

**Trapline foraging by bumble bees: VI. Behavioral alterations under speed-accuracy trade-offs**

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## ABSTRACT

Trapline foraging (repeated sequential visits to a series of feeding locations) has often been observed in animals collecting floral resources. Past experiments have shown that bumble bees cannot always develop accurate (i.e., repeatable) traplines to a sufficient level, despite their economic advantages in many situations. The bees' preference for short flights works against developing accurate traplines when plants or patches are distributed in zigzag fashion. How should bees cope with such situations in nature? We conducted laboratory experiments with artificial flowers to test 2 nonexclusive hypotheses: bees may travel faster to compensate for low traplining accuracy, and when local landmarks are available, bees may be able to develop traplines by remembering external spatial information in addition to the locations of flowers. As predicted, foragers on a zigzag-shaped floral array traveled faster, with lower route repeatability, than those on a triangular lattice where distance and angle could be chosen independently, suggesting that bees trade off accuracy for speed when it is more feasible. In contrast, bees traveled more slowly with unchanged traplining accuracy when landmarks were added into both arrays, possibly because the landmarks caused information load or visual distraction. Finally, bees on the zigzag array with additional landmarks made a quicker decision to switch from accurate traplining to fast traveling. If landmarks helped the bees to grasp the overall array geometry in our experiments, they may also permit bees in nature to select a distribution of plants or patches that aids accurate traplining.

*Key words:* *Bombus*, foraging, landmark effect, route learning, speed-accuracy trade-off, spatial use, trapline, travel speed

## INTRODUCTION

Patterns of movement or "spatial-use strategies" are considered key factors in the success of most animals collecting food that is unevenly scattered in space and time. Spatial-use strategies are especially interesting when foragers are pollinators because they also affect gene flow or reproductive success of plants (Levin and Kerster 1968; Waddington 1981; Schmid-Hempel 1986; Cartar and Real 1997; Cresswell 2000). Although researchers have often focused on simple movement rules as determinants of successive flights of pollinators (i.e., choices of distance and turning angle) as if the bees are "searching" for unknown locations in unfamiliar habitats, growing evidence suggests that other aspects of spatial use are important (reviewed by Ohashi and Thomson 2009). Specifically, certain pollinators return faithfully to small foraging areas (Ribbands 1949; Manning 1956; Gill and Wolf 1977). Within those areas, moreover, they sometimes visit a particular set of plants in repeatable sequences, referred to as "trapline foraging" (Manning 1956; Janzen 1971; Heinrich 1976; Ackerman et al. 1982; Dressler 1982; Lemke 1984; Gill 1988; Tiebout 1991; Thomson 1996; Thomson et al. 1997; Garrison and Gass 1999).

Recent laboratory experiments with nectar-collecting worker bumble bees have clarified several important aspects of trapline foraging. First, a bee tends to establish a trapline with geometric features that directly improve foraging performance, such as an increased return interval (Ohashi et al. 2007; Saleh and Chittka 2007), an approximation of the shortest possible route (Ohashi et al. 2007; Lihoreau et al. 2010, 2011, 2012), and priority for visits to higher-reward locations (i.e., visiting them first) (Lihoreau et al. 2011). Moreover, a bee increasingly repeats a particular sequence more accurately with each foraging circuit, producing periodical returns to each location with less variable intervals (Ohashi and Thomson 2005; Ohashi et al. 2007). Thus an increased repeatability or "accuracy" in traplining indirectly improves a bee's foraging, by (1) increasing its chances of encountering accumulated nectar before its competitors (Ohashi and Thomson 2005; Ohashi et al. 2007), by (2) discouraging others from intruding by keeping nectar standing crops low (Possingham 1989; Ohashi and Thomson 2005), and by (3) getting to feeding locations before the slowing refilling rate diminishes too much (Ohashi and Thomson 2005; Ohashi et al. 2008).

