20% sucrose solution

50% sucrose solution

Small and large bumblebees invest differently when learning about flowers

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Summary

Honeybees [1] and bumblebees [2] perform learning flights on leaving a newly discovered flower. During these flights, bees spend a portion of the time turning back to face the flower when they can memorise views of the flower and its surroundings. In honeybees, learning flights become longer, when the reward offered by a flower is increased [3]. We show here that bumblebees behave in a similar way and we add that bumblebees face an artificial flower more when the concentration of the sucrose solution that the flower provides is higher. The surprising finding is that a bee's size determines what a bumblebee regards as a 'low' or a 'high' concentration and so affects its learning behaviour. The larger bees in a sample of foragers only enhance their flower facing when the sucrose concentration is in the upper range of the flowers that are naturally available to bees [4]. In contrast, smaller bees invest the same effort in facing flowers, whether the concentration is high or low, but their effort is

less than that of larger bees. The way in which different sized bees distribute their effort when learning about flowers parallels the foraging behaviour of a colony. Large bumblebees [5, 6] are able to carry larger loads and explore further from the nest than smaller ones [7]. Small ones with a smaller flight range and carrying capacity cannot afford to be as selective and so accept a wider range of flowers.

Results and Discussion

Bumblebees forage individually for flowers that can supply nectar and pollen. In contrast to honeybees, which communicate the location of rewarding flowers to each other within the hive [8], each bumblebee keeps the results of its exploration to itself [9]. On encountering a flower, honeybees and bumblebees decide for themselves whether the flower is worth exploiting, and, if it is, they learn the flower's appearance and location. Some features of the flower and its surroundings are learnt during the bee's approach [10, 11], but whether this information is worth retaining can only be determined after the bee has sampled what the flower offers. The bee's assessment of the flower influences the learning flights that occur in both honeybees and bumblebees after leaving a flower. Bumblebees during these learning flights turn back to face the flower [1, 2, 12, reviewed by 13]. From this vantage point they can record views of the flower's appearance and the flower's visual surroundings for guidance on their return to it [14]. Honeybees [3], perform longer learning flights for greater rewards. The situation in bumblebees turns out to be complex in that the bee's size determines how it responds to flowers offering different rewards.

The size of *Bombus terrestris* workers varies considerably (thorax width: 2.5-6.9mm [6]), with bees of different sizes operating within different constraints [reviewed by 15]. Small bees tend to be involved more with tasks inside the nest [16-18]. Those that do forage return to the nest with lighter loads than do larger bees [6] and have on average a lower nectar foraging rate than that of larger ones [5]. Estimates of flight capacity across different species of bees indicate that larger bees have a larger foraging range and can home from greater distances than smaller ones [7]. It is likely that the same holds true across foraging bumblebees of different sizes. Larger bumblebees also have the benefits of resisting the cold better [19] than small ones, and of bigger and more sensitive eyes [20, 21], which improves the visual range over which they can detect floral patches and individual flowers [22, 23]. Potentially, these attributes also allow large bees to forage early in the day, at low light levels, and exploit the abundant nectar to be found then [24, 25]. Taken together, these attributes mean that large bumblebees are predisposed to be the main contributors to a colony's store of nectar, thus outweighing the costs to the colony of raising them [26]. The data presented here argue that large bees learn the locations and features of highly rewarding flowers, but tend to ignore less profitable ones. In contrast, small bees learn equally well about flowers of varying profitability, but expend less effort when doing so than large bees.

Learning flights and flower facing of bumblebees sampling different concentrations of sucrose

Experiments were conducted in a greenhouse [2] on bees that left their nest for the first time. After bees had performed a learning flight at the nest they were caught and placed on an artificial flower that contained sucrose of one of four concentrations (10%, 20%, 30% or 50% w/w). The bees' learning flights when they left the flower after drinking from it were recorded with a downward-facing video camera that captured a scene comprising the bees, the flower and three black cylinders that marked the flower's position. These recordings focus on the initial part of a learning flight when bees are likely to memorise the appearance of an individual flower and its immediate surroundings [12]. Outside the recording area bees, fly much further and higher and may record the broader surroundings of a flower patch at which they have foraged [27].

A sample flight from a bee that drank 50% sucrose solution (Figure 1A) shows the bee turning back and flying towards and facing the flower several times before leaving the area surveyed by the camera. In this flight, most flower facing occurred close to the flower, when the bee was flying directly towards it. Frames during which the body is facing within ± 10^{o} of the centre of the flower, which we term 'flower facing', are emphasised by yellow circles in plots of the bee's trajectory (Figure 1A), and in plots of its body orientation relative to the flower and its distance from the flower (Figures 1B, C).

The duration of the bees' learning flights increased with the concentration of the sucrose that the bees drank (Spearman Ranks, rho = 0.24, p = 0.009, n = 115). The proportion of a learning flight in which bees faced the flower also increased with the concentration of sucrose, as we show by plotting for each concentration the distributions of the bees' body orientation relative to the centre of the flower (Figure 2A). Flower facing was greatest when bees had drunk 50% sucrose solution and dropped at lower sucrose concentrations. To prevent later confusion, we note that we avoided using small bees in this initial experiment.

After additional experiments had alerted us to the significance of bee size, we explored the details of flower facing more fully using just two sucrose concentrations (20%

and 50%), but with a larger sample of bees of varying sizes, as measured by the width of the bee's thorax. As in Figure 2A, the pattern of flower facing varied with sucrose concentration (Figure 2B). There was a prominent peak in the direction of the flower when bees had drunk 50% sucrose solution, but a broad plateau instead of a peak after drinking 20% solution. Despite this striking visual difference, bees were too variable for the difference to be significant when each bee provided one data point for each bin (Figure 2B).

This larger data set also confirmed the indication from the example flight (Figure 1A) that most flower facing occurs when the bees are close to the flower. Irrespective of sucrose concentration, the frequency of flower facing was high when bees were within 10 cm of the flower and then fell steeply (Figure 2C). This clustering reassures us that the video records capture most of the flower facing. Flower facing increased with learning flight duration, but the relationships were similar for 20% and 50% sucrose solutions (Figure S1, Table S1).

Differences in the bees' responses to sucrose concentration emerged when we segregated bees according to their size (Figure 3A). Bees were classified as 'small' or 'large' according to whether they were below or above the midpoint of the species size range (4.5 mm thorax width) [6]. Learning flights are longer (Wilcoxon Rank Sum test, z = -2.71, p = 0.007) and flower facing is more frequent in large bees that drank 50% sucrose solution than in large bees that drank 20% (z = -2.64, p = 0.0083, Figure 3A). There is no difference between small bees that drank 50% sucrose solution and those that drank the lower concentration (flight duration z = 1.55, p = 0.12, flower facing z = 1.195, p = 0.232, Figure 3A).

In the example flight (Figure 1A) most flower facing occurs in bouts during which the bee pivots around or approaches the flower. Each bout provides a separate opportunity for a bee to record views of the flower. Since the duration and number of bouts (see Methods) may be more closely related to learning performance than raw flower facing, we analysed the properties of bouts across the four groups. Unsurprisingly, the pattern of bout duration and number resembled the differences in the number of flower facing frames (Figure 3B). They are greater in large bees drinking 50% sucrose than in those drinking 20% sucrose (Wilcoxon Rank Sum, bout duration z = -3.32, p = 0.001, bout number z = -2.80, p = 0.005), but do not differ between small bees drinking the two concentrations (bout duration z = 1.59, p = 0.11, bout number z = 1.36, p = 0.17). These distributions of bouts emphasise one significant difference between the small and large bees: bout length and number are significantly smaller for small bees drinking 50% sucrose than for large bees drinking that concentration (bout duration z = -2.68, p = 0.007, bout number z = -2.60 , p = 0.009). This difference suggests that, although small bees spend similar amounts of time facing flowers dispensing 20% and 50% sucrose solution, overall they spend less effort in this endeavour than do large bees drinking 50% sucrose.

A further question is which of the four groups (20% small, 50% small, 20% large, 50% large) face the flower more than would be expected by chance, given the length of their learning flight. The four histograms (Figure 3C), one for each group, show the proportion of the flight that each bee spent facing the flower (± 10^o). With no preference for flower facing, the expected proportion is 20/360, as shown by the vertical dotted line. Large bees drinking 20% sucrose solution were the only group in which the proportion of flower facing did not exceed chance, emphasising that larger bees were less likely to invest in learning about a flower of low value.

