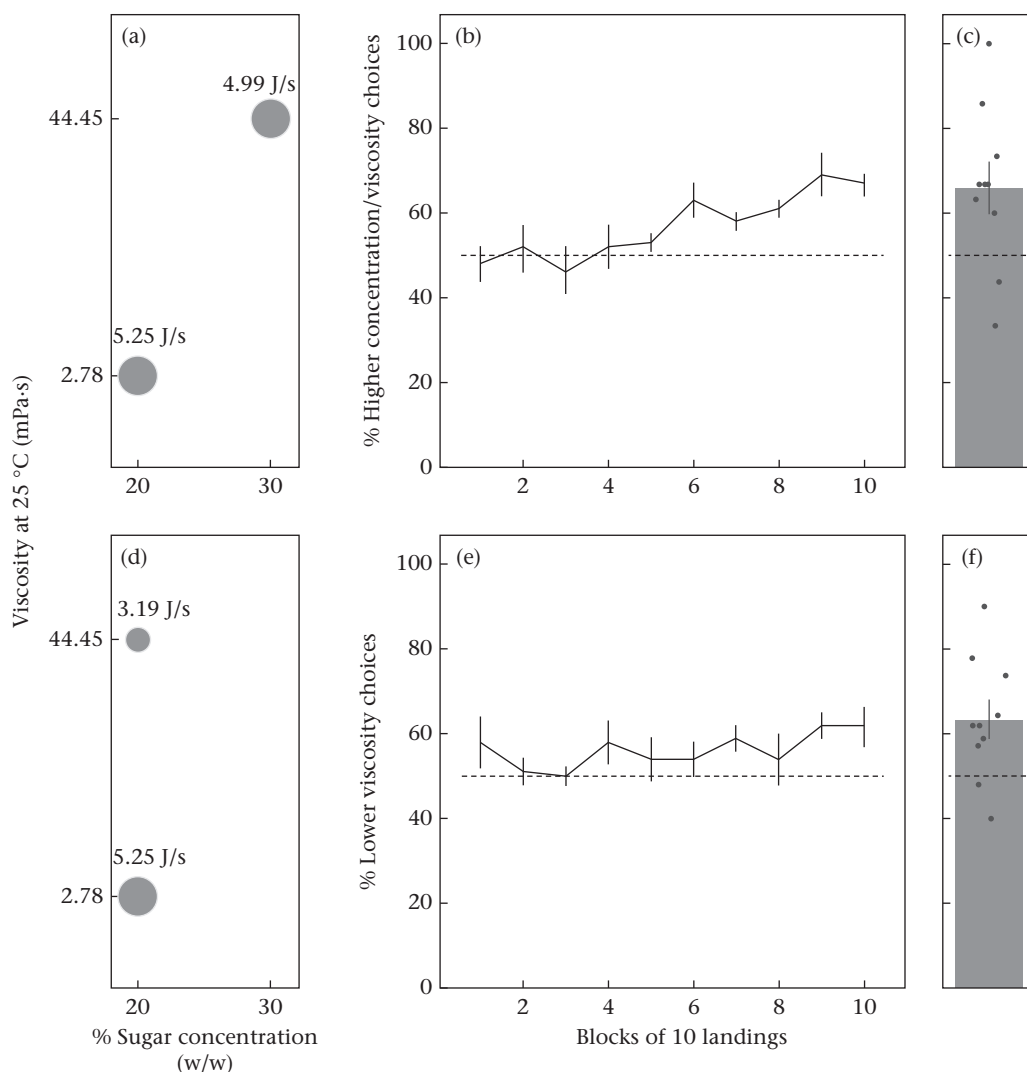


Figure 3. Bumblebees' preferences in relation to the offered energy intake rates of different options. (a, d) The sugar concentrations, viscosities and estimated energy intake rates for the two feeding options for experiments 1 and 2, respectively. (b, e) Percentage ($N = 10$) of choices for the option with higher sugar concentration (and higher viscosity) during training in experiment 1 and for the option with lower viscosity in experiment 2, respectively. (c, f) Percentage ($N = 10$) of choices for the higher sugar concentration (and higher viscosity) option during the unrewarded test of experiment 1 and for the less viscous option in experiment 2, respectively. Size of, and values adjacent to, circles in (a) and (d) indicate estimated energy intake rates. Vertical lines: mean \pm SEM. Horizontal dashed lines: 50% chance level, i.e. no preference for either option.

