Trading off short-term costs for long-term gains: how do bumblebees decide to learn morphologically complex flowers?

Felicity Muth [a, *], Tamar Keasar [b], Anna Dornhaus [a]

[a] Department of Ecology & Evolutionary Biology, University of Arizona, Tucson, AZ, U.S.A.
[b] Department of Biology, University of Haifa at Oranim, Tivon, Israel

Many animals learn skills that can take a long time to acquire. Such learned skills may have high payoffs eventually, but during the period of learning their net profitability is low. When there are other options available, it is not clear how animals decide to learn how to perform tasks that initially have low or no benefits. Bees in particular visit many types of flowers that vary in the time required to learn how to access their food rewards. We used bumblebees (*Bombus impatiens*) to address how individuals decide to persevere with learning to handle 'complex' flowers. We tested two hypotheses: (1) individuals have unlearned preferences for more complex flowers; (2) individuals use the absolute reward value of the flower to decide whether to learn to handle a particular flower type. We presented individual bees with mixed arrays of colour-distinct 'simple' and 'complex' flowers, either containing the same value of reward, or where the complex flowers contained twice the concentration of sucrose as the simple flowers. Foragers did not show any unlearned preferences towards the complex flowers, but instead preferred the simple flowers. The strongest initial preferences were for flower colour (purple over pink). Our second hypothesis was supported, because when the purple complex flowers contained a higher reward than the simple flowers, more bees persevered with visiting them, foraging on them exclusively by the end of the test period. There was significant variation between individuals in whether they learned to handle, and how much they visited, complex flowers. These results highlight the complex interplay between unlearned biases and environmental feedback in making decisions about what to learn.
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Much learned behaviour in animals is not initially beneficial to the animal as it learns it, but instead benefits the individual in the long run. This is particularly evident in foraging behaviour. Animals may spend numerous attempts refining foraging techniques that may only be beneficial once the forager has learned how to extract the food in question effectively, so that the cost of the time invested in accessing the food is smaller than the benefit gained. For example, capuchin monkeys, *Cebus paella*, can take years to perfect their ability to crack nuts open (Fragaszy & Adams-Curtis, 1997). These animals likely suffer a cost in foraging efficiency, at least at first. Bumblebee foraging is another notable example of this. When flowers require the same amount of time to handle but differ in nectar rewards associated with a floral feature, bumblebees can rapidly learn to visit the most highly rewarding flower (e.g. Cnaani et al., 2006). However, in other cases, bumblebee individuals visit flowers that may contain high rewards but yield low initial rates of net energy gain due to the many trials needed to learn how to access nectar efficiently (Laverty, 1980, 1994). Bumblebee foragers will sometimes persevere with learning to handle such flowers even when there are other flowers nearby containing higher net rewards (i.e. immediate rewards of lower-quality that do not require learning; Heinrich, 1979).

In the examples above, even though learning pays in the long term, it is not always clear what mechanisms influence the decision to persevere with learning a task in the short term when a more immediately rewarding option is available. In short, how does an animal decide to choose a more 'difficult' option if there is a simpler one available? One possibility for how animals might persevere with learning a task that is not immediately profitable is that there is an unlearned tendency for them to do so. This would mean that a particular behavioural strategy had been favoured by natural selection and that consequently the animal carries out the respective behaviour even if it is costly in the short term, as it pays in the long term. This is the case for many learned behaviours that do not become efficient until the individual reaches a certain level of proficiency or until it is of a particular age. For example, play behaviour

* Correspondence and present address: Department of Biology, University of Nevada, Reno, NV 89557, U.S.A.
    E-mail address: fmuth@unr.edu (F. Muth).

in mammals and some species of birds is costly and yields no im-mediate tangible reward, however it improves future adult motor skills and thus presumably pays in the long term (Caro, 1988). Other examples include the development of song in birds (Catchpole & Slater, 2008) and certain courtship rituals (Diamond, 1986). Alter-natively, animals might not have unlearned preferences to perform a particular task, but instead use feedback from the task to deter-mine whether it is worth persevering with it. This could be either through assessing the absolute value of the reward, the net value once handling costs have been accounted for, or via an estimation of how long it will take them to learn to perform the task effectively.

As generalists, bumblebees (*Bombus* spp.) sample many different species of flower that differ in structure and in the amount of learning that is required to extract their nectar, before special-izing on one or a few types (Chittka, Thomson, & Waser, 1999). Bumblebees forage not only for themselves but also for their colony, and individuals may make thousands of flower visits per day (Goulson, 2003). This means that a slight increase in the amount of handling time per flower might incur a large time penalty overall. Thus it is important that individuals specialize on the type of flower that yields the most reward per time spent to handle it. Learning to handle a structurally complex flower is costly both in terms of the time invested and because it can interfere with learning how to handle another flower of a different morphology (Chittka & Thomson, 1997; Gegear & Laverty, 1995). This means that it is important for the forager to make the 'correct' choice in deciding which flowers to learn to handle.