Finally, we asked whether increasing the duration of learning flights does in fact improve learning. For several reasons (see caption to Figure S2), this question is best answered by examining the flights of bees leaving their nest for the first time. Analogous to learning flights from flowers, the amount of nest facing increases with flight duration (Spearman Rank, rho = 0.81, $p < 0.001$). We found that the bees' precision in locating their nest site on their return is correlated positively both with the length of their previous learning flight ($n = 17$ bees, Spearman Rank , one-tailed, rho = -0.542, $p = 0.013$) and with the number of nest facing frames in the learning flight (rho = -0.646, $p = 0.0025$, Figure S2).

Interactions of bumblebee size, sucrose concentration, drinking volume and learning flights

The previous section shows what can be learnt from classifying bees as small and large, but in reality there is a continuous gradation in the size of bees and we wanted to see both how the gradient of bee size is related to learning flights when bees drink different concentrations of sucrose and how drinking volume varies with bee size and sucrose concentration. To get this information, we performed several supplementary experiments to work out how drinking time, which is easy to record, is related to drinking volume. For that we needed to know a bee's drinking speed and how that speed varied with sucrose of differing viscosities and with proboscis length [28] (see Methods and Figure S3).

The estimated volume that bees drank increased with their size and the slope was significantly steeper at the higher concentration (Figure 4A, Table S1). This plot shows once more the preference of larger bees for 50% sucrose over 20% sucrose. If size is ignored, then the average amount that bees drank was about the same for the two concentrations of (20%

median volume 54.1µl, IQR 31.2µl, n = 95 bees; 50% median volume 59.0µl, IQR 41.0µl, n = 84; Wilcoxon Rank Sum, z = 1.218, p = 0.223). The volume that bees drank in these experiments is consistent with that reported for naturally foraging bumblebees when they return to the nest after a foraging trip [5, 6]. This similarity is striking since drinking patterns in the two cases are quite distinct, with bumblebees visiting perhaps a hundred or more flowers during a normal foraging trip [27] and in this experiment consuming the sucrose in one sitting.

A bee's size had a strong effect on the amount of flower facing during learning flights. When the sucrose concentration was 20%, the length of learning flights and the amount of flower facing tended to drop with increasing bee size (Figures 4B, S4A). This trend reversed at the higher concentration: the length of learning flights and the amount of flower facing increased with the bee's size. The regression coefficients differ significantly between the two concentrations (Table S1). Again, we find that as size increases bees spend more time learning about flowers dispensing 50% sucrose than they do about flowers with the lower concentration and that smaller sized bees spend similar times learning about flowers dispensing the two concentrations. The drinking data (Figure 4A) also indicate that the value that both smaller and larger bees assign to a flower depends more on the content of the nectar than the amount of nectar that the bees consume. We also examined the relation between drinking volume and learning flight duration for each of the four groups considered in the previous section (small 20% sucrose, large 20% sucrose, small 50% sucrose, large 50% sucrose). There was no systematic relation between learning flight duration and drinking volume in the groups (Figure S4B). Foraging honeybees are similar in that the value honeybees give to a visited flower depends on the rate of sucrose intake rather than the volume that they collect [29].

Taken as a whole, the upshot of this analysis is that smaller bees invest equally in learning about relatively low and highly rewarding flowers, whereas larger bees focus primarily on highly rewarding flowers and may learn little about flowers delivering sucrose of low concentration. To make sense of these data in ecological terms, it helps to know the concentration of sugars in the nectar of flowers that *B terrestris* commonly visit. A large scale review [4] of the sucrose strength of the different flowers from which bees forage gives 40% w/w sucrose as the median concentration with 50% as an optimal level and 20% just adequate. The low value that larger bees assign to flowers delivering 20% is likely to be a reflection of their propensity to explore for very high yielding flowers. Even if it takes larger bees longer to find such flowers on the first occasion, the cost of initial exploration is met by the greater amount that they can harvest when they find suitable flowers. The benefit-to-cost energy balance will improve on the bees' subsequent visits as, with no need to explore, the trip to the flower patch is shorter. In natural foraging, each flower generally holds a tiny fraction of a full load, so that carrying capacity is not lost by drinking a little (e.g. Figure 4A) on encountering a weakly rewarding flower and then exploring further to find flowers worth revisiting.

Small bees are less discriminating than large ones, but are still likely to have a threshold below which they are reluctant to feed from a flower. Individual honeybees differ in the lowest concentration of sucrose that they accept. Bees that forage primarily for pollen have a lower threshold than those that forage for nectar [30-32]. Bumblebees may also have varying sensitivity to sucrose with small bees having lower thresholds than large ones, as an adaptation to their more limited carrying capacity, flight range and ability to explore. Perhaps an additional reason for smaller bees to accept a wider range of flowers and to invest less in learning about them is that they are more prone than large ones to switch back to performing tasks within the hive. In this case they would be unable to recoup the costs of exploration or learning through further visits to those flowers. It seems that the effort that small and large bees expend in learning about flowers providing different rewards matches closely the diverse foraging strategies of differently sized bees.

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Author contributions

T.S.C. and N.H.I. conceived the project and acquired funding. E.F., T.R., P.K.Y.C., N.H.I. developed the methodology and provided supervision. E.F., T.R., P.K.Y.C., B.S., S.G., N.M. planned the experiments, collected the data and conducted initial analysis, with inputs from T.S.C. and N.H.I. and using code written by A.P. Data were curated by E.F., T.R., P.K.Y.C. and N.H.I. Further analysis and statistical testing were carried out by T.R., T.S.C. and N.H.I. T.S.C. and N.H.I. wrote the original draft. All authors reviewed and discussed the manuscript.

Declaration of interests

The authors declare no competing interests.

Figure Legends

Figure 1. Learning flight of a bumblebee after drinking 50% sucrose from an artificial flower.

A. Trajectory showing bee's position (o) and body orientation (|) every 20 ms frame of the recorded flight. Grey disks represent cylinders that help bees locate the 5 cm diameter flower (+).

B. Time course of bee's body orientation relative to the flower during the flight.

C. Time course of bee' distance from the flower during the flight. In A-C, yellow circles mark frames in which the bee's body faced the flower (± 10°).

Figure 2. Some properties of learning flights after drinking from flowers of different concentrations.

A. Flower facing during learning flights from four samples of bees. The bees in each sample had drunk the same concentration of sucrose solution (10% n = 27 bees, 20% n = 31 bees, 30% n = 33 bees or 50% n = 24 bees). For each concentration, frames from all the bees are pooled and the bees' body orientation relative to the centre of the flower is expressed as the mean number of frames per bee in each 40° bin.

B. Flower facing during learning flights from two samples of bees. Bees of different sizes (between 3.3 and 5.7 mm thorax width) performed a learning flight after drinking from the artificial flower (20% n = 69 bees, 50% n = 68 bees, see also Figures S1 and S2). The bees' body orientation relative to the flower is expressed as the median number of frames for each bee per 40° bin. The dotted lines give the interquartile range. Bees drinking 50% sucrose solution tended to face (± 10°) the flower more often, but the difference was not statistically significant (Wilcoxon Rank Sum, z =1.11, p = 0.268).

C. Distances from which bees face the flower. Frames in which bees face the flower (± 10°) are collected in 5 cm bins from the learning flights of bees that had drunk 20% (n = 69 bees) and 50% sucrose solution (n = 68 bees).

Figure 3. Flower facing during learning flights

A. Median (± IQR) amount of flower facing of the small and large bees drinking 20% (n = 35, 34 bees) or 50% sucrose (n = 34, 34 bees).

B. Number of bouts of flower facing per bee vs bout duration for small and large bees drinking 20% and 50% sucrose. A bout was defined as a sequence of at least 4 consecutive frames of flower facing [12]. Where bouts were separated by <= 3 frames without flower facing, they were merged.