(mean = 62%; GLM: $N = 10$, $Z = 2.376$, $P = 0.018$; Fig. 3e) and in the unrewarded test (mean = 63.35%; GLM: $N = 10$, $Z = 3.088$, $P = 0.002$; Fig. 3f), confirming that bumble bees are able to discriminate and form preferences for flowers based on viscosity differences of nectar.

Sugar Concentration- and Viscosity-sensitive Preferences

In experiments 1 and 2 we demonstrated bees could develop preferences for the type of flowers with higher sugar concentration or lower viscosity. Therefore, we should expect that there exists a trade-off between bees' preferences for these two properties. In other words, we should be able to produce two nectar options where different levels of viscosity and sugar concentration result in no preference, even though the options provide nectar with different energy intake rates. To test this, in experiment 3, we trained another group of bees ($N = 10$) on a two-colour discrimination task with two flower options of the same viscosity difference as in experiment 1 (2.78 versus 44.45 mPa·s) but only a 2% difference in sugar concentration (20 versus 22% w/w), to (presumably)

balance the small perceptual difference between these two qualities (experiment 2). We found that bees had no preferences throughout training (GLMM on performance over training blocks: $N = 10$, $Z = -0.407$, $P = 0.684$; mean percentage of choices on the last block of training: 54%; GLM: $N = 10$, $Z = 0.799$, $P = 0.424$; Fig. 4b), and showed no preference in the unrewarded test (mean = 51.23%; GLM: $N = 10$, $Z = 0.337$, $P = 0.736$; Fig. 4c), despite the clear difference between options in energy intake rates (5.25 versus 3.54 J/s; Fig. 4a). These results further indicate that bumble bees' preferences for feeding options are based on a combined valuation of sweetness and resistance.

Our interpretation of the results of experiment 3 relies on the assumption that bumble bees are able to discriminate and form preferences for flowers with only a 2% sugar concentration difference. Although we assumed this to be true based on the work of others (Whitney et al., 2008), we wanted to ensure this was the case in our study. Therefore, in experiment 4, a group of bees ($N = 10$) was trained on two options with a 2% sucrose concentration difference (20 versus 22% w/w) and very similar (i.e. imperceptibly different) viscosities (1.71 versus 1.86 mPa·s; Fig. 4d). There was no

