

# 'X' marks the spot: The possible benefits of nectar guides to bees and plants

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## Summary

1. Many floral displays are visually complex, transmitting multi-coloured patterns that are thought to direct pollinators to nectar rewards. These 'nectar guides' may be mutually beneficial, if they reduce pollinators' handling time, leading to an increased visitation rate and promoting pollen transfer. Yet, many details regarding how floral patterns influence foraging efficiency are unknown, as is the potential for pollinator learning to alter this relationship.

2. We compared the responses of bumblebee (*Bombus impatiens* Cresson) foragers to artificial flowers that either possessed or lacked star-like patterns. By presenting each bee with two different foraging scenarios (patterned flowers rewarding/plain flowers unrewarding, plain flowers rewarding/patterned flowers unrewarding) on different days, we were able to assess both short- and long-term effects of patterns on bee foraging behaviour.

3. Bees discovered rewards more quickly on patterned flowers and were less likely to miss the reward, regardless of whether corollas were circular or had petals. Nectar guides' effect on nectar discovery was immediate (innate) and persisted even after experience, although nectar discovery itself also had a learned component. We also found that bees departed patterned flowers sooner after feeding. Finally, when conditions changed such that flowers no longer provided a reward, bees visited the now-unrewarding flowers more persistently when they were patterned.

4. On the time-scale of a single foraging bout, our results provide some of the first data on how pollinators learn to forage efficiently using this common floral trait. Our bees' persistent response to patterned flowers even after rewards ceased suggests that, rather than being consistently mutually beneficial to plant and pollinator, nectar guide patterns can at times promote pollen transfer for the plant at the expense of a bee's foraging success.

**Key-words:** *Bombus*, constancy, efficiency, foraging, handling time, learning, nectar discovery, pattern

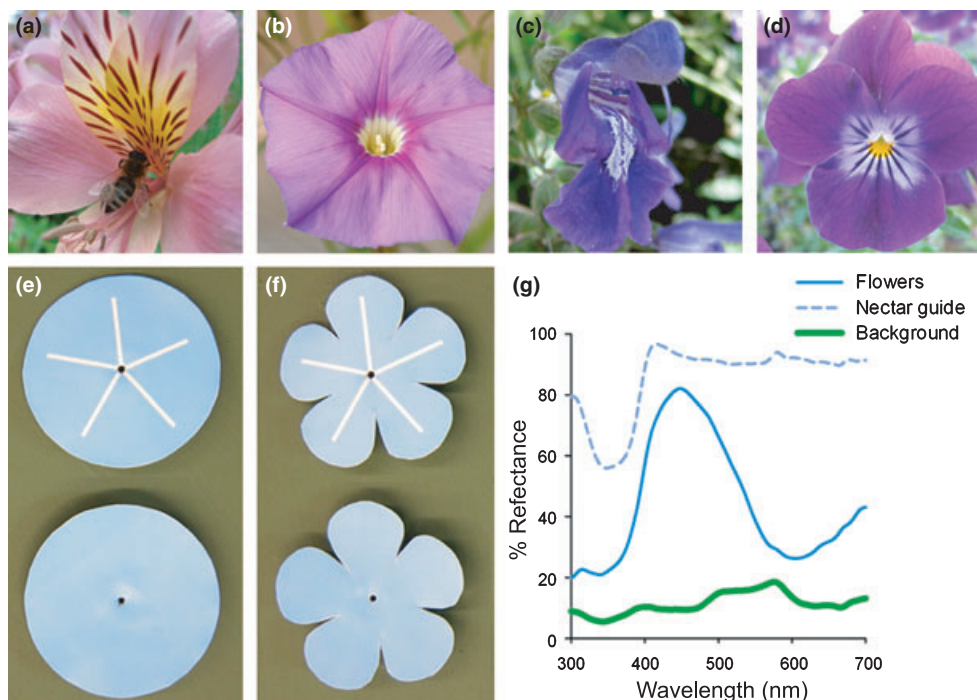
## Introduction

Flowers display a remarkable variety of colourful patterns (Dafni, Lehrer & Kevan 1997; Fig. 1a–d). Theories on the function of this visual complexity date at least to the time of Sprengel (1793), who proposed that *Saftmale* (a German word translating as 'juice marks') guide pollinators towards the flower's nectary. Despite the ubiquity of such floral patterns (Kugler 1966; Penny 1983; Chittka *et al.* 1994), a good understanding of what features make them attractive (Knoll 1926; Daumer 1956, 1958; Manning 1956; Free 1970; Jones & Buchmann 1974; Lunau *et al.* 2006; Shang *et al.* 2010) and evidence that pollinators perceive quite subtle aspects of their form (such as symmetry,

rotation, and configuration: Giurfa, Eichmann & Menzel 1996; Horridge 2000; Plowright *et al.* 2001; Avarguès-Weber *et al.* 2010), surprisingly few studies address whether and how the presence of a nectar guide benefits plants and/or pollinators.