C. Percent flower facing of small and large bees after drinking 20% or 50% sucrose solution. For each bee in each category, the Y-axis gives the number of bees and the X-axis the percent of flower facing during the learning flight. Dotted line (20/360) is the proportion of flower facing on the assumption that flower facing is at chance level. Small bees emphasise flower facing after drinking 20% and 50% sucrose. Large bees emphasise flower facing after drinking 50% sucrose, but not after 20% sucrose (Wilcoxon one-sample test, $M_0 > 0.056$, 20% large $z = 0.759$, $p = 0.24$, 50% large $z = 3.26$, $p < 0.001$, 20% small $z = 2.429$, $p = 0.008$, 50% small $z = 1.825$, $p = 0.034$).

Figure 4. The relation between bee size, sucrose concentration, drinking volume and learning flights.

A. Relation between drinking volume and bee size as given by thorax width for bees drinking 20% ($n = 95$ bees) or 50% ($n = 84$ bees) sucrose solution (see also Figures S3 and S4). Correlation is tighter when bees drink the more concentrated solution (Spearman Rank, 20% rho = 0.393, $p < 0.0001$, 50% rho = 0.615, $p < 0.0001$). A multiple regression analysis performed to predict drinking volume based on sucrose concentration and body size explained 28.4% of the variance ($F(3,175) = 24.51$, $p < 0.001$, Table S1), and the regression slopes for the two concentrations differ significantly ($ß_0 = 0.025$, $ß_1 = -0.25$, $ß_2 = 0.02$, $ß_3 = 0.018$, $t(3,175) = 2.86$, $p = 0.005$).

B. Relation between amount of flower facing and bee size for bees drinking 20% ($n = 69$ bees) or 50% ($n = 68$ bees) sucrose solution. The association between body size and the amount of flower facing was significant for 50% but not for 20% sucrose solution (Spearman Ranks 20% rho = -0.200, $p = 0.099$, 50% rho = 0.338, $p = 0.0049$). The interaction between flower facing and body size was significant between the two concentrations (Hurdle model with zero-truncated negative binomial regression with log link, $ß_0 = 4.389$, $ß_1 = -0.205$, $ß_2 = -4.148$, $ß_3 = 0.957$, $z = 3.34$, $p < 0.01$, Table S1).

STAR METHODS

RESOURCE AVAILABILITY

LEAD CONTACT

Further information and requests should be directed to and will be fulfilled by the Lead Contact, Natalie Hempel de Ibarra (N.Hempel@exeter.ac.uk)

MATERIALS AVAILABILITY

This study did not generate unique reagents.

DATA AND CODE AVAILABILITY STATEMENT

The research data supporting this publication are openly available from the University of Exeter's institutional repository at: https://doi.org/10.24378/exe.2864

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The experiments were conducted in 2016 and 2017. Supplementary experiments and tests took place in 2014-15 and 2018. In total, individual foragers from 17 commercially reared colonies were tested (*Bombus terrestris audax*, Koppert UK). Where bees were not removed after their first foraging flight, they were individually marked with numbered queen-marking tags. Before and during the experiments, the experimental colony was provisioned with daily rations of sugar syrup (Koppert UK) and honeybee-collected pollen (W. Seip, Germany) inside the nest. Feeding took place in the evenings to encourage forager activity during the day. Between experimental sessions the colony was kept in the lab. Bees could move freely in and out of the colony experiencing daylight but were enclosed in the exit box that was attached to the nest box.

METHODS DETAILS

Setup and experimental procedures

Experiments were conducted in a greenhouse (8 by 12m floor area) on the University of Exeter's Streatham Campus. A colony was placed beneath a table (1.5 x 1.8 m, 1.5 m height) with the nest-box connected to a hole in the centre of the table via a series of tubes (see also [12]). The arrangement allowed a controlled exit and re-entrance of individual bumblebees and made it possible to reduce the chances that the bees would interfere with each other. Bees fed at a second 'flower' table, about 5 m away. Both tables were covered with white gravel that was frequently raked. The artificial flower from which bees drank consisted of a flat, purple plastic ring (5 cm outer diameter) with, in the centre, a small transparent centrifuge tube containing sucrose. This flower was placed on the gravel in the centre of the food table. The flower was cleaned and filled with fresh sucrose solution just before a bee was released. Three black cylinders (17 cm high x 5 cm wide) were placed equidistantly around the flower in a 120° arc at a radial distance of 24.5 cm from the flower. A video camera (Panasonic HC-V720, HD 1080p, 50 fps) was hung 1.35 m above the table to record a bee's drinking behaviour and its learning flight on departure from the flower over an area of appx 60 by 100 cm on the table surface. The bees had never left the nest before to forage. After completing a learning flight at the nest, they flew within the greenhouse until caught with a butterfly net. They were then transferred into a tube and placed gently on the artificial flower. Most bees started to drink within a few seconds of their placement and drank *ad libitum*. The moment when drinking began was noted on the audio channel of the video. When a bee stopped drinking, it started moving again. The camera above the flower recorded the bees' behaviour throughout their time on the flower and when they left it and performed a learning flight. To examine the relation between the sucrose concentration drunk and the subsequent learning flights, the flower contained one of four concentrations (10%, 20%, 30% or 50% w/w) with a different concentration chosen each day in varying order over a few weeks of experiments. After each bee had completed its learning flight at the flower, it was caught and removed. Five colonies were used in the first experiment.

Subsequently, bees from six more colonies were tested in the same way with 20% and 50% (w/w) solutions. In this experiment, we wished to have similar numbers of small and large bees and selected the appropriate size as they emerged from the nest into a transparent exit box before being allowed to walk through the transparent tubing from the nest to the exit hole under the table. After bees had completed the procedure, the width of each bee's thorax (intertegular span) was measured with digital callipers (Axminster, UK) under a dissecting microscope. Intertegular span correlates well with other measures of body size in many species of bees, including bumblebees [33, 34].

Test after the first learning flight

To test whether the duration of a learning flight influences the precision of a bee’s search on its return, we analysed data obtained in a separate, so far, unpublished experiment. Learning flights at the nest are more suited to this question than those from flowers as they are longer and more varied in duration and the bees' subsequent test searches are more persistent [2, 12]. We recorded learning flights, in the way described above, of individually marked bumblebees from three further colonies on their first departure from the nest. The bees then had their first opportunity to view three cylinders arranged in a 120° arc and 14.5 cm away from the nest that marked the location of the nest hole.

After a bee had finished its flight and flew off, it was caught and placed on a sucrose feeder, it could take several hours after feeding before the bee returned and searched for the nest hole. This interval, during which the bee flew in the greenhouse or rested, is of comparable length to the bee's first foraging flight that often follows its first learning flight [9]. When a bee eventually decided to search for its nest, it found the array of cylinders displaced a few cm from the nest position and the nest hole covered up with a plastic sheet inserted under the gravel. The bee was allowed to search for several minutes until it gave up, flying far away from the table. It was then caught and placed inside the nest. The distance of its first landing relative to the virtual position of the nest was determined using custom-written code in Matlab from video footage recorded at 50 fps (Fig S2).

Supplementary experiments to translate drinking time into an estimate of drinking volume

In the first of these experiments, bees from a different colony were weighed before they had drunk sucrose solution (20% or 50% w/w) from the standard artificial flower. Their drinking time was recorded, and they were weighed again after they had performed a learning flight. Each bee was tested only once. and its thorax width measured after the procedure. The volume each bee drank was determined from the increase in its weight and the measured density of the sucrose solution. From these data we plotted, for the two sucrose concentrations, the relation between volume drunk and thorax width (Figure S3A).

Because the precision balance (Ohaus Pioneer TM, USA) for weighing bees could not be used in the greenhouse, this experiment was performed in a laboratory room (3.5 x 5 m, 3.5 m height) lit with high frequency daylight-type fluorescent tubes. There was only space for one table in the centre of the room. The table was covered with white cotton-loop bath rugs. The artificial purple flower in the centre of the table was marked by the standard array of three black cylinders. To provide a visual panorama and stabilise flight, the walls of the room were covered with high-contrast patterns.

The colony was placed in one corner of the room, and as in the greenhouse experiments, bees without any foraging experience were released individually. Each bee was caught after its learning flight at the nest, carefully transferred into a tube and weighed. The bee was then placed on the flower, and its behaviour recorded from above on video (50 fps) to monitor its drinking duration and learning flight. The bee was weighed a second time after it had drunk its fill and had performed a learning flight.