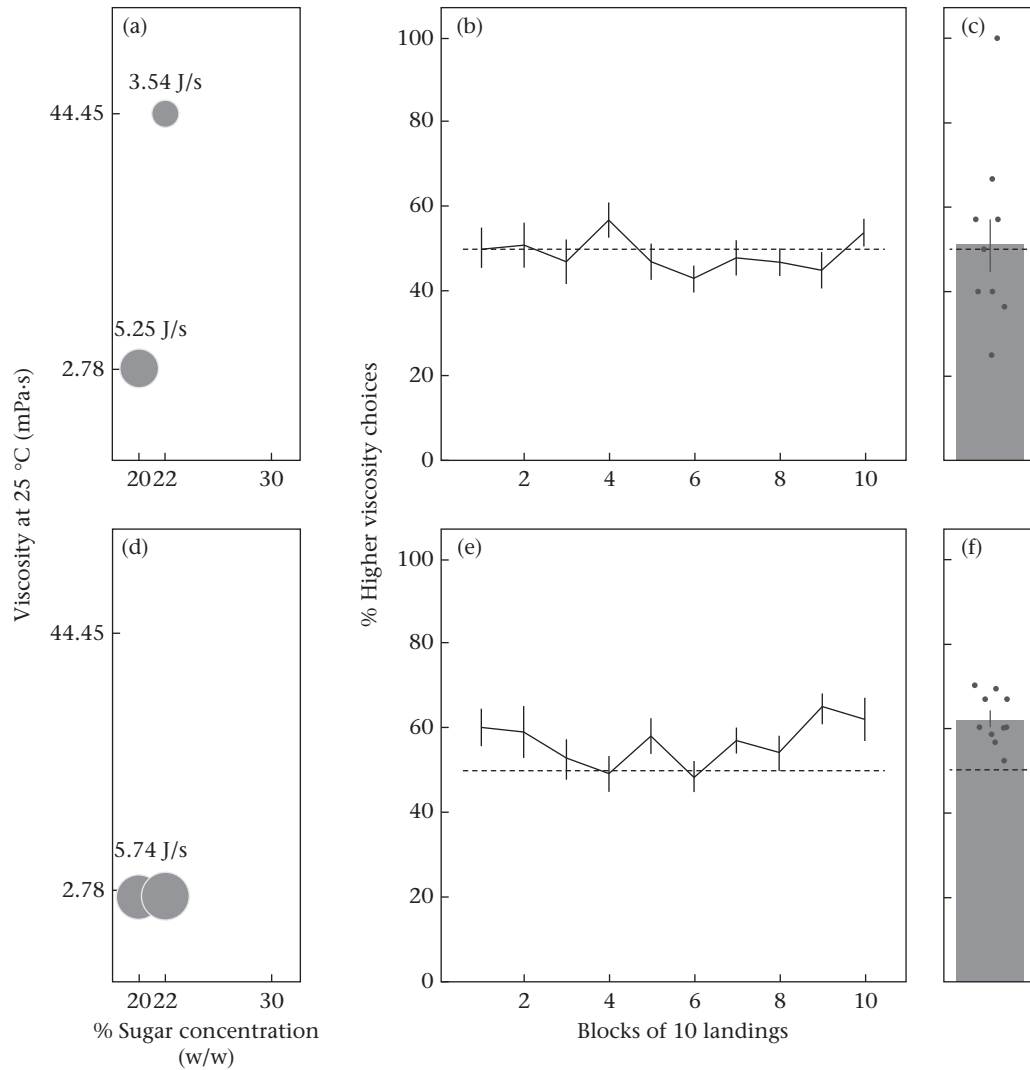


Figure 4. Bumblebees' preferences in relation to their joint valuation of nectar sugar concentration and viscosity. (a, d) The sugar concentrations, viscosities and estimated energy intake rates of the two feeding options in experiments 3 and 4, respectively. (b, e) Percentage ($N = 10$) of choices for the option with higher sugar concentration during training in experiments 3 and 4, respectively. (c, f) Percentage ($N = 10$) of choices for the higher sugar concentration option during the unrewarded test of experiments 3 and 4, respectively. Size of, and values adjacent to, circles in (a) and (d) indicate estimated energy intake rates. Vertical lines: mean \pm SEM. Horizontal dashed lines: 50% chance level, i.e. no preference for either option.

significant effect over blocks of training on preference (GLMM: $N = 10$, $Z = 0.878$, $P = 0.380$; Fig. 4e), indicating that the 2% sugar concentration difference was, as expected, perceptually small for bumble bees. However, bees showed a significant preference for the sweeter option in the last block of training (mean = 62%; GLM: $N = 10$, $Z = 2.376$, $P = 0.018$; Fig. 4e) and the unrewarded test (mean = 61.96%; GLM: $N = 10$, $Z = 2.299$, $P = 0.022$; Fig. 4f).