Often, it is assumed that floral patterns are mutually beneficial to both plant and pollinator. Optimal diet theory (Pyke, Pulliam & Charnov 1977; Sih & Christensen 2001) predicts that foragers should be sensitive to the costs of acquiring particular food items (nectar sources), adjusting their choices based not only upon energetic rewards, but also on the time or energy it takes to access those rewards. If pollinator fitness depends upon maximizing the rate of nectar collection (as in social bees: Pelletier & McNeil 2003; Burns 2005), then all else being equal, they should select flowers that have shorter handling times. Pollinators tend to spend less time using flowers that possess nectar

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**Fig. 1.** Animal-pollinated plants display a variety of floral patterns, which often function as nectar guides (a) *Alstroemeria* sp. with honey bee, *Apis mellifera* (b) *Ipomoea ternifolia*, (c) Lamiaceae, (d) *Viola* sp., (e) Circular and (f) Petaloid artificial flowers and patterns used in experiment. (g) Reflectance spectra for components of artificial flowers used in experiment; our colours mimic a natural situation in which foliage provides a grey-green background, flower petals are blue and nectar guide patterns are UV-blue. Photographs: A.S. Leonard.

guides, suggesting that these patterns are indeed associated with foraging efficiency (Waser & Price 1983, 1985; Dinkel & Lunau 2001; but see West & Laverty 1998).

Yet, we lack many basic details regarding how floral patterns influence pollinator behaviour. For example, rather than exploring how patterns affect the sequence of behaviours on the flower, previous studies have focused on overall flower handling time, or even travel time between flowers. Additionally, the long-term benefits of a guide remain unclear, because pollinators may acquire handling skills (e.g. Laverty 1994a) that allow them to eventually forage equally efficiently without a guide. Although a naïve bee's preference for landing on patterned flowers is well-established (e.g. Lehrer, Wehner & Srinivasan 1985; Dafni, Lehrer & Kevan 1997; Simonds & Plowright 2004), the effects of patterns on foraging efficiency have apparently not been assessed beyond the timeline of a single foraging bout. Perhaps not surprisingly, nothing has been reported about how experience shapes the guiding function of floral patterns, if at all.

Understanding how the on-flower effects of a nectar guide change with experience would provide a more precise description of the function(s) of this common floral trait. It might also yield a more informed perspective on the possible benefits of guides to plant and pollinator. In general, nectar guides are presumed to benefit both plant and pollinator. A reduction in nectar discovery time, for instance, might increase the rate at which a bee acquires energy in the form of nectar (of benefit to the bee) and simultaneously improve the rate at which the bee transfers pollen (of benefit to the plant). Yet, most studies examine pollinator responses to nectar guides on the time-scale

of a single foraging trip, where a pattern is always associated with a reward. Because nectar availability of any one plant species can fluctuate (because of temporal changes in production or depletion by floral visitors: Heinrich 1979a), our multi-day experiment allowed us to examine how a floral pattern affected the capacity of bees to switch floral types when a previously rewarding type becomes unrewarding. In this scenario, the interests of the bee are in potential conflict with those of the plant. The bee stands to benefit if the nectar guide on the once-rewarding floral type facilitates switching to a novel type, whereas the plant might benefit if the nectar guide impedes switching. Does experience using a nectar guide to locate a reward thus facilitate – or hinder – foraging on flowers that lack guides?

Our study explored two general aspects of the relationship between floral patterns and bumblebee (*Bombus impatiens*) nectar foraging. First, we focused on possible handling time benefits of a pattern. We examined the effects of a floral pattern not only on nectar discovery but also on a bee's decision to leave the flower. Secondly, we also determined how pollinator experience affected nectar discovery in the context of floral patterns. Whereas we hypothesized that a nectar guide would facilitate naïve bees' nectar discovery, we developed two opposing predictions for how the benefit of a guide might change with experience. If nectar guides aid bees in learning how to locate nectar more effectively, then the relative time savings of a nectar guide should grow over the course of a foraging trip. Alternatively, if guides primarily benefit naïve bees, the relative benefit of a guide should decrease.

## Materials and methods

### SUBJECTS AND PRE-TRAINING

We used 30 *B. impatiens* workers as subjects, selected from a colony obtained from Koppert Biological Systems (Romulus, MI, USA). The colony was provided with pollen *ad libitum* and housed in a plastic box (L × W × H: 22.0 × 24.0 × 12.0 cm). Experiments occurred in a room-sized experimental chamber (L × W × H: 3.05 × 1.92 × 1.55 m), fitted with a screen door to permit observation. The experimental chamber was connected to the colony via a gated buffer box (L × W × H: 35.0 × 22.0 × 15.0 cm) that allowed us to release individual foragers, fitted on the thorax with numbered tags (E.H. Thorne Ltd., Wragby, Lincolnshire, UK) for identification. The chamber was illuminated by fluorescent lighting (see Fig. S1a, Supporting Information; Sylvania Cool White 34 Watt bulbs, # F40CW1SS, Osram Sylvania, Danvers, MA, USA 560 lux measured at centre of array).