The morphology of wild flowers has generally been defined in terms of how structurally 'complex' the flower is (Heinrich, 1976, 1979; Laverty, 1980). This morphological 'complexity' of a flower can be a somewhat subjective description: 'complex' flowers have a closed corolla with the nectar either concealed or in an unusual location, and they often possess bilateral symmetry such as the flower *Chelone glabra*, but can also be radially symmetrical, as in the case of flowers in the genus *Aquilegia*. 'Simple' flowers are usually radially symmetrical with an open corolla, and their nectar may be detectable to insects that land on them through sight or smell (Heinrich, 1979; Laverty, 1980, 1994), for example, *Taraxacum offi-cinale* flowers. However, as these are rather human-subjective definitions of complexity, from the pollinator's perspective, floral complexity can be described as how long it takes an individual to learn how to effectively extract nectar from the flower. 'Simple' flowers require little or no learning according to this working definition, and 'complex' flowers require more learning (Laverty, 1994). As there may be variation between forager species in the amount of learning required to effectively handle a given flower species, how 'simple' or 'complex' a flower is will depend on the species of forager in question. However, in the majority of cases the amount of learning required to extract nectar from a flower directly relates to the more subjective view of how 'complex' the flower structure appears (Heinrich, 1979; Laverty, 1980, 1994). Note that in this paper 'complex' always refers to the complexity of handling behaviours required, not to the stimulus complexity, such as whether the floral signal belongs to multiple modalities (Leonard, Dornhaus, & Papaj, 2011).

Both field and laboratory studies demonstrate costs of extracting a nectar reward from structurally complex flowers, including increased handling time (Macior, 1966; Ohashi, 2002), making more errors (Laverty, 1980) and often failing to gain any reward during the earlier visits (Heinrich, 1979; Laverty, 1980, 1994). In a controlled field study by Heinrich (1979), inexperienced *Bombus vagans* bumblebees had 100% success at accessing nectar from simple flowers with open inflorescences (*Aster novae-angliae* and *Solidago* sp.) Their initial success on the more complex species of jewelweed, *Impatiens biflora*, was around 45%, improving to 90% only after 60

visits to that flower type. Despite this, most bees became constant to the complex jewelweed that required the most learning but had the highest nectar reward (measured as sugar per flower; Heinrich, 1979). Similarly, individuals of *Bombus* species tested on natural flowers manipulated simple flowers effectively from their first visit, whereas they took 60−100 visits to reach 90% accuracy at handling the complex flowers *Impatiens biflora* and *Chelone glabra* (Laverty, 1980). In another study, four species of naïve bumblebees (*Bombus fervidus*, *Bombus impatiens*, *Bombus rufocinctus* and *Bombus vagans*) had 100% initial success on simple flowers, but only 29−45% success on complex flowers, taking 30−60 visits to reach the level of experienced bees (Laverty, 1994). From the flower's perspective, complexity may be advantageous as it can result in specialist for-agers, increasing the likelihood of the insect forager visiting the same species of flower successively and thus pollinating effectively. Because floral complexity thus leads to a small proportion of specialist pollinators being able to access the nectar reward, this selects for higher nectar production rates compared to simple flowers (Cohen & Shmida, 1993; Heinrich, 1979; Peleg, Shmida, & Ellner, 1992; Potts et al., 2003; Warren & Diaz, 2001). Therefore, the payoff for bees that learn how to effectively handle these flowers is predicted to be greater in the long term. Individuals that learn to handle complex flowers may also benefit through reduced intra- and interspecific competition (Chittka et al., 1999; Heinrich, 1979; Stout, Allen, & Goulson, 1998). While there are clear advantages then to persevering with learning how to handle complex flowers in the long term, it is not clear how individual bees decide to persist with learning them in the short term.

Here we investigate when and how *B. impatiens* bumblebees persevere with learning how to handle a flower that is morpho-logically complex when there is a simpler option available. Specif-ically we ask (1) whether individuals have unlearned biases that lead them to forage on the more complex option in spite of initial low rewards. We also ask (2) whether bees make their foraging decisions based on the reward value (i.e. nectar concentration), despite a high cost of handling. To address these two questions, we presented bumblebee foragers with arrays of artificial flowers that were either 'simple' or 'complex' and that contained sucrose solu-tion as a reward. Both types of flowers were radially symmetrical, but the simple flowers had the sucrose solution visible to the bee upon or even prior to landing, whereas the complex flowers had the nectar hidden inside them. Thus the bees were required to learn how to access the reward in the complex flowers, which involved pushing open the petals, crawling inside the corolla and locating the sucrose solution inside. In one treatment, the two types of artificial flowers contained identical rewards, whereas in the other, the su-crose reward in the complex flowers was twice as concentrated as in the simple flowers. Bees were able to distinguish the two types of flower by colour. Hypothesis (1), an unlearned predisposition to visit complex flowers, predicts initial preferences for the artificial complex flowers over the simple flowers. Hypothesis (2), that bees use reward value as an estimate of future profitability, predicts that bees offered flowers with equal rewards should specialize on the simple flowers. On the other hand, individuals that forage on complex flowers that contain twice the reward of the simple flower should invest in learning how to access the nectar effectively from this flower type, as its eventual profitability should be higher than that of simple flowers, even if this is not the case initially.

METHODS

Subjects and Maintenance

We obtained five colonies of *B. impatiens* from Koppert Biological Systems (Howell, MI, U.S.A.) and marked all bees using numbered

tags (E.H. Thorne Ltd, Wragby, Lincolnshire, U.K.) fitted on to their thorax with superglue. We housed each colony in a wooden nestbox measuring $39 \times 10.5 \times 23$ cm (length $\times$ height $\times$ width) with a clear plastic Perspex roof. From there the bees accessed an experimental chamber via a plastic tube, which could be restricted by inserting a cardboard 'gate' that blocked the foragers' path. The experimental flight chamber measured $90 \times 40 \times 60$ cm (length $\times$ height $\times$ width) and was also wooden with a clear Perspex roof. Bees were maintained on 25% sucrose solution (by volume) throughout the experiment, which was transferred into their honeypots at the end of each day with a syringe used to fill all honeypots. Nestboxes were also provided with pollen ad libitum.