A second experiment was designed to measure how fast bees drink 20% and 50% (w/w) sucrose solution (Figure S3B). We recorded individually-marked bees from two further colonies in a small test chamber as they drank from a vertically-oriented conical tube (the same as the one placed in the centre of the artificial flower). The tube containing sucrose was removed and weighed before and after each bee was tested to determine the volume the bee had drunk.

To give more detail: The tube was inserted from below into a tightly-fitting hole in the floor of the chamber and raised about 1 mm above the floor. The tube was fixed in place to avoid spillage. A small transparent box with one open side and an open floor was placed over the tube, forcing the bee to approach the tube from one direction. The test chamber was connected directly to the bee's nest box, with access to the chamber controlled by sliding doors. In order to record proboscis movements, the video camera was positioned to face the bee. After reaching the sucrose, bees drank continuously from it in a single bout, and then stopped drinking. Thirteen of these bees were tested with both solutions, but on different days and counterbalancing the sequence. Prior to and between test days the colony was fed with commercially supplied syrup and pollen inside the colony. All the tested bees, apart from two, gave reliable data (n = 34).

To relate drinking time to the volume drunk, we calculated each bee's drinking speed for sucrose concentrations of 20% and 50% from the bee's first drinking test with each of the two sucrose concentrations, excluding an outlier with a thorax width of only 3 mm. These data show that there is a linear relation between bee size and drinking speed (Figure S3B).

QUANTIFICATION AND STATISTICAL ANALYSIS

Videos were examined with video-editing software (Adobe CS6) to determine the durations of the bees' learning flights and their drinking behaviour. We discarded the few flights in which bees landed during the learning flight or flew directly away from the flower.

To analyse the details of the learning flights, the positions and orientations of the bees' body were extracted from the videos using custom-written code in Matlab. Most of the flights were recorded at 50fps (n = 137 bees), but on two experimental days the camera was mistakenly reset to 25fps. These slower recordings (n = 13 bees) could not be included in some of the comparisons but were included in Fig 3C.

A particularly significant part of these learning flights is when bees orient their body to face the flower (± 10°). This flower facing mostly happens in bouts of several frames. We defined as a bout a sequence of at least 4 consecutive frames of flower facing [12] and merged bouts that were separated by <= 3 frames without flower facing,

Drinking volumes of bees of known size were estimated from the video recordings of the duration of drinking and a calibration curve (see Figure S3B) that gave the drinking speed of different sized bees. The duration of drinking was taken to be the interval between the audio record of the start of drinking and the bees' first movement on the flower.

Statistical tests on the data were performed in Matlab and R (version 3.6.1) for comparisons of medians, regression and correlation analyses. R packages 'pscl' [35] and 'betareg' [36] were used to run hurdle models [37, 38] as data for flower facing were overdispersed and contained zeros.

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Figure 1

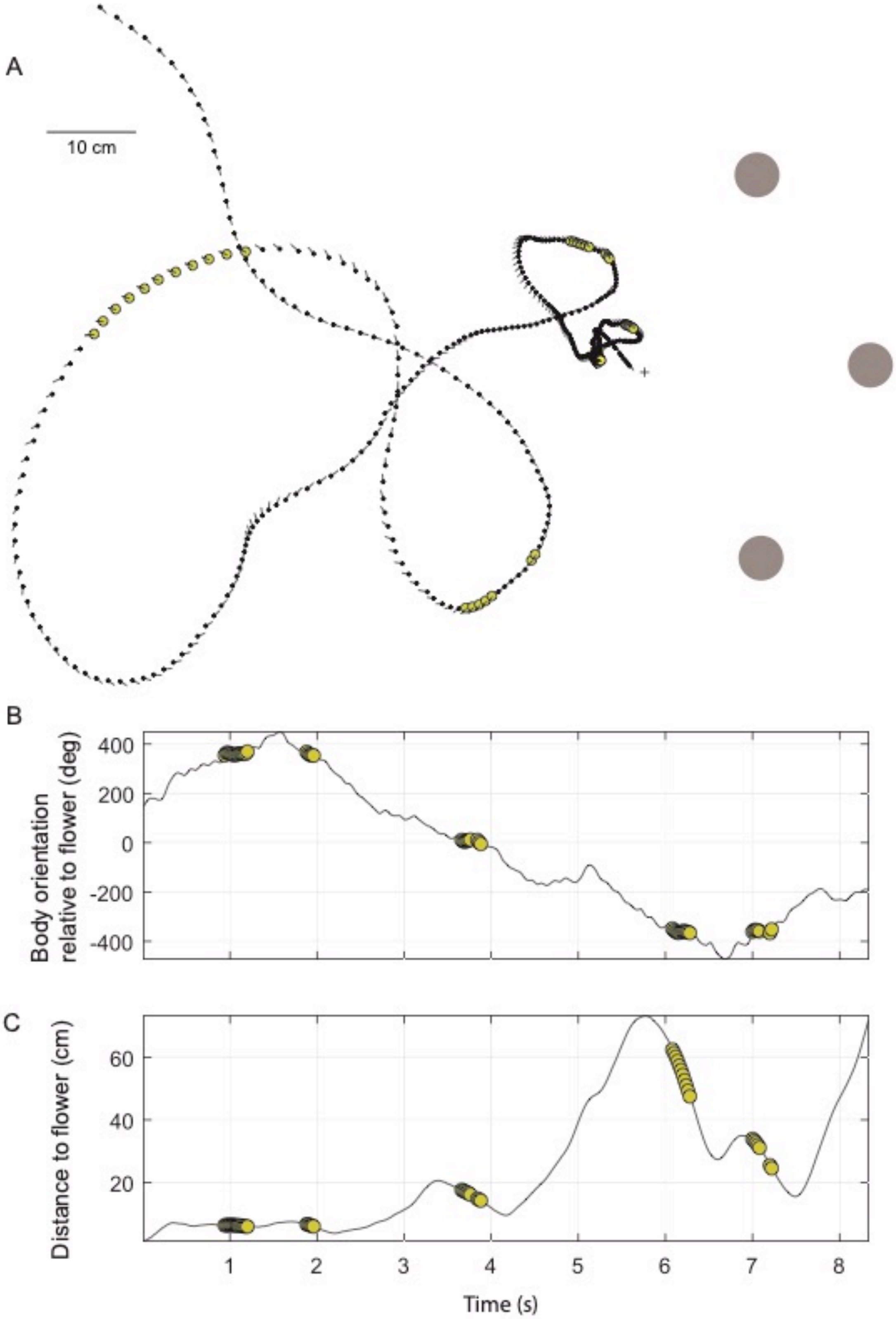

A
10 cm
B
Body orientation relative to flower (deg)
400
200
0
-200
-400
C
Distance to flower (cm)
60
40
20
1
2
3
4
5
6
7
8
Time (s)