In order to train bees to visit the experimental chamber and forage at the floral array, we allowed the colony free overnight access to a pre-training array. The pre-training array offered nine feeders, each providing 10 mL of 30% w/w sucrose through a cotton wick. The pre-training array was similar to the experimental array, and it consisted of a green horizontal board (60 × 45 cm, DecoArt acrylic paint, 'Avocado' #DA052) with feeders spaced 10 cm apart. Each white cylindrical feeder base (height: 5.5 cm; diameter: 1.7 cm) was topped with a light grey artificial flower, the same size (5.0 cm diameter) and shape (circle,  $N = 4$  or petaloid,  $N = 5$ ) as the flowers used in foraging experiments. No patterns were present on pre-training flowers. We varied the wick's position relative to the centre or edges on each of the nine flower surfaces to prevent bees from learning to feed in a particular flower region. The floral arrays used in pre-training and experiments were positioned on a stool in the centre of the experimental chamber, at a height of 50 cm above the ground.

### FLORAL ARRAY

The floral array held 12 artificial flowers (Fig. 1e,f), arranged at 10 cm intervals in a 3 × 4 grid. Flowers consisted of a white cylindrical base (height: 5.5 cm; diameter: 1.7 cm) connected to a flower top (5.0 cm diameter). Flower tops were light blue, printed on water-resistant paper (Adventure Paper, National Geographic, Margate, FL, USA), using a Canon Pixma MX860 inkjet printer, and laminated (Xyron matte laminate, Scottsdale, AZ, USA). Figure 1g shows the reflectance spectra for colours present on the floral array. Because humans and bees are sensitive to different wavelengths of light, these colours can be represented in bee colour space (Chittka 1992) using the reflectance data, information about the lighting conditions, as well as the sensitivity of *B. impatiens*' three photoreceptors (Skorupski & Chittka 2010) (Fig. S1, Supporting Information). Using this model of bee colour space, we determined that our flower colours offered substantial green contrast against the background, as well as green contrast between corolla and guide (Fig. S1c, Supporting Information). Green contrast is critical for bees' long-range detection of flowers (Giurfa *et al.* 1996; Kevan & Backhaus 1998; Spaethe, Tautz & Chittka 2001). The flower top had a small central hole (d: 1.5 mm) through which bees accessed a well hidden in the white plastic base. Depending on treatment, this well contained either 10 µL of 50% w/w sucrose (rewarding flowers) or 10 µL of deionized water (unrewarding flowers). Rewarding and unrewarding flowers were distributed haphazardly across the grid, and their position was changed between foraging trips.

Flowers were either circular ( $N = 16$  bees) or petaloid ( $N = 14$  bees). The petaloid flower had 19.8% less surface area, but 51.6% more

perimeter than the circle (surface area of circle: 19.63 cm<sup>2</sup>; petaloid: 15.75 cm<sup>2</sup>; perimeter of circle: 15.70 cm; petaloid: 23.80 cm; measured with Adobe Photoshop CS3, Adobe Systems, San Jose CA, USA). Shape differences may have altered the detectability of flower targets, as honey bees detect circular targets at a longer distance than petaloid targets (Ne'e-man & Kevan 2001). On any given foraging trip, six of these flowers were plain, and six had a light (human white; bee UV-blue), radially symmetrical, nectar guide pattern. The pattern was identical for both circular and petaloid flowers. Although our artificial flowers were not modelled on a specific species, many bee-pollinated flowers present a blue corolla with light, UV-reflective radiating lines (e.g. *Iris*, *Salvia*, *Ipomoea*). Generally, chromatic contrast between pattern and corolla is important in guiding bumblebees' orientation towards flowers (e.g. Lunau, Wacht & Chittka 1996; Lunau *et al.* 2006). Likewise, both circular- and star-shaped flowers commonly display star-shaped guides (Dafni & Kevan 1996).