Artificial Flowers

All bees were exposed to two types of flower to forage on, 'simple' and 'complex'. These were made from artificial flowers bought from a craft store (Michael's) and resembled tulips. The same artificial flower type was used to make both experimental flower types, to ensure that they were the same in all aspects (size, material, etc.) except for the ones that we manipulated. Both types had 5 mm Eppendorf tube tips glued into them with hot glue to hold the 'nectar' (sugar solution) reward. Simple flowers had their petals glued to the tube at the top, such that the tube opened upward (thus the sucrose solution was exposed and accessible to a bee sitting on top of the flower). Complex flowers had the tube glued onto the inside base of the flower. In these flowers, the nectar was thus not visible from outside the flower, and bees had to go inside the flower to access the reward (see Fig. 1 for diagram and Supplementary Material for photographs of the artificial flowers). We used pink and purple flowers for both types, and measured the reflectance spectra of their 'petals' using a single beam UV-VIS spectrophotometer (Ocean Optics, Dunedin, FL, U.S.A.). The spectrophotometer was connected by means of a USB cable to a computer running SpectraSuite software (Ocean Optics). Both the pink and purple flowers had reflectance peaks around 440 nm. In addition, pink reflected strongly around 600 nm and beyond, whereas purple reflected strongly at 700 nm. For further details on reflectance spectra see Fig. S1 in the Supplementary Material. We used two colours to give the bees a cue to differentiate between the two flower types.

General Protocol

Foragers were identified in the training (see below) and pseudorandomly assigned to one of four treatment groups for testing,
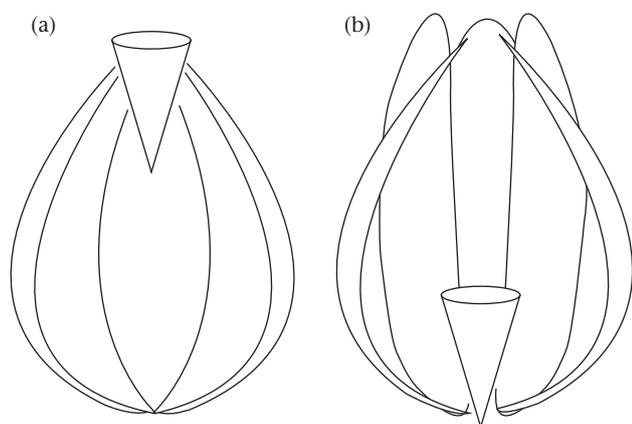

Figure 1. A cross-section diagram of a (a) simple and (b) complex artificial flower used in the current experiment, each containing a cone-shaped Eppendorf tube tip that held the sucrose reward.

such that bees from three colonies were represented in each treatment group, with at least two colonies being included across all treatment groups (see Supplementary Material). Bees were then allowed to forage on the test flowers, and if they landed on either of the flower types they were included in the experiment. All treatment groups were exposed to both types of flower (simple and complex) to forage on, all containing 20 µl of unscented sucrose solution. This volume of reward has been used in artificial flowers in previous experiments with *Bombus* (e.g. Leadbeater & Chittka, 2007). The energy reward of floral nectar depends on both its volume and its sugar concentration. In our experiment we manipulated sugar concentration rather than volume, as bees respond more strongly to this (Cnaani et al., 2006), with the aim of seeing a more pronounced flower preference after sampling. In two of the treatment groups, the reward value (sucrose concentration) was equal in both flower types (50% sucrose:water by volume), whereas in the two other groups the complex flower had twice the concentration of sugar as the simple flower (50% versus 25%). In each of these two treatments, one group was tested on purple complex flowers and pink simple flowers, and one on pink complex flowers and purple simple flowers, allowing us to control for bees' colour preference biases. This resulted in four colour–morphology–reward combinations.

A total of 54 bees were used in our experiment over a 6-month period (between January and June 2013). We used eight bees in each of the pink complex/purple simple groups, and 16 and 22 bees in the equal reward and unequal reward purple complex/pink simple groups, respectively. For an explanation of sample sizes used, see Supplementary Material.

Training

Bees were trained on a feeder placed in the same location as the experimental flower array to encourage them to forage in the experimental arena. This training feeder was a single multiport glass feeder containing 25% unscented sucrose solution (by volume). It was placed on a platform with equal numbers of pink and purple 'petals' taken from the artificial flowers glued around its outside. This feeder was used as it was sufficiently different from both types of flower to prevent any motor learning that might be used in manipulating the two experimental flower types. Thus the pretrained bees were naïve to the handling technique of the experimental flowers when they first encountered them. We added the coloured petals to the training feeder to encourage the bees to visit the artificial flowers through a learned association with colour. The bees were given access to the experimental arena and training feeder for between 30 min and 2 h per day, to allow individuals that foraged successfully and returned to the colony to be identified. Once such individuals had been identified, they were generally tested within the next 2 days. Bees did not encounter the artificial flowers used in the experiment until testing.

Testing

Each forager bee being tested was given access to the experimental arena where 10 artificial flowers (five simple and five complex) were presented. To speed up the selection of foragers for the experiment, multiple (up to four) foragers that had been seen feeding on the training feeder were let into the arena at a time (for more information see Supplementary Material). Once one of these landed on a flower, the others were removed without disturbance to the foraging bee. Each bee was tested in a total of seven trials, generally conducted within a single day ($N = 50$ bees; $N = 4$ bees were tested over 2 days). Each trial consisted of the bee entering the experimental arena and visiting flowers in a free-choice assay. The

trial was stopped when the bee left the arena and returned to the colony or when 10 min had elapsed (at which point we removed the bee and returned it to the nest). The exception to this was the first trial, where the bee was given unlimited time to visit the first flower. At the end of each trial, all flowers were replaced with clean ones to avoid possible effects of any scent marks on the next trip or individual. The flower array was always placed in the same location in the flight chamber, but the location of simple and complex flowers within the array was randomized for each trial. At the end of each day of experiments, all flowers were soaked in hot soapy water for about 6 h, and air dried overnight.