Figure 2

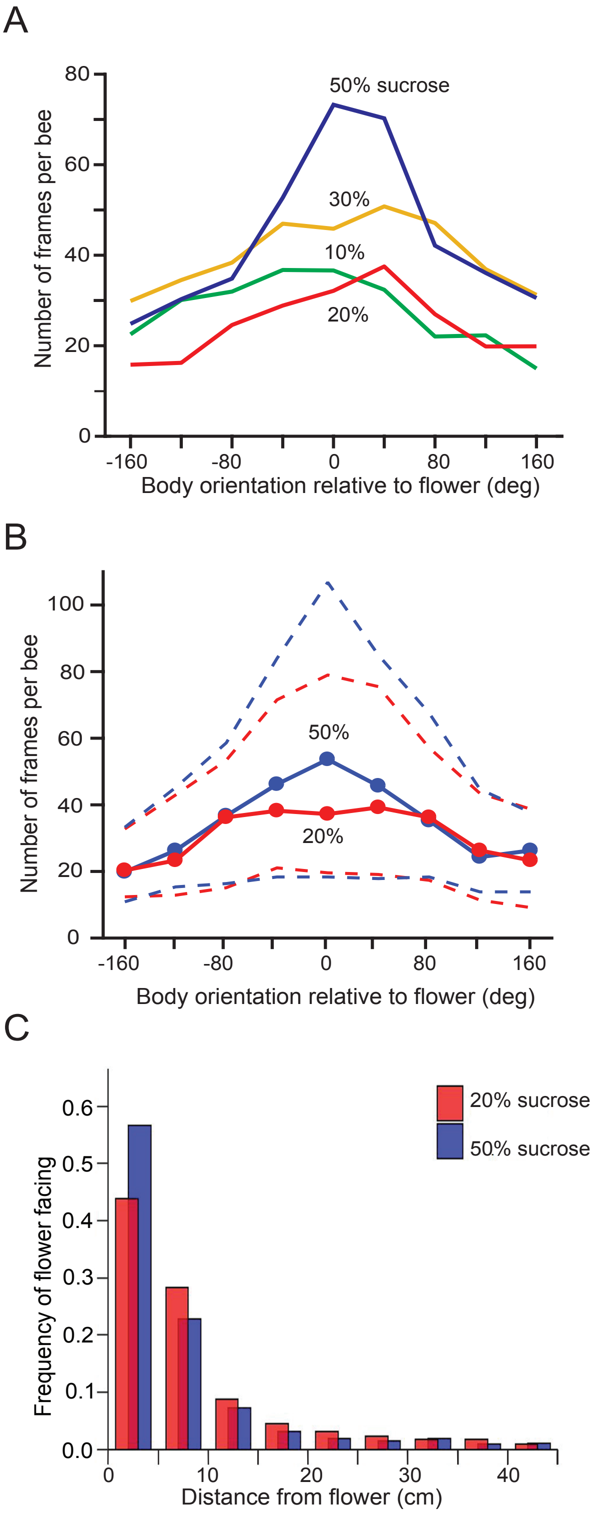

A
Number of frames per bee
0
20
40
60
80
-160
-80
0
80
160
Body orientation relative to flower (deg)
50% sucrose
30%
10%
20%
B
Number of frames per bee
0
20
40
60
80
100
-160
-80
0
80
160
Body orientation relative to flower (deg)
50%
20%
C
Frequency of flower facing
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
10
20
30
40
Distance from flower (cm)
20% sucrose
50% sucrose

Figure 3

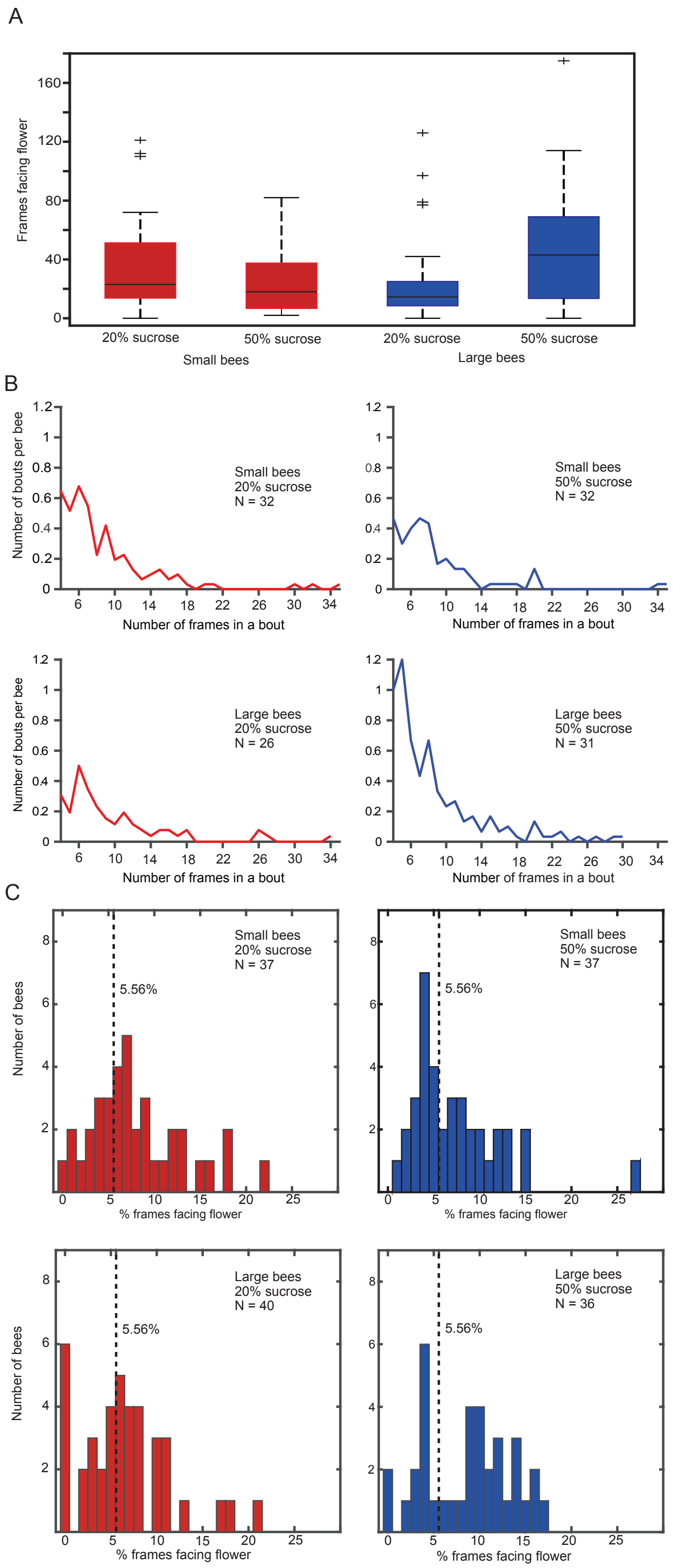
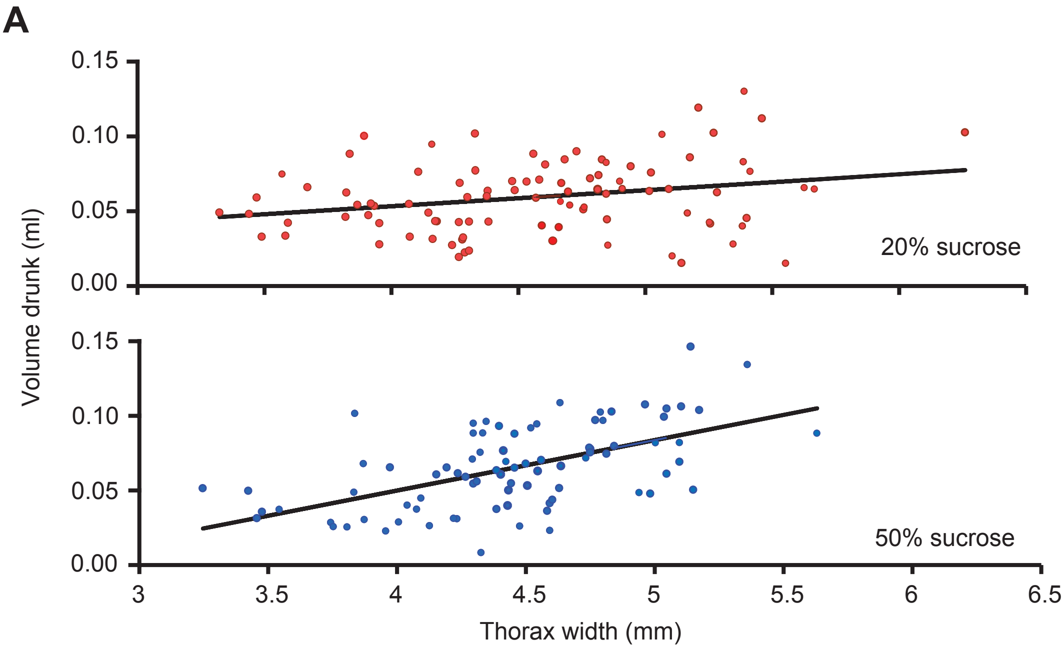
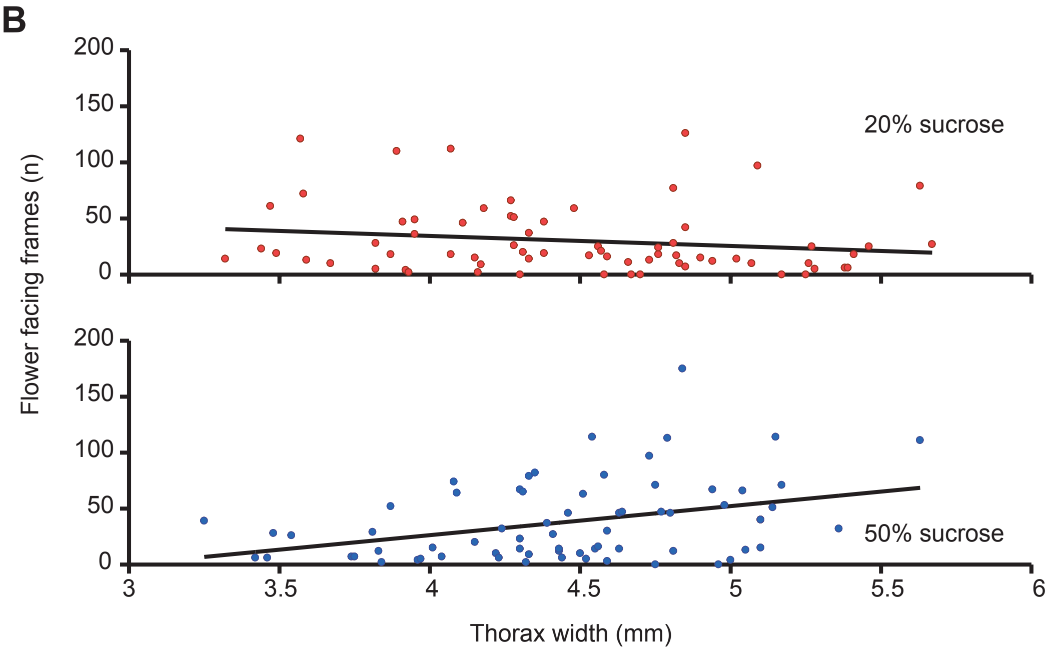