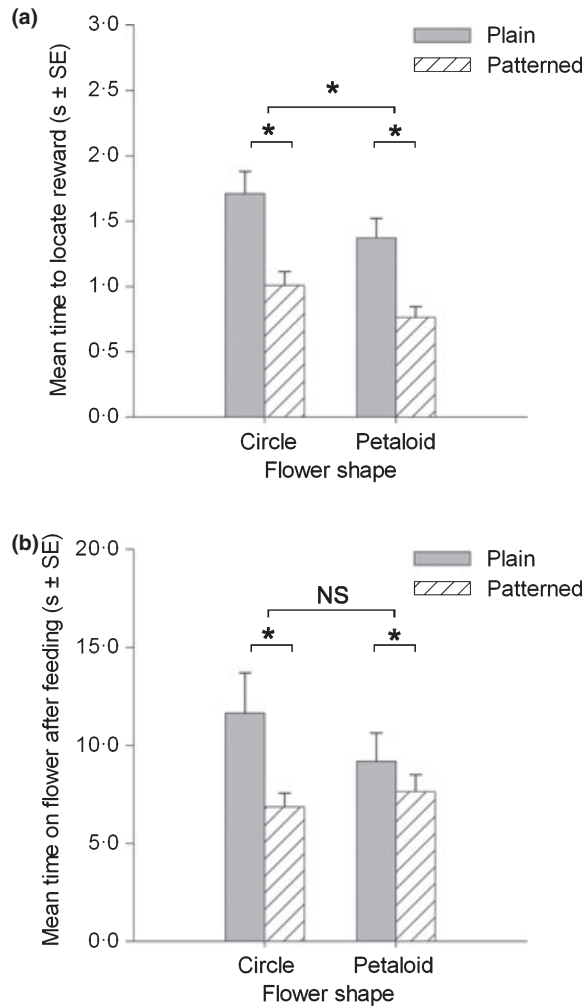
Each bee was videotaped (30 frames/s; Sony DVM-60PR Mini DV cassettes) during two foraging trips, occurring 2 days apart. During each trip, the bee encountered both plain and patterned flowers; one type was rewarding and one type was unrewarding, but this relationship was switched for each individual bee across foraging trips. Repeatedly assaying the same forager allowed us to determine whether handling times were shorter when rewarding flowers had patterns, even if individual bees varied in their foraging speed. On Day 1, 14 bees had plain flowers rewarding/patterned flowers unrewarding, followed by patterned flowers rewarding/plain flowers unrewarding on Day 3. For seven of these bees, all flowers were circular, and for seven of these bees, all flowers were petaloid. Conversely, 16 bees on Day 1 had patterned flowers rewarding/plain flowers unrewarding, followed by plain flowers rewarding/patterned flowers unrewarding on Day 3. For nine of these bees, all flowers were circular, and for seven of these bees, all flowers were petaloid. Between foraging trips (Day 2), bees were provided access to the pre-training array described above. During a foraging trip, bees were allowed to visit flowers until they had drained all six of the rewarding flowers, or until they did not visit the array for 3 min, at which point they were collected and returned to the colony. After each foraging trip, flowers were cleaned with 30% ethanol to remove any scent marks deposited by foragers.

We used iMovie 8.0.6 (Apple Computer Inc., California, USA) to record the sequence of landings and measure the frame-by-frame details of initial landings on up to 12 flowers (six rewarding, six unrewarding) within a foraging trip. If a bee departed its first visit to a rewarding flower without having located the reward, we scored the visit as a 'miss' and used data from its first successful revisit to that flower. During a typical visit to a rewarding flower, a bee landed, searched on the surface of the flower, located the reward, consumed all the reward and then searched again on the surface of the flower before leaving. We measured the time spent searching for the reward after landing, as well as the time spent searching on the flower after feeding. Our sample sizes for certain comparisons were <30 because some bees did not feed on their Day 3 rewarding flower type ( $N = 2$ ). Sample sizes for comparisons of post-feeding search time on the final flower visited were similarly reduced because a few bees ( $N = 3$ ) remained motionless for longer than 30 s and were therefore excluded from this analysis.

## Results

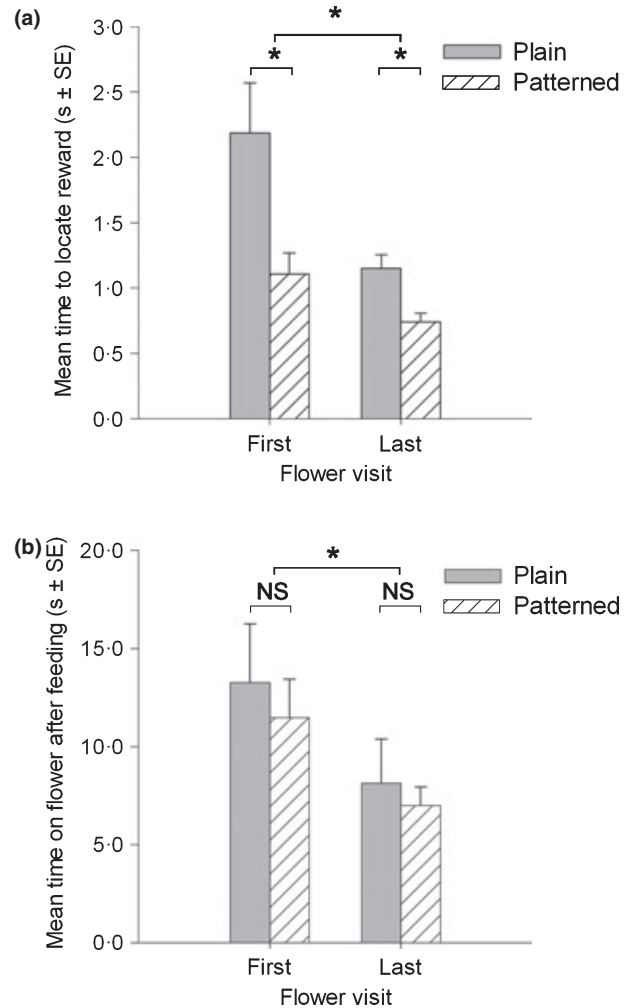
### PATTERNS REDUCED SEVERAL COMPONENTS OF HANDLING TIME