We recorded which flowers the bee landed on and whether the bee gained the reward. We also filmed all test trials on a Panasonic camcorder positioned above the experimental arena.

Behaviour Coding

We defined bees as having visited a flower if they landed on it. If a bee located the sugar solution in the tube in the artificial flowers and was seen to feed on it (identified as the abdomen moving while the bee had its head positioned over the tube), we recorded this as the bee taking the reward. If the bee stuck its head (and presumably extended its proboscis) into the tube only briefly (usually <5 s), then we defined this as tasting and rejecting the reward. If a bee found the location of the tube in the artificial flower, and stopped over it (presumably ejecting its proboscis) but the tube had already been emptied by the bee, we coded this as the bee finding the location, but the flower being 'empty'. If the bee landed on the flower but did not extend its proboscis at the location of the tube (e.g. if the bee was seen walking over the tube but not putting its head in it), we coded this as the bee not finding the reward.

Behavioural observations were made during trials and double-checked by comparison with the recorded video afterwards, at which point we also recorded how long (to the nearest second) the bees spent on the flower in total, from the first contact of their front legs with the flower at landing until all legs completely left the flower. We also recorded how long it took the bee to gain the reward (from first landing on the flower to stopping over the reward tube). Visits that took less than 1 s were recorded as 'less than 1 s'. In two cases bees stopped on flowers without moving (apparently resting) and these data were excluded.

Body Size Measures

We measured thorax width of all individuals used in the experiment as an indicator of body size. We did this because larger foragers tend to learn more rapidly than smaller ones (Mares, Ash, & Gronenberg, 2005; Worden, Skemp, & Papaj, 2005). We then tested whether body size correlated with the bees' foraging choices.

Data Analysis

We carried out all analyses in R version 2.15.1 (R Development Core Team, 2010). GLMMs were carried out using the lmer() function in the lme4 package (Bates & Maechler, 2010) that gives z values and $\mathrm{Pr}(>|z|)$, an estimation of a P value, for each level of testing. LMMs were carried out using the lme() function in the nlme package, specifying type three sum of squares and sum contrasts in cases where there were interactions (Pinheiro, Bates, Debroy, & Sarkar, 2010). For all models, maximal models were run initially, and then nonsignificant interactions were removed in a stepwise fashion. In cases of significant interactions, simplified models were rerun to determine the significance of the individual factors in these interactions. To do this, we divided the data into two groups by one of the two-level factors in the significant interaction, and ran

two separate analyses in order to determine the significance of the remaining main effects.

To ascertain whether our complex flowers took longer for bees to learn to handle than the simple ones, we looked at the cumulative time taken for bees to access the nectar on each flower type. We defined 'cumulative time' as the total time bees spent on a flower type (potentially across several landings and different individual flowers) before accessing the nectar. Using the data from bees that gained equal ($N = 10$) and unequal ($N = 10$) rewards from both flower types, we calculated a linear mixed model (LMM; 'Model 1') with the response variable 'cumulative time until reward'. We tested for the effects of the following fixed factors: treatment (equal/unequal rewards); flower (purple complex/pink simple); the continuous variable 'rewarded visit number' (1–15; this is the number of times a bee accessed the reward in a particular flower type, and thus reflects the amount of experience the bee has gained across all trials of the experiment); and the random factor 'bee' (1–20).

To ascertain whether there were any unlearned preferences for simple or complex flowers, we looked at the first flower that bees landed on (regardless of whether they gained the reward from it). We pooled the data from the two different reward treatments because at the bees' first choice they had not yet encountered the reward within the flowers, and thus flower choice was presumably unaffected by it. However, the same results held when the treatments were considered separately.

To determine whether the value of the reward affected bees' tendency to persevere with learning, we carried out a GLM analysis with the response variable 'number of bees choosing flower type' and the factors 'flower type' (pink simple or purple complex), 'treatment' (equal or unequal rewards) and the continuous variable 'visit number'.

To determine why individuals differed in the flower type they persevered on, we addressed individuals' tendency to reject lower-rewarding, simple flowers in the unequal rewards treatment group. To see whether bees that rejected more simple flowers were more likely to then take a reward from a simple flower, we ran an LMM with the response variable 'number of simple flowers probed and rejected before choice' with the explanatory factor 'choice of flower' (complex/simple) and the random factor 'bee'.

RESULTS

Unlearned Preferences

When purple simple flowers and pink complex flowers were presented as options, there was a clear preference for the purple simple flowers as all bees visited these flowers first (chi-square test: $\chi^2_1 = 16, P < 0.0001$; Fig. 2). When purple complex flowers and pink simple flowers were presented as options, there was no difference in the overall preference for one flower type over the other (chi-square test: $\chi^2_1 = 0.9, P = 0.343$). This indicates that initial preferences of bees are for the colour purple over pink and for simple over complex flowers.