A
Frames facing flower
0
40
80
120
160
20% sucrose
50% sucrose
Small bees
20% sucrose
50% sucrose
Large bees
B
Number of bouts per bee
Small bees
20% sucrose
N = 32
Small bees
50% sucrose
N = 32
Large bees
20% sucrose
N = 26
Large bees
50% sucrose
N = 31
Number of frames in a bout
6
10
14
18
22
26
30
34
0
0.2
0.4
0.6
0.8
1
1.2
C
Number of bees
Small bees
20% sucrose
N = 37
Small bees
50% sucrose
N = 37
Large bees
20% sucrose
N = 40
Large bees
50% sucrose
N = 36
5.56%
% frames facing flower
0
5
10
15
20
25
2
4
6
8

Figure 4


A
Volume drunk (ml)
0.15
0.10
0.05
0.00
20% sucrose
0.15
0.10
0.05
0.00
50% sucrose
3
3.5
4
4.5
5
5.5
6
6.5
Thorax width (mm)


B
Flower facing frames (n)
200
150
100
50
0
20% sucrose
200
150
100
50
0
50% sucrose
3
3.5
4
4.5
5
5.5
6
Thorax width (mm)

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: Organisms/Strains		
Bombus terrestris audax	Koppert UK	Bombus terrestris audax
Software and Algorithms		
'betareg', R	[36]	DOI: 10.18637/jss.v034.i02
'pscl', R	[35, 37]	https://github.com/atahk/pscl/ DOI: 10.18637/jss.v027.i08
Other		
Sugar	Silverspoon, UK	British granulated sugar

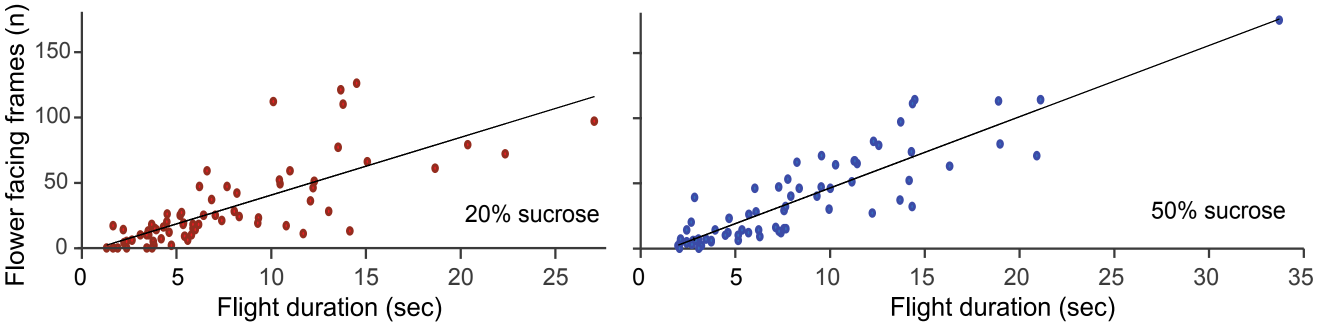


Figure S1. Flower facing *versus* flight duration. Related to Figure 2.

The relation between the duration of an individual's learning flight and the number of its flower facing frames for bees that drank 20% (n = 69 bees) or 50% sucrose solution (n = 68 bees). The regression coefficients between the duration of the flight and the number of flower facing frames are significant (z = 7.496, $p < 0.001$, Table S1) but there is no difference between coefficients of the two concentrations (z = 0.78, p = 0.436) and no difference between the intercepts (z = -0.517, p = 0.605).

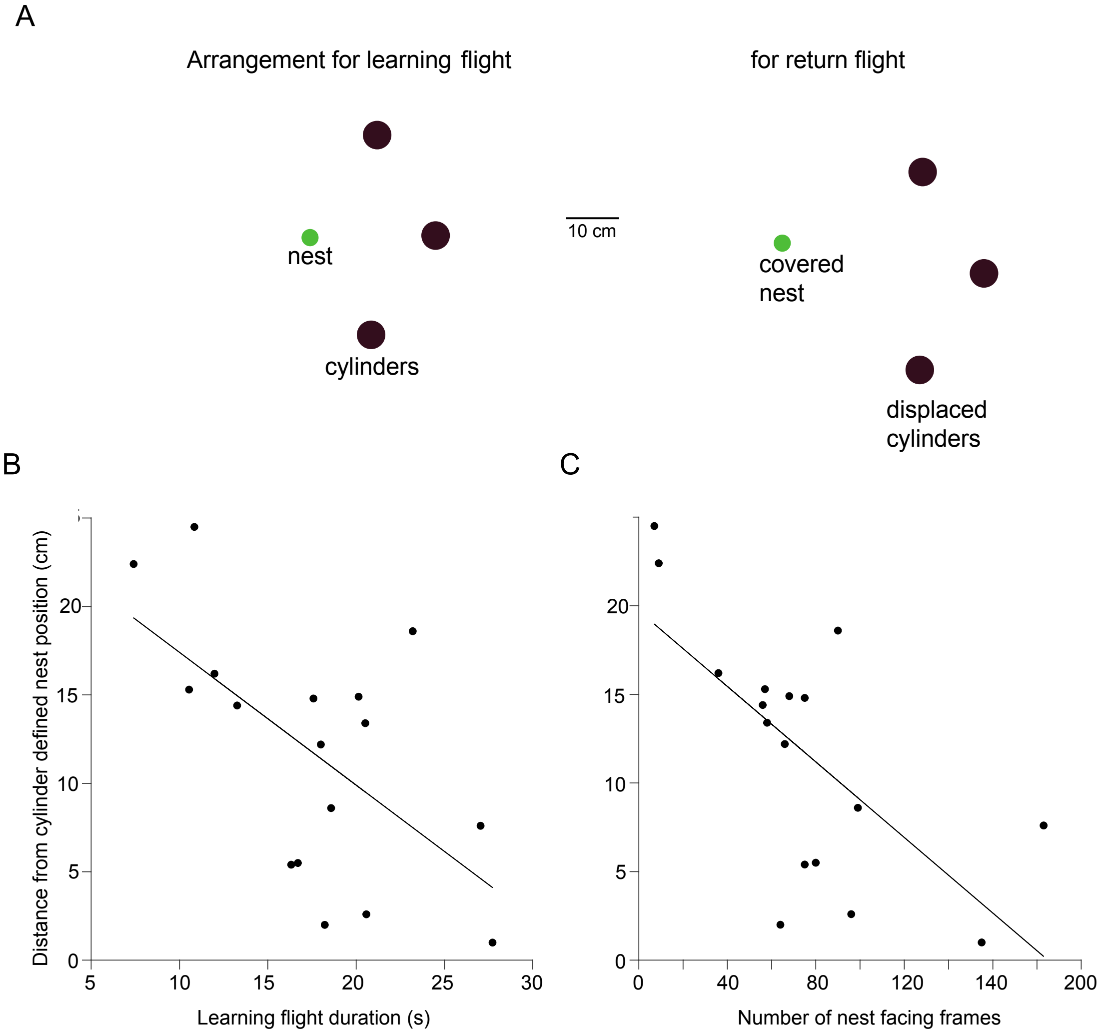


Figure S2. Landing precision on a bee's first return after the first learning flight. Related to Figure 2.