Disruption of Consummatory Behaviour

When feeding successively from options with downshifted reward quality, many animals including bees, can experience a negative incentive contrast effect indicated by a disruption of consummatory behaviour (Bitterman, 1976; Couvillon & Bitterman, 1984; Daniel, 2020; Hemingway & Muth, 2022; Wendt et al., 2019; Wiegmann et al., 2003). It is common to report bees' simple choice behaviours (e.g. landings/visits), and there are studies investigating how and when bees decide to leave a particular area of flowers in search of a more profitable area (Dreisig, 2012; Taneyhill & Thomson, 2007). However, disrupted drinking and early/partial

rejection of an individual flower's available nectar (i.e. leaving a flower before completely drinking its nectar contents) has not been considered outside of studies specifically exploring incentive contrast. Here, we noticed instances of disrupted drinking in each of the experiments by all bumble bees (Fig. 5). From videos, we recorded instances of incomplete drinking when any volume of the nectar droplet on a flower was left unfinished. Strikingly, we found that the incomplete drinking episodes were in accordance with the preferences bumble bees developed in each of the four experiments. In experiments 1, 2 and 4, bees demonstrated a clear difference in their incomplete consummatory behaviour for the two different options (GLMM: N for each group = 10, $P < 0.001$; Fig. 5a, b, d), and had fewer instances of aborted drinking for the option that they eventually preferred. For example, bees preferred the option with higher sugar concentration but higher viscosity in experiment 1, and so they aborted drinking this option less often than the alternative option (Fig. 5a). The only group of bees that showed no significant preference between options (experiment 3) also showed no difference in disrupted feeding episodes between the two options (GLMM: $N = 10$, $Z = 0.968$, $P = 0.333$; Fig. 5c).