To determine whether bees might have an unlearned tendency to persist with attempting to access the nectar in complex flowers after having landed on them without gaining the reward, we assessed their next flower choice after their first visit to a complex flower (which was unrewarded in the majority of cases). Of the 16 bees tested on purple simple flowers and pink complex flowers, nine landed on complex flowers but none of them gained the reward on their first visit there. There was no preference for complex flowers in the next choice of these bees: five chose complex flowers and four chose simple flowers on their second landing (chi-square test: $\chi^2_1 = 0.111, P = 0.739$). Of the 40 bees tested on pink
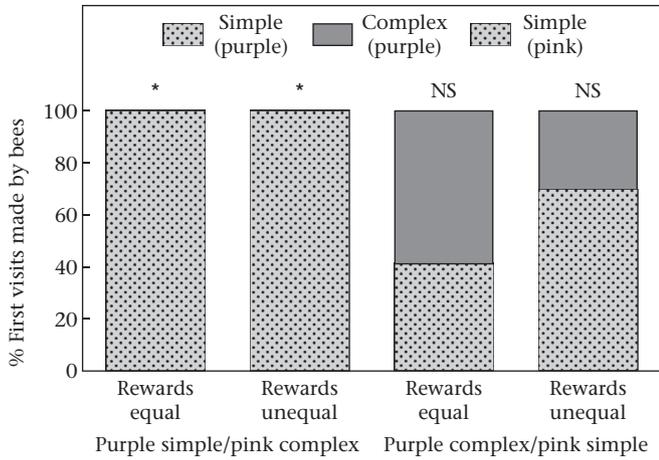
Figure 2. Percentage of bees that chose simple or complex flowers as their first choice, when choosing between pink and purple flowers. The first and third bars from the left are the choices made when the flowers contained equal sucrose rewards, and the second and fourth are when the complex flowers contained twice the reward as the simple flowers.

simple flowers and purple complex flowers, all visited complex flowers at some point: three gained the reward on their first visit and 37 did not. These 37 bees did not show a preference for complex flowers in their next visit: 23 visited complex flowers and 17 visited simple flowers (chi-square test: $\chi^2_1 = 0.900$, $P = 0.343$).

Do Complex Flowers Require More Learning Than Simple Flowers?

To determine whether our 'complex' artificial flowers met the criterion of learning difficulty, we ascertained (1) whether reward was initially more difficult for bees to locate in complex versus simple flowers, and (2) whether a longer period of learning was required for bees to reach a constant level of performance. We tested this second question for the purple complex flowers only, as bees never accessed the nectar in the pink complex flowers.

It was harder for bees to locate the nectar in the complex artificial flowers than in the simple flowers: of all the bees across all treatments, 46 of 50 bees were successful at obtaining nectar from the simple flower type on their first visit to it. In contrast, only 3 of 49 bees that landed on the complex flower type were successful at

gaining the reward from it on their first visit (chi-square test between percentage success with each flower type: $\chi^2_1 = 65.478$, $P < 0.0001$).

The bees' speed and success at handling complex flowers improved with learning (Fig. 3). It took bees longer overall to access rewards on complex flowers compared with simple flowers (LMM: flower type: $F_{1,167} = 31.357$, $P < 0.0001$). Bees showed more improvement in time to access rewards in complex flowers with increased experience (more successful rewarded visits) compared with simple flowers (LMM: flower type ∗ rewarded visit number: $F_{1,167} = 5.904$, $P = 0.016$). The time to access the complex flowers was not explained by the treatment group (equal or unequal rewards; $F_{1,18} = 0.003$, $P = 0.957$), but this comparison was limited as fewer bees persevered with choosing complex flowers in the unequal rewards group. The same findings (a decrease in time over trials and effect of flower type) were reflected in the bees' success rate using the number of trials, rather than the number of rewarded visits, as a measure of foraging experience (see Supplementary Material and Fig. S1).

To better compare the initial response to complex flowers in the two treatments, we also ran post hoc tests comparing the first three rewarded visits a bee made, while the majority of bees were still visiting both flower types (see data for first three rewarded visits in Fig. 3). Bees in the equal rewards treatment did not become significantly faster at accessing complex flowers over their first three successes (repeated measures ANOVA: $F_{1,8} = 1.966$, $P = 0.199$), while those in the unequal rewards treatment did ($F_{1,7} = 11.101$, $P = 0.027$).

We also analysed how many rewarded visits to complex flowers the bees needed before they handled them as quickly as the simple flowers. To this end, we compared handling times of simple and complex flowers in the unequal rewards treatment group, where bees persevered with choosing the complex flowers over many visits. These bees were equally fast at accessing complex and simple flowers by the 11th rewarded visit (post hoc t test: $t_5 = 1.90$, $P = 0.117$; Fig. 3b).

Does the Value of the Reward Affect Bees' Tendency to Persevere with Learning?

Fewer bees persevered with choosing the complex flowers when the simple flowers contained the same reward than when the flowers had unequal rewards (GLM using data from the first 15
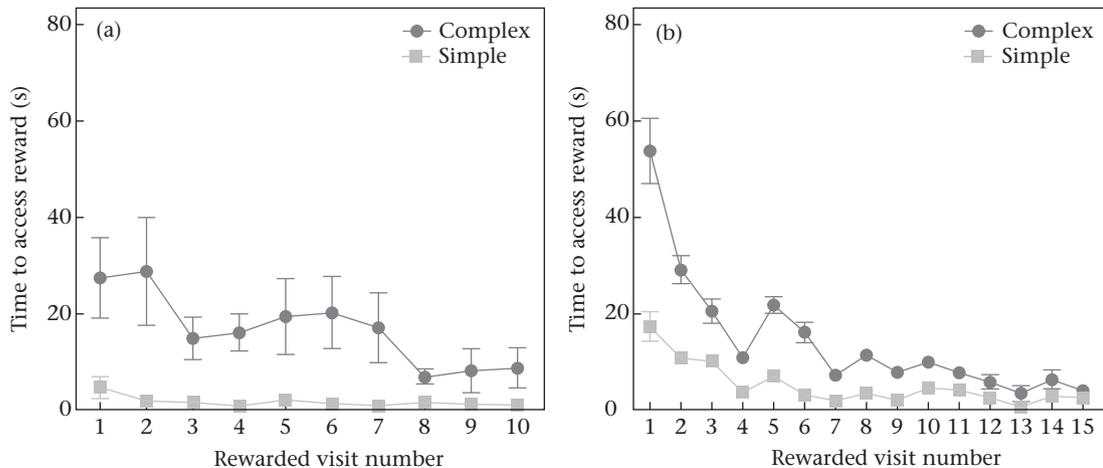

Figure 3. Mean ± SE cumulative time required for the bee to access the reward on purple complex flowers and on pink simple flowers when (a) the flowers contained equal rewards and (b) the complex flower had twice the reward as the simple flower.

rewarded visits: visit number∗flower type∗treatment (equal/un-equal): $F_{1,52} = 72.117$, $P < 0.0001$; Fig. 4). However, there were differences between bees, both in whether they accessed the reward in complex flowers at all, and in what they specialized on once they did access both rewards. This variability is described, and potential reasons for it are addressed, in the following sections.