Previous studies have also shown that the advantageous features of traplines develop over hours and cannot be reproduced by simple rules of movement between successive visits, such as choosing short

distances and straight moves (Ohashi et al. 2007; Saleh and Chittka 2007; Ohashi et al. 2008; Lihoreau et al. 2010, 2011, 2012). This suggests that traplining requires a long-term spatial memory of locations, reward values, a sequence of locations, or a sequence of motor patterns. In addition, foragers tend to develop repeatable circuits with experience even in the absence of others, although most of the benefits associated with accurate traplining show up only in the presence of competitors or potential intruders (Ohashi and Thomson 2005; Ohashi et al. 2008). In fact, solo bees developed accurate traplines more readily than those gaining experience in competitive situations. Because bumble bees in most field conditions compete for limited resources and it often takes hours for spatial learning, it is probably advantageous for a forager to make such a "prior investment" in an accurate trapline even if it finds itself working alone (Ohashi et al. 2008).

Even if accurate traplining is an efficient foraging tactic and bumble bees have sufficient cognitive ability to employ it, bees in nature may not always enjoy the benefits of it. Past experiments have suggested that trapline foraging has at least one limitation in its implementation: route repeatability or traplining accuracy is reduced if the spatial configurations of plants or patches such as zigzags require sharp turns to make nearest-neighbor flights, although the realized visit sequences are still more repeatable than those generated by pure searching behavior (Ohashi et al. 2007). This is because bees prefer to choose short distances over straight moves and show little plasticity in this regard, unless circuitous routes involve heavy penalty costs (Ohashi et al. 2007; Lihoreau et al. 2010). If a difficult plant configuration does not permit a bee to select a set of plants where nearest neighbors are consistent with those of directional movements, therefore, the bees are likely to suffer from inaccurate traplining. Considering that bumble bees tend to avoid intensive overlap of their foraging areas with competitors (Thomson et al. 1987; Makino and Sakai 2005) and that this would limit available plant options for an individual, such geometrical difficulties in traplining may often prevail in nature.

One possible response to difficult configurations is to increase travel speeds to compensate for the inaccuracy in traplining. Ohashi et al. (2008) have shown that fast travel improves a bee's foraging performance through effects very similar to those of periodic returns, i.e., by taking accumulated nectar in feeders more often than competitors, as well as by returning to feeders before the refilling rate tapers off.

Pushing travel speed above a certain level, however, may sacrifice the accuracy of traplining; in an array of feeders where choices of nearest neighbors are consistent with those of directional movements, Ohashi et al. (2008) discovered that experienced bees followed less repeatable routes in foraging trips during which they traveled faster, while speed and accuracy both contributed to their competitive performance. When confronted with zigzag configurations, therefore, such a "speed-accuracy trade-off" in traplining may encourage bees to actively choose higher speeds at the cost of accurate traplining. Another solution may come from choosing habitats with rich visual landmarks close to focal plants or patches. It is well known that animals can learn more precise locations or routes when visual landmarks are available near the goal or intermediate goals (reviewed by Collett et al. 2003). Although bees were provided with a few potential cues such as the observer and the nest box in our previous studies (Ohashi et al. 2007), they might have been able to trapline with greater accuracy (i.e., repeatability) or speed if more or better landmarks had been available. Therefore, we revisited Ohashi et al.'s (2007) experiments, adding very salient landmarks and a system for measuring speed.

As before, we conducted laboratory experiments on the patterns of spatial use by bumble bees (*Bombus impatiens*) collecting nectar from multiple feeders ("flowers") in a flight cage. We allowed naive bees to accumulate foraging experience singly in different learning conditions, by crossing the 2 arrangements of flowers and the presence or absence of additional salient landmarks in a 2 x 2 design. Using a LED-based monitoring system, we recorded all the flower visit sequences that each bee followed during foraging, as well as its travel speed between flowers. We asked these specific questions: (i) When flowers are distributed in ways that make accurate traplining difficult, do bees actively switch to fast traveling? (ii) When bees can use visual landmarks to learn the locations and sequences of flowers, do they perform more accurate traplining? Or do they further increase their travel speed? (iii) How do the effects of flower distribution and landmark availability on bees' spatial-use patterns interact with one another?