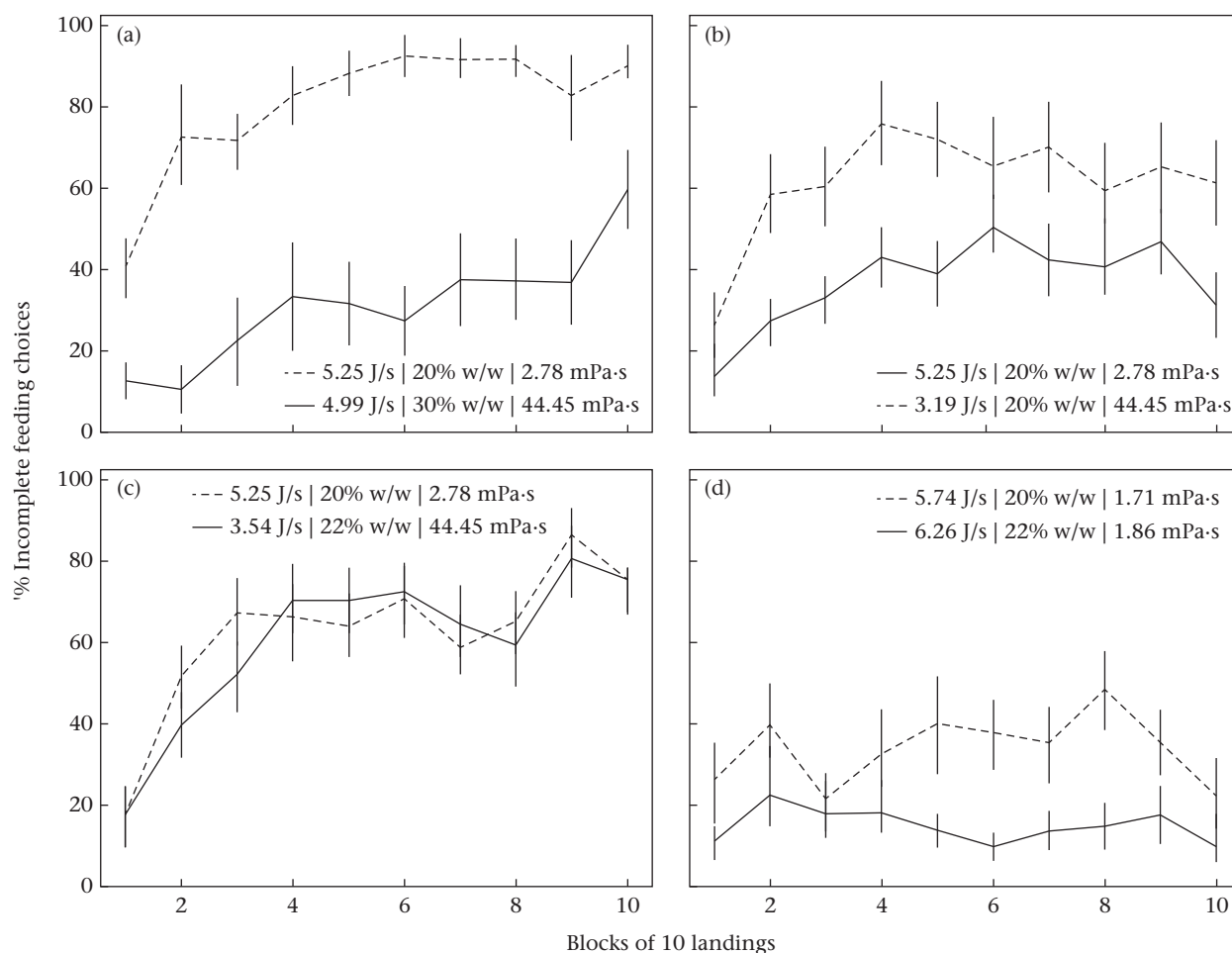


Figure 5. Bumblebees' flower preferences in relation to valuation of nectar resistance and sweetness. (a–d) The percentage of incomplete feeding episodes on different options during blocks of training in experiments 1–4, respectively. Vertical lines indicate mean \pm SEM.

These results suggest that bumble bees might have built up their floral preferences through continuous comparisons of the two sensory nectar properties over successive flower visits, each only several seconds apart.

DISCUSSION

Which currencies animals are intent on maximizing is crucial to our understanding of decision making across species and contexts. Physical modelling of the feeding mechanics of bumble bees has predicted that both sugar concentration and viscosity can affect their energy intake rates (Kim et al., 2011; Lechantre et al., 2019, 2021; Patrick et al., 2020). Our results extended those predictions, demonstrating that bumble bees' learned flower preferences can be explained by the joint valuation of nectar sweetness and resistance. The rapid rejections of feeding options we observed also suggest that bumble bees sampled and decided on whether to continue feeding or stop feeding and leave a flower as a result of very quick (a few seconds) sensory comparisons. Given bumble bees' short flower visits, and the disrupted feeding outcomes, our observations suggest that bees use pre-absorptive over potential postabsorptive information to form preferences for flowers and their offered nectar. However, further research is needed to determine whether bumble bees use any potential postabsorptive mechanisms to guide their preferences and whether they can override the pre-absorptive mechanisms suggested here.

Note that as flowers do not mix Tylose or similar compounds into their nectar, so the natural nectar viscosities will always depend on nectar sugar concentrations. Most of the offered nectar sugar (total carbohydrate) concentrations by bee-visited flowers are well below 50% (w/w; Pamminger et al., 2019). The corresponding nectar viscosities might thus not be impactful enough. Therefore, in natural settings, sugar concentration (sweetness) alone may work as a good rule-of-thumb of energy intake for bumble bees. However, it is also possible that the two sensory properties are rapidly integrated to form an overall perceptual impression of a feeding option (Forde & Graaf, 2022) even for those options with relatively lower concentrations, which may affect the food's palatability (i.e. its positive hedonic value; McCrickerd & Forde, 2016) and collectively determine feeding preferences (Levy & Glimcher, 2012; Polanía et al., 2019; Sugrue et al., 2005; Vlaev et al., 2011).