Why Did Only Some Bees Learn to Successfully Forage on Complex Flowers?

When the complex flowers were pink, the bees' initial strong preference for purple simple flowers meant that the sucrose reward in pink complex ones was never accessed by any bee ($N = 16$). However, when the complex flowers were purple, 10 of 16 bees in the equal rewards treatment and 10 of 22 bees in the unequal rewards treatment accessed the nectar in the complex flowers, and all bees accessed the nectar in the simple ones.

Perhaps the very first visit made by a bee affects its later choices. Indeed, 14 out of 18 bees that only gained rewards from pink simple flowers had visited a simple flower first (chi-square test: $\chi^2_1 = 5.556$, $P = 0.018$). Bees that gained rewards from complex flowers were equally likely to have first visited either flower type ($\chi^2_1 = 0.800$, $P = 0.371$).

There were no differences in body size (thorax width) between the bees that did and did not learn to access nectar in complex flowers (unpaired *t* tests: equal rewards treatment: $t_{14} = 0.220$, $P = 0.829$; unequal rewards treatment: $t_{20} = -0.371$, $P = 0.715$).

Which Flower Type Did Bees Eventually Focus on in the Different Treatments?

Bees that encountered flowers of equal rewards differed in their final choices from those that encountered flowers of unequal rewards (Fisher's exact test of the choices of the 20 bees that gained access to both flower types in their final trial: $P = 0.028$; Fig. 5). In the equal rewards treatment, six bees specialized on simple flowers while four were still foraging on both types. However, in the unequal rewards group, six bees specialized on choosing complex flowers while three specialized on simple ones, and one bee still foraged on both. Thus, only when complex flowers contained twice the reward of the simple flowers did bees continue to forage from them.
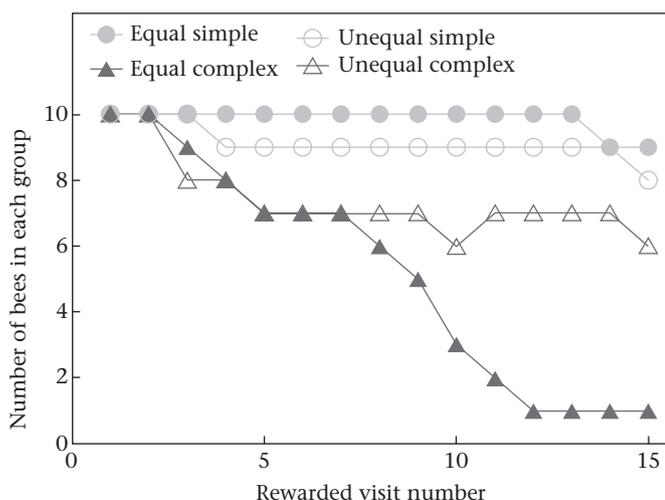

Figure 4. Number of bees that continued to choose simple or complex flowers across rewarded visits, in both the equal and unequal rewards treatment groups.
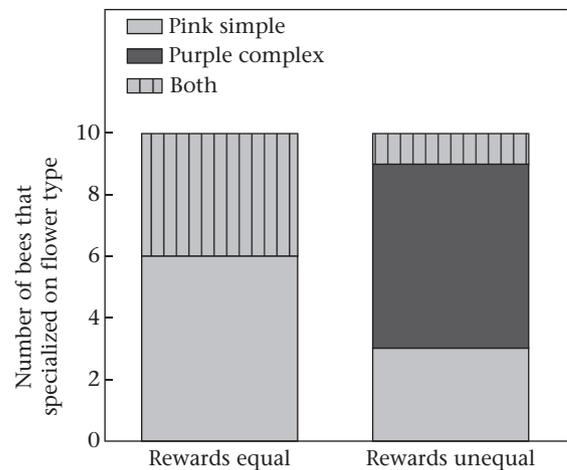

Figure 5. Number of bees that chose to specialize on simple flowers, complex flowers or both types of flower by the end of the seven test trials in the equal and unequal rewards treatments. Data are for bees that foraged on pink simple flowers and purple complex flowers only.

What Explains These Differences Between Individuals?

The bees' specializations on different flower types were not explained by their initial preferences, the first flower they gained a reward from, their speed at accessing the reward on complex flowers, their success rate on complex flowers, the time spent on each complex flower visited, or their body size (see Supplementary Material).

The only variable we found that explained whether individuals eventually foraged on simple or complex flowers when the complex flowers contained twice the reward was the bee's tendency to reject simple flowers. We observed 8 of 10 bees in the unequal rewards treatment rejecting a reward (i.e. extending their proboscis into a flower but then immediately leaving without taking in the sucrose solution). This did not occur when bees were foraging on flowers of equal rewards. Thus bees in the unequal rewards treatment group displayed this behaviour for a higher proportion of simple flowers than did bees in the equal rewards treatment (unpaired *t* test: $t_{18} = -4.795$, $P < 0.001$).