Comparing the mean time bees took to locate the reward on the six rewarding flowers available in a given trip (Fig. 2a), reward



**Fig. 2.** Mean time spent by bees on flowers. Regardless of whether flowers were circular or petaloid, when flowers were patterned, bees (a) located the reward more quickly and (b) spent less time on the flower after feeding.

discovery was faster when flowers were patterned ( $F_{1, 26} = 19.62$ ,  $P < 0.001$ ); bees also located the reward more quickly on petaloid flowers than on circular flowers ( $F_{1, 26} = 6.65$ ,  $P = 0.016$ ). There was no interaction between flower shape and pattern presence ( $F_{1, 26} = 0.208$ ,  $P = 0.652$ ), suggesting that patterns enhanced foraging to a similar extent on circular and petaloid flowers. On circular flowers, bees located the reward an average of 41.1% faster in the presence of a pattern; on petaloid flowers, bees located the reward an average of 44.5% faster with a pattern present. Similarly, a pattern reduced the time bees spent searching on the flower after feeding (Fig. 2b:  $F_{1, 26} = 4.26$ ,  $P = 0.049$ ). However, flower shape did not influence this post-feeding search time ( $F_{1, 26} = 0.625$ ,  $P = 0.436$ ), nor was there a significant interaction between pattern and shape ( $F_{1, 26} = 1.592$ ,  $P = 0.218$ ). It is worth noting that we found no significant differences between the time bees spent feeding from flowers of different types (Pattern:  $F_{1, 26} = 2.744$ ,  $P = 0.108$ ; Flower shape:  $F_{1, 26} = 2.834$ ,  $P = 0.104$ ; interaction:  $F_{1, 26} = 0.128$ ,  $P = 0.723$ ). Bees were



**Fig. 3.** Effect of foraging experience on flower handling. (a) On their first landing, bees located nectar more quickly when flowers were patterned; yet, over the course of the trial, bees showed similar decreases in time to locate nectar when flowers were plain and patterned. Floral shape did not affect either of these two measures. (b) Bees spent similar amounts of time on their first flower after feeding regardless of pattern; this post-feeding search time declined over the course of a trial, but did so similarly when flowers were plain and patterned. Floral shape had no effect on post-search time.

more likely to miss the reward on plain flowers ( $F_{1, 28} = 7.639$ ,  $P = 0.010$ ); unexpectedly, misses were also more frequent on petaloid flowers ( $F_{1, 28} = 5.907$ ,  $P = 0.022$ ). In short, we conclude from these results that a pattern of radiating lines functions as a nectar guide and does so regardless of the shape of the flower itself.

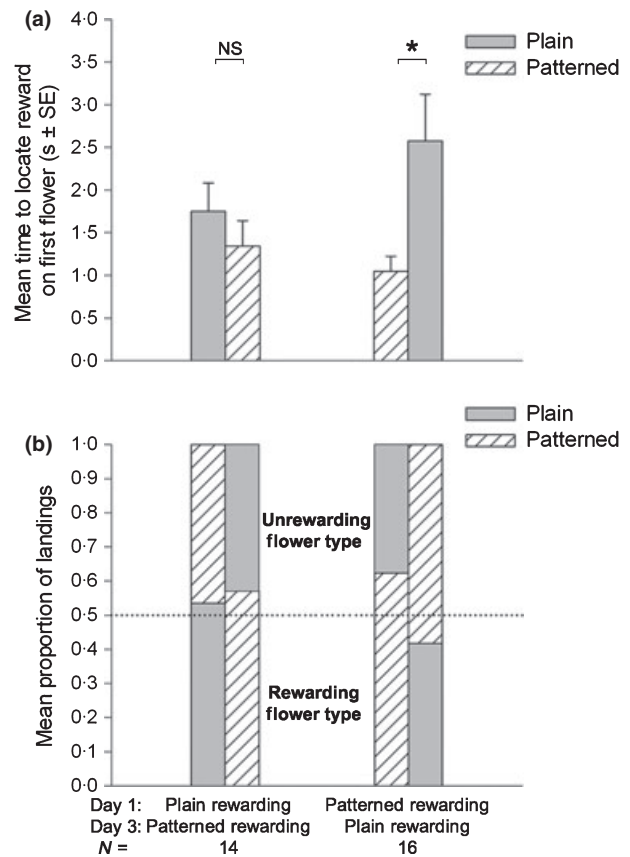
#### NECTAR GUIDES ARE INITIALLY HELPFUL, AND THEIR BENEFIT PERSISTS EVEN AFTER EXPERIENCE

If patterns save bees the cost of learning to handle the flower, then we expected that the benefits of a guide should be evident even on the bees' first landing. This expectation was met for reward discovery time (Table S1, Supporting Information; Fig. 3a): on both their first and last landing, bees located the

reward more quickly on patterned flowers, regardless of floral shape ( $P = 0.005$ ). If nectar guide patterns primarily benefit naïve bees, we expected that the relative benefit of a guide would be greatest at the start of a foraging trip. Alternately, if patterns facilitate bees' learning to locate nectar, then the relative benefit of a guide should be greater at the end of a trip. Although bees located sucrose progressively faster over the course of a trip ( $P = 0.001$ ), this decrease was independent of flower pattern or shape: patterns sped reward discovery similarly for naïve and experienced bees (Table S1, Supporting Information). The order in which bees experienced the two kinds of foraging trips (plain flowers rewarding on first trip vs. patterned flowers rewarding on first trip) did not have a significant effect or interaction with these factors, although we did note an interaction between pattern, flower position within a trip (first vs. last), flower shape and order of rewarded floral type across trips that bordered on statistical significance ( $P = 0.061$ ; Table S1, Supporting Information).