## METHODS

We worked indoors in a mesh flight cage measuring 788 (length) x 330 (width) x 200 (height) cm. The same cage was used in Ohashi et al.'s (2007) experiments, except that it was then set up in a rooftop greenhouse. Temperature ranged from 26 to 30°C. The room was illuminated during daylight hours with normal

fluorescent bulbs, while the windows were covered with cardboard. Our subjects were workers from 2 commercial colonies of *Bombus impatiens* Cresson (supplied by Biobest, Leamington, Canada; Figure S1). Colonies were maintained in nest boxes and connected to the cage with a transparent entrance tunnel fitted with gates, which allowed individual bees to be tested by restricting access of other bees. Pollen was supplied ad lib every day, directly to the colony. Sucrose solution was dispensed by artificial flowers, as detailed below.

### Artificial flowers

We used 10 morphologically identical artificial flowers for experiments. The design of the artificial flowers is described elsewhere (Ohashi, Thomson, and D'Souza 2007, Ohashi, D'Souza, and Thomson 2010; see also Fig. S1). Briefly, each flower consists of a vertical box of clear Plexiglas (67 cm tall), equipped with a small electric clock motor on its top. As the motor turns a thin axle (diameter = 3 mm) at 1/30 rpm, the axle winds up a thread that is clipped to one end of a 50-cm of flexible tubing (internal diameter = 3 mm) that contains 30% sucrose (w/w) solution ("nectar"). The nectar oozes out through the needle at the other end of the tube into a "nectar bucket" (diameter = 5.5 mm, depth = 7 mm) on a horizontal platform halfway up the box, and accumulates in the bucket at a constant rate ( $2.2 \mu\text{L}/\text{min}$ ). A thin plastic baffle prevents the bees from getting excess nectar directly from the steel needle hole. The depth of the nectar bucket is adjusted to the tongue length of *B. impatiens* workers so that they can empty the nectar accumulated at the bottom. Each nectar bucket is topped with a U-shaped block of plastic painted in blue, so that bees can easily find and learn to extract nectar from it. A piece of clear plastic canopy covers the block, so that a bee can approach the bucket only from the front opening through the tunnel.

An electronic monitoring system recorded all the visit sequences and the arrival/departure time at each flower. The opening of each U-shaped block houses an IR detector: an infrared light-emitting diode (LED) produces a beam that is sensed by a phototransistor. When a bee crawls through the tunnel, it interrupts the beam and produces a signal on the phototransistor output. The IR detectors are all connected to a central control box. The control box converts the analog signal received from the phototransistor to the appropriate electrical levels required for sending it to a PC through a digital input/output card. When the change of the

electrical level indicates that the bee has vacated the tunnel (i.e., the beam is reconnected), then the arrival and departure time (to 0.1 s) are logged to a data file. Similar IR systems have been often used to record the presence and the timing of bee visits to artificial feeders (Keasar et al. 1996; Chittka and Thomson 1997; Cnaani et al. 2006).

Between experiments, we used two training flowers to let bees learn where to find nectar. One of the flowers is made of a plastic vial and the other is made of a plastic Petri dish (Fig. S1). The vial is topped with a Plexiglas platform with a hole (diameter = 5.5 mm) and U-shaped blue plastic block. The lid (platform) of the Petri dish also has a hole, topped with a U-shaped blue plastic block. In either training flower, the hole on the platform was plugged with a 3-cm wick made from a cotton dental roll, the other end of which was dipped in 20% (w/w) sucrose solution in the container. Bees could extract nectar from the surface of the wick. We lowered nectar concentration in the training flowers to 20%, so that bees could become more motivated for foraging when they encountered 30% nectar in the artificial flowers.

### **Spatial arrangements of flowers and additional landmarks**

We tested 2 configurations of floral arrays to simulate variation in available spatial distribution of plants or patches in nature (Fig. 1). These arrays varied with respect to 2 general preferences of bees: the preference to make short flights and the preference to continue moving in one direction. In the “independent” array, flowers always had 2-6 equidistant nearest neighbors in different directions, and bees could choose movement distance and turning angle independently. In the “negative” array, on the other hand, proximity and directionality were negatively linked, i.e., a bee had to turn to choose the nearest neighbor. For the negative array, Ohashi et al. (2007) showed that *B. impatiens* foragers produced less repeatable circuits than those in the independent array, suggesting that the negative link between proximity and directionality hampered the establishment of more repeatable traplines. In both arrays, the interflower spacing of 0.95 m was chosen to be far enough that neighboring flowers would be distinguishable to bees as different “patches” or “plants” (Thomson et al. 1982; Burns and Thomson 2006).