When the flowers had unequal rewards, bees that consecutively probed (and then rejected) more of the simple flowers were then more likely to next take a reward from a simple flower. Likewise, those that probed and rejected simple flowers but next took a reward from a complex flower did this after fewer probes and rejections of simple flowers (LMM with the response 'number of simple flowers probed and rejected before choice' and the explanatory factor 'choice of flower' (complex/simple) and random factor 'bee': $F_{1,33} = 2.042$, $P = 0.049$). Bees that took more rewards from simple flowers than from complex flowers after consecutive probing and rejections of simple flowers were then more likely to later specialize on simple flowers (comparison of the proportions of the rewards taken from simple flowers for bees that specialized on simple or complex flowers: Mann–Whitney *U* test: $U = 18.000$, $N_1 = 3$, $N_2 = 6$, $P = 0.024$).

DISCUSSION

When presented with artificial simple and complex flowers, bumblebee foragers only persevered with choosing the more complex flower when it contained twice the value of reward as the simple flower. Therefore our second hypothesis was supported, as

bees chose flowers with higher rewards despite gaining no reward from many of them at first. However, bees only accessed the reward in the complex flower when it was the preferred colour (purple), indicating that an initial bias towards the complex flower due to another cue (i.e. colour) may be necessary for the bee to even persevere with finding the reward to begin with. Our first hypothesis was not supported, as bees did not show any unlearned preferences towards the complex flowers; instead, they were more likely to land on the simple flowers first. The combination of bees' preferences for both simple and purple flowers meant that when the artificial flowers contained both of these features, no foragers ever visited the pink complex flowers enough to gain reward from them. Bees tested on purple complex flowers and pink simple flowers had a lower success rate on the complex flowers and took longer to learn to handle them, as has been found for bumblebees foraging on natural flowers. The quality of rewards also affected the speed of learning, as bees that encountered complex flowers that had higher rewards than simple flowers became faster at learning how to handle these flowers than bees that encountered complex flowers that had the same reward as simple flowers.

One hypothesis for how bees persevere with learning to handle complex flowers despite initial low rewards is through an unlearned tendency to manipulate complex flowers. This was not directly supported by our data: naïve bees were in fact more likely to visit the simple flowers first when given a choice between simple and complex flowers. This may be because as the bees flew around the arena searching for food, they were more likely to see the nectar in the open, exposed simple flowers. Other evidence against an unlearned preference for complex flowers was that once a bee landed on a complex flower and failed to find a reward, it was not more likely to return to a complex flower than to switch to a simple one. This lack of an unlearned preference for complexity contrasts with the bees' unlearned preference for colour: all bees tested on purple simple flowers chose them on their first visit above the pink complex ones, and bees tested on purple complex flowers were as likely to land on them as on pink simple ones, despite not being able to see the nectar reward inside. Unlearned colour preferences are well documented in bumblebees, in particular for colours in the violet–blue range of the colour spectrum (Forrest & Thomson, 2009; Ings, Raine, & Chittka, 2009; Raine & Chittka, 2007a; Raine, Ings, Dornhaus, Saleh, & Chittka, 2006; Simonds & Plowright, 2004). In this experiment, the bees' innate preference for the colour purple was a factor in encouraging the bees to continue visiting these flowers when they were of a more complex morphology. This finding suggests that perhaps colour could be used by plants to encourage a bumblebee forager to keep returning to a flower and persevere with learning how to access the nectar inside. Thus, one might expect that complex flowers might use this ability to attract specific foragers. Whether this is the case is not clear. Although bees have preferences for particular colours that are correlated with higher nectar rewards within certain areas (Chittka, Ings, & Raine, 2004; Giurfa, Nunez, Chittka, & Menzel, 1995; Raine & Chittka, 2007), it does not seem that a particular structure of flowers is associated with a particular colour in general (Gumbert, Kunze, & Chittka, 1999; Kevan, 1972; Smith, Ané, & Baum, 2008; Waser, 1983). However, flower colour may be correlated with the flower structure (or complexity) within a particular area (Dukas & Shmida, 1989), possibly reflecting local selection for the area's native pollinators.

If foragers use the value of the reward from the complex flower once they gain access to it as the main determinant as whether to persevere with learning how to handle it, then bees should persevere on complex flowers only when they contain a reward large enough to outweigh the cost of learning. This was indeed the case, although with considerable variation between individuals.

This finding makes sense from an optimal foraging perspective, because if more time is needed to learn how to extract the reward effectively, this will only be worthwhile if the reward is of a higher value. Furthermore, the initial value may be a cue to the bee for the expected payoff from learning: even though the payoff may be low initially because of high handling time, once the bee learns how to handle the flower effectively the higher value of reward is likely to be worth the cost of learning. As it took bees the same amount of time to access the reward in the complex flower as in the simple one by the 11th rewarded visit (but with multiple unrewarded visits), it would be more profitable to choose this flower exclusively at this point, if not before. Surprisingly, not all bees that encountered the higher-rewarding complex flowers foraged exclusively on them by the end of testing. This individual variation in foraging specializations, despite having the same flower choices, agrees with multiple laboratory studies of behaviour in bumblebees (e.g. Keasar, Motro, Shur, & Shmida, 1996; Worden et al., 2005), as well as in a study of their foraging behaviour on wild complex flowers (Laverty, 1994). Similar findings have also been reported for honeybees tested under similar conditions, where individuals varied in what flower type they specialized on when choosing between artificial flowers with high rewards but high handling times and low rewards and low handling times (Cakmak et al., 2009). Flower preferences were also heavily influenced by unlearned colour preferences in that species, as bees would restrict their visits to either blue or white flowers regardless of reward or cost of accessing the reward.