In contrast, although post-feeding search time declined between the first and last flower visited ( $P = 0.014$ , Table S1, Supporting Information; Fig. 3b), initial post-feeding search time was not lower on flowers with nectar guides ( $P = 0.631$ ), nor was there a significant interaction between pattern presence and flower order (first vs. last). Likewise, floral shape had no significant effect on post-feeding search time ( $P = 0.398$ ). We did note a non-significant interaction between pattern and shape ( $P = 0.061$ ), suggesting that differences in post-feeding search time (on both first and last flowers) between plain vs. patterned flowers may be smaller on petaloid flowers. As above, the order in which bees experienced the different flower patterns as rewarding did not have a significant effect on post-feeding search (Table S1, Supporting Information). However, we did find a significant interaction between the order of rewarded floral types across trips and flower position within a trip (first vs. last;  $P = 0.036$ ). Regardless of whether flowers were patterned or plain, bees that had plain flowers rewarding on Day 1/patterned flowers rewarding on Day 3 tended to spend less time searching on their last flower after feeding than on their first flower. Bees who experienced the two trips in the opposite order (patterned flowers rewarding on Day 1/plain flowers rewarding on Day 3) tended to spend a relatively similar amount of time on their first and last flower after feeding.

Our analysis also shows that using a guide to locate rewards had a long-term negative effect on handling of plain flowers. Figure 4a shows the mean time bees took to locate the sucrose reward on their first flower, for both foraging trips. Bees that on Day 1 collected sucrose from plain flowers took a similar amount of time to locate their first reward on Day 3, when rewarding flowers had patterns ( $P = 0.269$ , Table S2, Supporting Information). However, bees that on Day 1 collected sucrose from patterned flowers were slower to locate their first reward on Day 3, when rewarding flowers were plain ( $P = 0.015$ , Table S2, Supporting Information). Bees' nectar discovery was thus less disrupted by the appearance of a novel pattern on a rewarding flower than it was by the disappearance of a pattern previously useful in finding nectar.



**Fig. 4.** Whether bees fed from plain or patterned flowers on Day 1 affected foraging behaviour on Day 3. (a) Bees that collected sucrose from the first plain flower on Day 1 located the reward on the first patterned flower at a similar speed on Day 3. However, other bees that collected sucrose from the first patterned flower on Day 1 took longer to locate the reward on the first plain flower on Day 3. Flower shape was not a significant factor in this analysis. (b) While bees that collected sucrose from plain flowers on Day 1 made a similar proportion of landings on rewarding patterned flowers on Day 3, bees that collected sucrose from patterned flowers on Day 1 persisted in landing on (now unrewarding) patterned flowers on Day 3. Dotted line at 0.50 indicates random choice.

#### NECTAR GUIDES AFFECT BOTH INITIAL AND LONG-TERM LANDING PREFERENCES

Bees showed an initial bias towards landing on flowers with patterns. On Day 1, regardless of whether patterned or plain flowers were rewarding, bees were more likely to make their first landing on a patterned flower (no significant effect of shape  $z = -0.276$ ,  $P = 0.784$ ; 2-tailed binomial test on combined data:  $P = 0.0014$ ).

Interestingly, experience feeding on patterned and plain flowers had different long-term effects on bees' landing preferences (Fig. 4b). We compared the relative proportion of landings on rewarding flowers, asking whether the order in which bees experienced the two foraging trip types (Trip order as between-subjects factor with 2 levels) interacted with the effect of day (Day as within-subjects measure with 2 levels: 1; 3). We found a significant effect of Day (Table S3, Supporting Information;  $P = 0.007$ ) but not of Trip order ( $P = 0.294$ ); the interaction of

interest between these two factors was, however, significant ( $P < 0.001$ ). Note this analysis did not find a significant effect of shape (Table S3, Supporting Information). We did note a trend towards statistical significance for an interaction between Shape, Day and Trip order ( $P = 0.062$ ): bees that fed from plain circular flowers on Day 1 tended to make a higher proportion of landings on patterned circular flowers on Day 3, whereas bees whose flowers were petaloid showed a more similar proportion of landings on rewarding types across both days. In general, these results show that although bees that on Day 1 fed from plain flowers readily switched to feeding from patterned flowers on Day 3, bees that on Day 1 fed from patterned flowers persisted in landing on these flowers on Day 3, when they were unrewarding.