The design of our flower, with its tower behind the platform, might limit bees' departure directions even though its transparency is likely to allow bees to see through it. To minimize such effects,



therefore, we arranged all flowers so that their backs faced outside of the array where there was no other flower. In the independent array, moreover, we rotated the center flower every 10-12 trips in a clockwise direction, so that its back faced each of the three edges in turn.

We performed 2 types of experiments for each array to simulate variation in landmark availability among habitats. In the treatment experiment, we added 4 artificial landmarks with different combinations of color and shape; yellow and purple columns (50 cm tall and 32 cm wide), and yellow and purple T-shaped columns (71 cm tall and 32 cm wide). We set out each of them within 1 m from the array (Fig. 1). Positions of these landmarks were kept constant throughout trials in each array. Considering that the maximum distance between a landmark and a flower is approximately 5.2 m (Fig. 1), the minimum visual angle required for detecting a 50 cm tall landmark is  $5.5^\circ$ . We thus assume that *B. impatiens* on flowers could perceive all the 4 landmarks, as they possess a minimum resolvable angle of  $1.39^\circ$  (Macuda et al. 2001). In the control experiment, artificial landmarks were absent. Without additional landmarks, the only potential spatial cues for bees, except the feeders themselves, were the bees' entrance hole on the cage screen (1.2 m from the floor) visually emphasized with ivory-colored paper cut out into a star-shaped polygon, and other stationary objects external to the cage.

### Experimental procedures

Before running an experiment, we opened the gate to the cage and let bees forage freely on the 2 training flowers. These flowers were placed within 1.5 m from the entrance, away from any locations of the artificial flowers. The vial flower was placed halfway up a Plexiglas box resembling the artificial flowers (Fig. S1). The Petri dish flower was placed on the floor. During this training phase, the 10 electric artificial flowers were turned off and covered with dark brown cloth bags to prevent the bee's access. None of the 4 artificial landmarks was added in the cage. On non-experiment days, we left the gate open typically between 1000 and 1700. This procedure allowed bees to associate the U-shaped blue plastic block with nectar reward, but they remained naive to the spatial array of flowers and additional landmarks.

On experiment days, we let bees forage on the training flowers for 30-60 min in the morning to refresh their experience of being rewarded on the flowers with the U-shaped block. When several bees began

"regular foraging" (i.e., visiting the flower directly after entering the cage, returning to the nest briefly to deposit their nectar loads, and repeating the same process), we prevented the other bees from reentering the cage once they returned to the hive. Among these regular foragers, we picked one for the trial that had accumulated more than two trips and uniquely marked its thorax with an oil-based paint marker. The training flowers and the cloth bags on the 10 artificial flowers were then removed. The 4 artificial landmarks were added only in the treatment experiment. With a syringe, we drained accumulated nectar from all nectar buckets so that the first visit to each flower would not fill a bee's honey stomach. At the beginning of a trial, therefore, only a trace of nectar was left in each flower. Thus, the first bee visit set the nectar amount to 0, after which it accumulated nectar with time while the motors were turned on.

We then released the focal bee only. Upon release, a bee would usually fly around in the cage but begin to forage systematically within a few minutes. We would catch a slow-starting bee in a plastic vial and guide it into one of the flowers, which often initiated active foraging. If more than 15 min elapsed in vain, we chose another bee for the trial. Because bees often started out sampling flowers slowly, we avoided nectar overflow by not turning on the electric motors until the bee had visited the first 5-6 flowers. When the bee finished its first foraging trip and returned to the hive, we turned off the motors until it reemerged. Throughout these trials, we switched the motors off except when bees were actively foraging. Therefore, we were simulating a situation in which foragers quickly deposited their collections in the nest and returned to foraging immediately. Such behavior is commonly, if not universally, shown by motivated bumble bee workers (Thomson et al. 1987). The trial was continued until the bee made 64 to 73 foraging trips, which would usually take 5-6 hours. We observed 23 bees in this way, assigning 5 bees to each of the 3 factorial combinations of landmark availability and array type (i.e., treatment-independent, control-independent, and treatment-negative experiments) and 8 bees to the control-negative experiment.