In our study, the only factor we identified that related to whether bees were more likely to specialize on simple or complex flowers when the complex flowers contained rewards of greater value was the bee's tendency to reject simple flowers. Individuals substantially differed in their response to a lower reward after having experienced a higher one. When sampling the less concentrated sucrose in simple flowers after having accessed the higher reward in complex flowers, bees demonstrated negative incentive contrast: a disruption in consummatory behaviour of an animal expecting a particular magnitude of reward if that expected reward is unrealized (Flaherty, 1982). This is well documented in bumblebees (Waldron, Wiegmann, & Wiegmann, 2005) and is generally followed by the bee switching to a new flower type (Wiegmann, Wiegmann, & Waldron, 2003). In the current study, some bees continued to sample and then reject the simple flowers over consecutive visits more often than other bees, and these bees were then more likely to take a reward from a simple flower next. Bees that took more simple flower rewards after displaying negative incentive contrast were then in turn more likely to specialize on simple flowers. Why bees differed in their degree of incentive contrast is not clear, but it is possible that bees differed in their perceptual abilities, with some bees being more receptive to the difference in higher and lower concentrations of sucrose and therefore less willing to accept a lower-rewarding flower (as has been found for honeybees; Scheiner, Page Jr, & Erber, 2001). For this or another reason, some bees may have formed a stronger learned association with the lower-rewarding simple flower that was then more difficult for them to override with new information, causing them to continue to persevere with returning to this flower type. It is also possible that repeated visits to simple flowers, even if just to taste and then reject, allowed those individuals to overcome their incentive contrast and eventually accept the lesser reward again. Regardless of their origin, differences in what flowers individuals specialize on may be maintained by selection as they reduce competition (Chittka et al., 1999; Heinrich, 1979; Stout et al., 1998).

In addition to the variation in foraging specializations among individuals that managed to gained access to both types of flower, there was also variability between individuals in whether they

accessed the nectar in the complex flower at all, with only half of them managing to do so. It is plausible that these differences are due to unlearned differences in individual preference for a particular flower type or colour. This is supported by our finding that the individuals that only ever gained rewards from pink simple flowers were more likely to have landed on the pink simple flower on their first visit than were bees that gained reward from both pink simple flowers and purple complex flowers. These initial differences in flower choices are most likely due to unlearned differences in colour preference (Ings et al., 2009; Raine & Chittka, 2007; Waddington, Allen, & Heinrich, 1981). A slight preference for one colour over another might then quickly become reinforced by finding the sucrose reward, leading to a specialization.

The artificial complex flowers we designed for use in this experiment reflected the findings from natural flowers (Heinrich, 1979; Laverty, 1980, 1994), in that bees had much higher failure rates on complex flowers (6% success) than on simple flowers (82% success) on their first visit. The 6% success rate on complex flowers is lower than what has been found for natural flowers (Heinrich, 1979; Laverty, 1980, 1994), but it is likely that natural complex flowers contain other cues to help bees locate the nectar, such as scent and nectar guides (Gegear & Laverty, 2005; Laverty, 1980, 1994; Leonard & Papaj, 2011). When the bees did access nectar in complex flowers, this initially took them much longer than to locate nectar in the simple ones. However, as bees learned the motor pattern needed to locate the nectar reward, this time decreased until it did not differ from the time taken to access the nectar in simple flowers, at least for the bees that persevered with learning to handle this flower type. Similarly, individual success was much lower on complex flowers than on simple flowers initially, but increased dramatically across the seven trials. In this experiment we addressed only the handling of flowers when foraging for nectar, but bees also collect pollen, and the handling skills involved in pollen collection may require an even larger learning investment than handling of flowers when collecting nectar, even on morphologically 'simple' flowers such as poppies (Raine & Chittka, 2007b).

Not only did bees in the current experiment take longer to access rewards on complex flowers than on simple ones on their first visit to a complex flower, this effect seemed to be larger in the unequal rewards treatment group than in the equal rewards treatment. The primary difference between these treatments that could explain this is the value of reward the bee had previously encountered in the simple flowers (as most bees gained the reward from simple flowers before they gained the reward from complex flowers). It is possible that bees that previously encountered rewards of higher value in the simple flowers were either more motivated or had more energy to 'solve' the complex flowers faster.

As our experiment preselected for individuals that were more likely to land on the artificial flowers to begin with, it is possible that individuals that discovered the novel food source (i.e. either flower type) faster were in fact more 'error-prone' individuals (Evans & Raine, 2014). However, it seems that even if we did inadvertently select for individuals that were more likely to discover a novel food source, this effect does not seem to be stronger than the preexisting colour preferences, as no bees that experienced purple simple flowers ever made the 'error' of sampling a pink complex flower.

The current study demonstrates how colour may act as an initial cue to attract bees to complex flowers and entice them to handle those flowers before the bee can assess the value of the flower's nectar reward. After accessing the nectar reward, its value is used by the bee to decide whether to continue choosing this flower type, also influenced by their unlearned colour bias. As natural complex flowers use a range of cues to attract and direct bees, including scent, nectar guides and bilateral symmetry (Laverty, 1980, 1994; Leonard & Papaj, 2011; Rodríguez, Gumbert, De Ibarra, Kunze, & Giurfa, 2004), future studies should encompass some of these into how bees learn to handle complex flowers. A greater number of cues could mean less variation between individuals as they can more readily identify a particular highly rewarding flower (Gegear & Laverty, 2005; Leonard et al., 2011). Alternatively, it could lead to more variation as differences in individuals' perceptual abilities are emphasized (Cakmak et al., 2009). Discerning this would help us better understand how individual differences in this context arise.

Supplementary data

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2014.12.024.

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