## Discussion

Floral patterns are often assumed to benefit both plant and pollinator by efficiently guiding foragers to nectar. On the time-scale of a single foraging trip, our experiment supports this view: when flowers had star-shaped patterns, bumblebees not only located rewards more quickly but also were less likely to miss the reward and to linger on the flower after feeding. Interestingly, although bees generally foraged more quickly on petaloid flowers than on circular flowers (perhaps because petals themselves provide spatial information about the position of the nectar well), patterns were similarly beneficial on both corolla shapes. We established that these benefits are evident on the first flower visited; nevertheless, speed in using a pattern to locate rewards improved across a foraging trip. Our experiment is the first, to our knowledge, to show that the nectar discovery benefit of a nectar guide persists even after bees gain experience in handling a flower.

We also found that experience using nectar guides to locate sucrose had long-term effects on foraging behaviour. For example, when we compared how long it took bees to locate the reward on the first flower of each trip, we found that, 2 days after collecting sucrose from patterned flowers, bees took much longer to locate a reward on a plain flower. In contrast, bees that initially found rewards in plain flowers located the reward on their first patterned flower after a similar amount of time. Additionally, bees that collected sucrose from patterned flowers on Day 1 continued to visit these flowers on Day 3 even when they no longer offered a reward. As a whole, these results raise the possibility that the long-term consequences of a nectar guide may be tilted in favour of the plant, which merely by producing a pattern can gain pollinator visits, regardless of reward status.

### HANDLING TIME BENEFITS OF NECTAR GUIDE PATTERNS TO PLANT AND POLLINATOR

Making a flower easy to handle may promote a plant's reproductive success in several ways. Apart from promoting visitation of a particular pollinator species via reduced handling time (e.g. bees, as documented in this experiment), easily accessed nectar might also broaden the range of potential pollinators (Ollerton *et al.* 2007). Further, like other floral traits that influence

on-flower behaviour (e.g. nectar concentration and volume: Thomson 1986; corolla depth: Harder 1983; structural complexity: Lavery 1980), nectar accessibility may also allow the plant to optimize the rate of pollen transfer. Our discovery that bees spend less time on flowers with nectar guides *after* feeding suggests that floral patterns might help to regulate on-flower time, thus minimizing pollen spillage, or simply controlling the amount of pollen distributed to individual visitors (e.g. Harder & Thomson 1989). If time spent by the bee searching on the flower increases the probability of self-fertilization, then the reduction in post-feeding time could be particularly beneficial to self-compatible species.

Why bees should spend less time after feeding on patterned flowers is an open question. Post-feeding behaviour generally consisted of searching on the flower surface, presumably for other nectaries or pollen sources, a strategy that may reflect the variety of floral architectures bees are likely to encounter (e.g. isolated flowers, inflorescences, composite flowers). Our results raise the possibility that a pattern facilitates the bee's learning about the number of rewards available per landing more effectively than a plain flower surface.

We also noted that bees showed a lower frequency of 'misses' when flowers had guides. For the plant, such misses are at best neutral. However, they may be costly if bees subsequently avoid visiting floral types that have been unrewarding. Although visits where bees failed to locate sucrose were generally brief in our experiment (mean duration:  $2.12 \pm 0.50$  s), Lavery's (1980) study suggests that under natural conditions, misses may have a larger impact on foraging efficiency, as bumblebees were observed to spend several minutes unsuccessfully attempting to access nectar from various plant species.

Flower handling will likely be influenced by multiple plant traits, yet how they interact to influence pollinator behaviour is largely unexplored (Fenster *et al.* 2004). To date, synergistic effects of patterns have been largely considered from the perspective of colour values and symmetry: perhaps not surprisingly, the effectiveness of a guide depends not only on pattern, but also on the degree of chromatic contrast between guide and petals (e.g. Lunau, Wacht & Chittka 1996; Dinkel & Lunau 2001). Additionally, noting a widespread correspondence between both internal (nectar guide) and external (corolla) floral contours, Dafni and collaborators (Dafni & Kevan 1996; Dafni, Lehrer & Kevan 1997) suggested that symmetry complementarity may itself be an important determinant of handling effectiveness. Because bees track the contours of flowers as they fly towards them (Lehrer, Wehner & Srinivasan 1985), any shape mismatch between corolla and guide might slow nectar discovery.

Having matched radially symmetrical guides with radially symmetrical corollas, our experiment allowed us to determine whether guide and corolla shapes interact to influence handling time. We anticipated that the guide might be either more effective on petaloid flowers (drawing the bee away from petal edges, which bees tend to follow and probe: Lavery 1980; Lehrer & Srinivasan 1993; Lehrer, Wehner & Srinivasan 1985) or less effective on petaloid flowers (because visual or tactile information provided by petals might direct pollinators towards the nectary: Goyret & Raguso 2006). In fact, we found that the

pattern was equally effective on both flower types. The ability of a nectar guide to reduce handling time thus appears to be robust across corolla shapes (and/or sizes). Even if a flower's petal shape and orientation provide significant information about the location of the nectary, a pattern still facilitates bee foraging.