After each trial, we immediately placed the focal bee in a clean plastic vial and froze it at -20 °C. For all experimental bees, we measured the average radial cell length of the left and right forewings of each bee as an index of body size (Bertsch 1984, Owen 1988, 1989). We also checked all the experimental bees through dissection and fecal screening and confirmed that none of them had the tracheal mite *Locustacarus buchneri* (Husband and Sinha 1970) or the intestinal trypanosome *Crithidia bombi* (Lipa and Triggiani 1988).

Infections by these parasites sometimes occur in commercial stocks and can affect behavior (Otterstatter et al. 2005).

### **Data analysis**

Using the flower visitation sequences (Table S1) and timing data recorded in the PC, we characterized 2 aspects of behavior for each bee: repeatability of foraging routes (i.e., traplining accuracy) and travel speed between flowers. For an index of foraging-route repeatability or traplining accuracy, we calculated the coefficient of variation (CV) of the "return cycle" (= number of flower visits a bee made before returning to any particular flower) for each trip made by a bee, multiplied by minus one. Larger (i.e., closer to zero) values of this "route repeatability" indicate more accurate traplining, because variation in return cycles would be small if a bee repeated a fixed circuit (Thomson et al. 1997; Ohashi et al. 2007). We also calculated the travel speed between flowers for each trip as the sum of all distances between successively visited flowers divided by the total time spent traveling the distance (hereafter, "travel speed"). For each bee, we computed an average of each behavioral measure during the initial 30 foraging trips (former half) and during all the remaining trips (latter half), respectively.

To examine whether and how bees changed their travel speed and route repeatability in response to varying learning conditions, we fitted general linear models (GLMs; Grafen and Hails 2002) with "array type" (independent or negative), "landmark availability" (treatment or control), and "body size" (radial cell length) as the explanatory variables and "array type x landmark availability" as the interaction term. We included body size as a potential covariate to adjust for its possible effects on bee behavior through correlations with physiological and cognitive abilities (Goulson et al. 2002; Spaethe and Weidenmüller 2002; Worden et al. 2005; Spaethe et al. 2007; Ohashi et al. 2008; Riveros and Gronenberg 2010).

To illustrate how quickly bees responded to differences of learning conditions, we also calculated least-squares means for route repeatability and travel speed, i.e. the expected values of an observed marginal mean if body size were fixed to the sample mean (Searle et al. 1980), in each of the 4 combinations of array type and landmark availability. To control for the repeated measurements from individual bees in calculating the least-squares means and their standard errors, we used a generalized linear mixed model (GLMM;

Crawley 2002) with "learning condition" (either of 4 combinations of array type and landmark availability), "phase" (former or latter), and "body size" as the fixed effects, the individual bees as the random effect, and "learning conditions x period" as the interaction term.

## RESULTS

The GLM models fitted to the data explained significant portions (43-58%) of the variance in route repeatability or travel speed (Table 1). We found 3 major trends in these analyses. First, there was a consistent effect of spatial arrangement of flowers throughout the experiments, such that bees followed more repeatable or accurate traplines at lower travel speed in the independent than in the negative array (Table 1, Fig. 2).

Next, addition of artificial landmarks did not change the above trend; route repeatability in the negative array never surpassed that in the independent array (Fig. 2a), while travel speed was consistently higher in the negative than in the independent array (Fig. 2b). On the other hand, the additional landmarks significantly reduced travel speed between flowers in both the independent and the negative arrays (Table 1, Fig. 2). This trend became more apparent during the latter half, where control-experiment bees in the negative array traveled faster than those in the independent array (Fig. 2b).

Finally, the interaction between array type and landmark availability was significant during the former half of the experiments (Table 1a), although this effect dissipated during the latter phase where bees had accumulated more experience (Table 1b). This is because the differences in the bee behavior between the array types became evident more quickly when additional landmarks were available (Fig. 2). In contrast, bees shifted their priority from traplining to fast traveling after they gained experience in the control experiment (Fig. 2).