A. Arrangement of cylinders during the learning flight when the bee departed the nest (left). When it returned (right), the nest entrance was covered and the whole cylinder array shifted in different directions.

B. Correlation between first landing distance relative to the cylinder-defined position of the nest and the duration of its first learning flight prior to the test.

C. Correlation between first landing distance and number of frames facing the nest (± 10^{o}) during its first learning flight.

Bumblebee's learning flight on leaving its nest for the first time gives the bee its first view of the surroundings of its nest. This first learning flight, which is typically much longer than learning flights from flowers [S1, S2], is often followed by a long foraging trip [S3, S4] so that the bee's ability to find its nest depends in large measure on the efficacy of its learning flight. Learning flights on first leaving a flower have several functions. Foraging bees mostly visit multiple flowers within a patch and several patches before filling their crop. Honeybees are known to learn the colour and shape of flowers during their learning flights as well as the flower's surroundings [S5, S6]. It is hard to guess which of the memories of these properties improves most from longer flights. Given this uncertainty and the greater range in duration of learning flights from the nest, we analysed pre-existing data to determine whether a bee's precision in localising its nest improves with the duration of its learning flight.

The video recordings of the bee's return were examined to find the first time that the bee landed relative to the fictive nest position specified by the displaced cylinders. One bee initially landed very far away (> 60cm), therefore its second landing was included.

There is a clear relation between the duration of the learning flight and the proximity of a bee's landing distance position from the nest (n = 17 bees, Spearman Rank Spearman Rank, one-tailed, rho = -0.542, p = 0.013). A similar relation is found between the number of nest-facing frames in the flight and the bee's landing position (Spearman rho = -0.646, p = 0.0025).

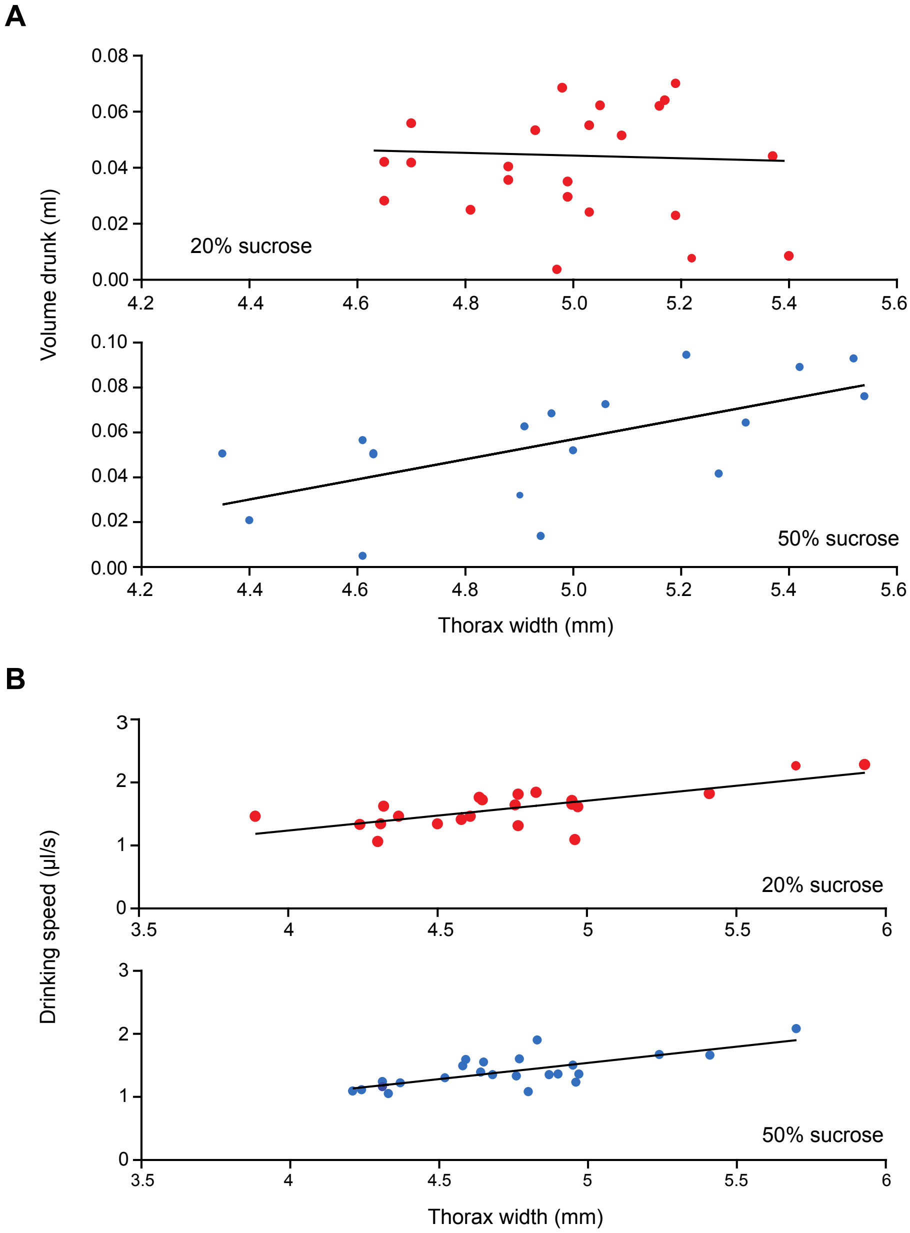


Figure S3. Drinking behaviour of differently sized bees. Related to Figure 4.

A. Relation between imbibed volume and thorax size. Most bees fell in the large category (> 4.5 mm, 20% $n = 23$ out of 23 bees, 50% $n = 16$ out of 18 bees). Bees drank similar volumes when the sucrose solution was 20% (Spearman Ranks, rho = 0.02, $p = 0.45$), but larger bees drank more 50% sucrose solution than smaller ones (rho = 0.69, $p < 0.0001$) ($t(3,37) = 2.03$, $p < 0.05$, Table S1).

B. Relation between a bee's body size and its speed of drinking 20% ($n = 22$ bees) or 50% ($n = 24$ bees) sucrose solution. Drinking speed was significantly higher in larger bees (Spearman, 20% rho = 0.61, $p = 0.0028$, 50%, rho = 0.64, $p < 0.001$). The rate of increase was 0.5 µl/s per mm of thorax width (20% ß = 0.00048, SE = 0.0001, $t(20) = 4.72$, $p < 0.001$, 50% ß = 0.00051, SE = 0.0001, $t(22) = 5.06$, $p < 0.001$). This relationship accounts for a significant proportion of the variance in speed for each concentration (20% $R^2 = 0.53$, $F(1,20) = 22.3$, $p < 0.001$, 50% $R^2 = 0.54$, $F(1,22) = 25.6$, $p < 0.0001$). These data allowed us to estimate drinking volumes from the duration of drinking that was recorded on video.