#### NECTAR GUIDES AND LEARNING

Although a large body of literature has established that bees both have naïve pattern preferences and can learn to discriminate between patterns that differ in particular characteristics (e.g. Kugler 1936; Barth 1985), very little is known about the related question of how experience with patterns affects nectar discovery time. This question has remained open, despite the fact that nectar-foraging bees are known to show experience-based changes in several aspects of foraging (e.g. choosing flowers on the basis of reward rather than display size: Makino & Sakai 2007; increases in floral constancy: Heinrich 1979b; Laverty 1980; establishment of traplines: Ohashi, Thomson & D'Souza 2007). We showed that although a pattern reduces nectar discovery time from the very start of a foraging trip, performance on flowers with guides improves with experience. Both relatively naïve and experienced foragers, then, benefit similarly from the presence of a floral pattern.

Interestingly, the asymmetry (Fig. 4a) in the degree to which Day 1 experience affected bees' initial location of nectar on Day 3 (when rewarding flower types were switched from plain to patterned or *vice versa*) suggests that learning to use a pattern to locate a reward may impair bees' performance on other floral types. Future studies could investigate whether this effect applies similarly to bees foraging on flowers with two different nectar guides or is specific to a pattern's presence vs. absence. Previously, such 'switching costs' have largely been considered in the context of floral morphology, rather than visual signals (e.g. Laverty 1994b); they have also been generally documented over relatively short time-scales. Our study indicates that such effects may be present even 48 h after a single foraging bout.

#### FORAGING EFFICIENCY VS. POLLEN TRANSFER

Given that the reproductive interests of partners in a mutualism are not always perfectly aligned (Bronstein 1994), it is possible that like other floral traits (e.g. signal complexity in general: Gegear 2005; Leonard, Dornhaus & Papaj 2011; repellants in nectar: Kessler, Gase & Baldwin 2008; pollinia that slow bee foraging: Morse 1981), patterns may sometimes increase the plant's reproductive success at the expense of the pollinator's foraging efficiency. Indeed, because nectar guides are signals, we expect that their effect on foraging efficiency may be more complex than morphological traits that also affect handling time, such as corolla depth (Inouye 1980) or petal microtexture (Whitney *et al.* 2009). This is because floral signals have the potential to deceive or exploit reward-seeking pollinators. For example, rewardless flowers may produce nectar guides but not nectar (Jersáková, Johnson & Kindlmann 2006; Schaefer & Ruxton 2010). Even in rewarding species, floral patterns may

exploit a pre-existing sensory bias, such as a preference for radiating lines that resemble a nest entrance (Biesmeijer *et al.* 2005; see also Naug & Arathi 2007). If pollinators' attraction to a pattern is persistent enough, it may allow the plant to limit rewards. For example, the presence of anther-mimicking radial lines or pollen-mimicking blotches increases the attractiveness of flowers to bees (Lunau 2000); this attraction is so strong that the mere presence of a (presumably pollen-mimicking) yellow dot interferes with bumblebees' (*B. terrestris*) ability to discriminate between unrewarding and rewarding artificial flower types (Pohl, Watolla & Lunau 2008). This work also shows that the degree to which patterns inhibit a shift to the rewarding flower type depends upon colour and placement; because we only observed bees on two foraging trips, we cannot comment upon whether or not our bees would have eventually learned to completely avoid unrewarding patterned flowers. Although we focus on bees, it is worth mentioning that other species respond to nectar guides (e.g. hawkmoths: Knoll 1926; hummingbirds: Waser & Price 1983; syrphid flies: Dinkel & Lunau 2001). Whether these distantly related species might show similar experience-based effects in use of and response to nectar guides remains an intriguing question for future research.

Previously, the connection between handling time, pollinator learning and plant vs. pollinator fitness has been explored in the context of Darwin's 'Interference Hypothesis' regarding the phenomenon of floral constancy (Laverty 1994b; Gegear & Laverty 1998; Chittka, Thomson & Waser 1999). According to this hypothesis, bees' tendency to selectively visit one or two flower species, even when equally rewarding other species are available, results from a limitation on their ability to learn how to efficiently handle multiple flower types simultaneously (Chittka & Thomson 1997). In this scenario, a plant may actually transport more pollen to conspecifics by producing a difficult-to-handle flower, because once a bee has invested in learning how to handle it, it may be more likely to restrict its visits to this type. With relevance to the current experiment, one implication of our findings is that a trait that makes a flower easier to handle (a nectar guide), rather than more difficult, may also manipulate pollinators' propensity to visit a particular flower type.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Figure S1.** Colors of our flower tops and nectar guides represented in bee color space.

**Table S1.** General linear models used to assess the effects of flower pattern, flower shape, flower order within a trip, and trip order on nectar discovery and post-feeding search time.

**Table S2.** General linear models exploring how the order in which bees fed from plain vs. patterned flowers affected nectar discovery on the first flower of a given foraging trip.

**Table S3.** General linear model used to assess how proportion of landings on rewarding flower type was affected by day, flower shape, and the order in which bees fed from plain vs. patterned flowers.

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