Also note that in experiment 3, we fortuitously observed that our estimated sugar concentration and viscosity parameters for the two feeding options resulted in a nonsignificant preference by bees. This outcome aligns well with our hypothesis suggesting a potential trade-off between sugar concentration and viscosity. However, it is crucial to acknowledge the possibility of a false negative in experiment 3. Although the selected parameters might not be balanced precisely, the results of experiment 3 are a proof-of-concept, especially in comparison with the other experiments, that a balance point does exist. Moreover, the absence of a clear

difference in the incomplete consummatory analysis in experiment 3 contrasts with the distinct preferences in the incomplete drinking shown in all the other experiments (Fig. 5), which further supports the idea that the combination of sugar concentration and viscosity difference here drives bees' floral preferences to the extent that when near balanced they can complicate bees' evaluation of the two options.

Importantly, bumble bees might not have remembered a particular 'value' for each sensory food property, but instead only encoded comparative information in each choice set (Solvi et al., 2022). In other words, instead of remembering that an option had a particular sugar concentration or viscosity, bumble bees may have only remembered that one option was better than the other. Nevertheless, how they make use of multiple comparisons on different attributes remains an open question. One possibility worth further exploration is that bumble bees could assign each option a binary outcome after comparison (good or bad in one attribute), but cumulate those single-attribute binary results for overall decisions (Bhui & Gershman, 2018; Stewart et al., 2006).

A potential concern is that it has not been established whether bumble bees can detect Tylose directly. Neural recordings would be necessary to determine whether bumble bees in our experiments responded to the taste of Tylose or to viscosity. However, recent work (Zhang et al., 2016) has shown that hydroxypropyl cellulose (HPC), another cellulose derivative very similar to hydroxyethyl cellulose (HEC/Tylose), does not trigger responses in *Drosophila*'s gustatory neurons. Further, the limited number of taste receptor genes in bees compared with flies would suggest an even more limited taste repertoire for bees (de Brito Sanchez, 2011). We therefore believe, like others who used Tylose in behavioural studies with different species (Josens & Farina, 2001; Köhler et al., 2010; Lois-Milevicich et al., 2021; Nicolson et al., 2013; Yang et al., 2023), that Tylose likely lacks both taste and odour for bumble bees.

Overall, we suggest that the investigation of how various sensory food properties drive animals' preferences could significantly add to our understanding, and future examination, of their decision making, and provide essential information on the fine-scale dynamics of their decision processes. This includes other qualities that do not affect energy intake rate as directly and as immediately as sugar concentration and viscosity. For example, the addition of salt to a flower's nectar can increase pollinator visit rate (Finkelstein et al., 2022) and the addition of caffeine can increase honey bees' foraging frequency and recruitment to those flowers (Couvillon et al., 2015). Other food properties, like volume and reward probability, also affect pollinator decision making (Shafir et al., 2003), as do innate and learned colour preferences (Schiestl & Johnson, 2013). In general, we believe our results underscore the potential multifaceted hedonic value that sensory food properties may provide for bumble bees and emphasize the need to consider the impacts of individual subjective experiences (Cnaani et al., 2006) of these animals and their implications for fitness.

Author Contributions

Yonghe Zhou: Conceptualization, Methodology, Formal analysis, Data Curation, Writing – Original draft, Writing – Review & editing, Visualization; **Shuyi Ding:** Investigation; **Caiying Liao:** Methodology, Validation; **Jianing Wu:** Methodology, Validation; **Lars Chittka:** Writing – Original draft, Writing – Review & editing, Supervision; **Cwyn Solvi:** Writing – Original draft, Writing – Review & editing, Supervision; **Fei Peng:** Conceptualization, Methodology, Formal analysis, Writing – Original draft, Writing – Review & editing, Visualization, Supervision, Funding Acquisition.

Data Availability

All data generated or analysed during this study are available on the Dryad Digital Repository: <https://doi.org/10.5061/dryad.g79cnp5tn>.

Declaration of Interest

None declared.

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