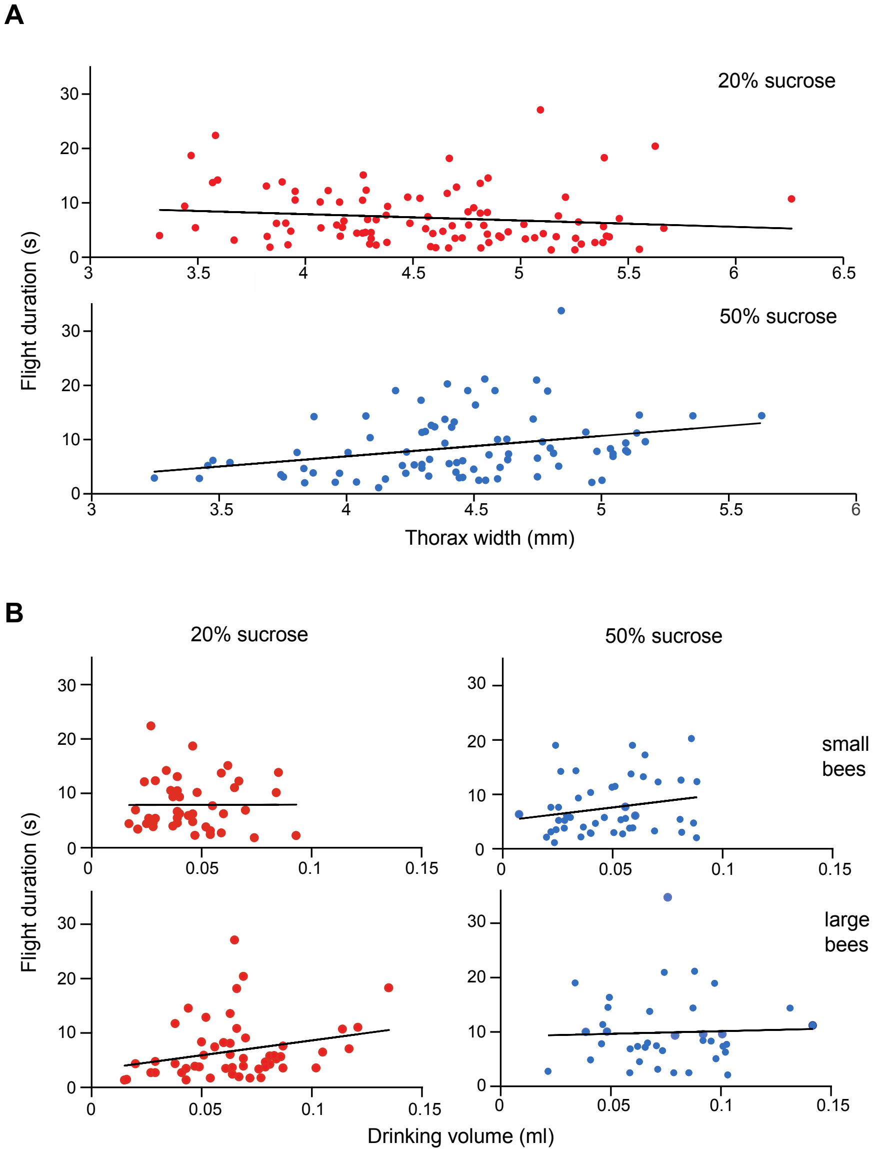


Figure S4. Learning flight duration, bee size and sucrose concentration. Related to Figure 4.

A. Duration of learning flights in different-sized bees. After drinking 20% sucrose solution, the duration of learning flights reduced a little with bee size (n = 95 bees, Spearman Rank, rho = -0.216, p = 0.035). The relation reversed with 50% (n = 84 bees, rho = 0.331, p = 0.0021). The two regression coefficients differ significantly (p = 0.001, Table S1). As expected, this pattern is similar to that in Figure 4B.

B. Drinking volume and flight duration in small and large bees. The volume of sucrose drunk had little effect on the duration of the subsequent learning flights. In small bees (thorax width < 4.5 mm) drinking 20% sucrose solution, there is no association between learning flight duration and volume drunk (n = 43 bees, Spearman Rank, rho = 0.038, p = 0.807). When small bees drank 50% sucrose solution, learning flight duration increased slightly with volume drunk (n = 46 bees, rho = 0.222, p = 0.138). The situation reversed in large bees (thorax width >= 4.5 mm). There was a slight increase in learning flight duration with increased drinking volume after drinking 20% sucrose solution (n = 52 bees, rho = 0.282, p = 0.043) but no change in duration with increasing volume after drinking 50% (n = 38 bees, rho = 0.0056, p = 0.974).

Figure	Dependent variable Model	Predictors Parameters	Coefficients	Error	z	F/t	df	P
4A	Drinking volume	Thorax width						
	Linear model	Adj R^2	0.284			24.51	3,175	<0.001
		Intercept	-0.025	0.02		-1.42		0.157
		20%	0.018	0.004		4.67		<0.001
		50%	-0.074	0.03		-2.57		0.011
		20% : 50%	0.018	0.01		2.86		0.005
4B	Flower facing	Thorax width						
	Hurdle model						8,128	
	Count model (Zero-truncated negative binomial with log link	Log-likelihood	-609.9					
		Intercept	4.389	0.84	5.24			<0.001
		20%	-0.205	0.19	-1.10			0.273
		50%	-4.148	1.28	-3.24			0.001
		50% : 20%	0.957	0.29	3.34			0.001
	Zero model (Binomial with logit link)	Intercept	7.017	3.73	1.88			0.060
		20%	-1.011	0.78	-1.3			0.196
		50%	5.562	9.05	0.61			0.539
		50% : 20%	-0.941	1.87	-0.50			0.615
S1	Flower facing	Flight duration						
	Hurdle model						8, 128	
	Count model (Zero-truncated negative binomial with log link)	Log-likelihood	-550.2					
		Intercept	2.228	0.17	13.45			<0.001
		20%	0.131	0.02	7.5			<0.001
		50%	-0.122	0.24	-0.52			0.605
		20% : 50%	0.019	0.02	0.78			0.436
	Zero model (Binomial with logit link)	Intercept	-2.813	1.56	-1.80			0.072
		20%	1.372	0.53	2.58			0.010
		50%	1.526	3.45	0.44			0.658
		20% : 50%	-0.076	1.26	-0.06			0.952
S3A	Drinking volume	Thorax width						
	Linear model (50% vs 20%)	Adj R^2	0.257			5.62	3,37	0.003
		Intercept	0.065	0.10		0.64		0.527
		20%	-0.005	0.02		-0.25		0.808
		50%	-0.231	0.12		-1.90		0.066
		20% : 50%	0.050	0.02		2.03		0.0495
S4A	Flight duration	Thorax width						
	GLM Gamma family (log link)	Intercept	2.626	0.55		4.77	3,175	<0.001
		20%	-0.141	0.12		-1.18		0.239
		50%	-2.766	0.90		-3.06		0.003
		20% : 50%	0.649	0.20		3.24		0.001

Table S1. Statistical analysis. Related to Figure 4.

Results are shown for multiple regression and hurdle models with interactions. All models were validated.

Supplemental references

S1. Robert, T., Frasnelli, E., Hempel de Ibarra, N., and Collett, T.S. (2018). Variations on a theme: bumblebee learning flights from the nest and from flowers. J. Exp. Biol. *221*, jeb172601.

S2. Robert, T., Frasnelli, E., Collett, T.S., and Hempel de Ibarra, N. (2017). Male bumblebees perform learning flights on leaving a flower but not when leaving their nest. J. Exp. Biol. *220*, 930-937.

S3. Hempel de Ibarra, N., Philippides, A., Riabinina, O., and Collett, T.S. (2009). Preferred viewing directions of bumblebees (*Bombus terrestris* L.) when learning and approaching their nest site. J. Exp. Biol. *212*, 3193-3204.

S4. Osborne, J.L., Smith, A., Clark, S.J., Reynolds, D.R., Barron, M.C., Lim, K.S., and Reynolds, A.M. (2013). The ontogeny of bumblebee flight trajectories: from naïve explorers to experienced foragers. PloS ONE *8*, e78681.

S5. Lehrer, M., and Collett, T. (1994). Approaching and departing bees learn different cues to the distance of a landmark. J. Comp. Physiol. A *175*, 171-177.

S6. Lehrer, M. (1993). Why do bees turn back and look? J. Comp. Physiol. A *172*, 549-563.