## DISCUSSION

### **Behavioral alterations under speed-accuracy trade-off in traplining**

Foraging bumble bees adopted different movement tactics depending on the spatial arrangement of flowers. As we have previously found (Ohashi et al. 2007), bees learned to repeat foraging circuits or traplines in the independent array—where choosing nearest neighbors could be achieved without making sharp turns,

whereas they failed to achieve similar levels of route repeatability even after they gained experience in the negative array—where choosing nearest neighbors conflicted with choosing straightest movements (Fig. 2a). In addition, bees in the negative array sped up after they gained experience, traveling faster than those in the independent array by further sacrificing repeatability of foraging routes (Fig. 2b). This seems consistent with our initial prediction that bees travel more rapidly between flowers to supplement for the lack of accuracy in traplining. To put it another way, accurate traplining is incompatible with the speediest travel. This trade-off has already been demonstrated by examining speed and accuracy in traplining with their effects on competitive performance in a larger independent array (Ohashi et al. 2008). However, our new results show that solo bees actively chose to travel faster at the cost of traplining accuracy when it was more feasible, providing another indirect support for the existence of the trade-off. Chittka et al. (2003) described other consequences of speed-accuracy trade-offs in bumble-bee foraging: bees could choose between flowers with different values wisely or rapidly, but not both at once. As discussed later by Burns (2005), in that example the benefits of higher speed outweighed the costs of more accurate discrimination. Such trade-offs between decision speed and accuracy have also been suggested in many ecologically relevant tasks (reviewed by Chittka et al. 2009). Although the underlying mechanism of the trade-off is unknown, it is possible that accurate route following requires some additional information such as the memory of sequential order of visual stimuli associated with particular movements (Collett et al. 1993; Zhang et al. 1996; Chameron et al. 1998), whereas fast movement only requires at most memory of flower locations or distributions. Such an explanation is consistent with our finding that the "negative" distribution of flowers hampered accurate traplining without limiting speed, together with our previous report that naive bees sped up but failed to increase route repeatability in the presence of experienced competitors (Ohashi and Thomson 2009). The requirement for such high levels of cognitive, perceptual, or motor skills for accurate traplining may therefore generate a speed-accuracy trade-off by increasing the time costs for memory retrieval and comparison.

Even though there was a trade-off between speed and accuracy in traplining, and even though both behavioral aspects could have positive effects on foraging performance (Ohashi et al. 2008), bees gave priority to traplining accuracy over fast movement unless it was hampered by the distribution of flowers (Fig. 2). Because it is quite unlikely that fast movements were less beneficial (or more costly) in the independent

than in the negative array, our results suggest that bumble bees are predisposed to accurate traplining when feasible, possibly because it often produces a greater net benefit than fast movements in field conditions. For example, accurate trapliners may acquire detailed knowledge of individual patches during their periodical returns, thereby reaping extra benefits, such as an increased selectivity for nectar-rich flowers within patches (Williams and Thomson 1998), quicker responses to newly arrived competitors (Gill 1988; Garrison and Gass 1999), or a more efficient avoidance of aggressive competitors or predators (Thomson 1989; Dukas and Morse 2003). Alternatively, faster flights may increase the frequency of wing collisions with vegetation and cause permanent wing damage (Foster and Cartar 2011). It has been suggested that wing wear results in a higher mortality rate in bumble bees, possibly through a decreased ability to escape from predators (Cartar 1992; Hedenström et al. 2001).

### **Landmark effects**

As opposed to our initial predictions, addition of landmarks on the periphery did not improve the bees' route repeatability in either spatial arrangement of flowers even after they gained experience; rather, these landmarks significantly reduced the bees' travel speed in both types of array (Fig. 2b). The lack of improvement in trapline accuracy seems inconsistent with the proposal that route-following insects store retinotopic views or "snapshots" of the surrounding landmarks and use them to get to a goal or intermediate points on the way (Cartwright and Collett 1983; Collett et al. 2003). The most probable explanation for this discrepancy is that our bees could produce stable traplines in the absence of additional landmarks. This may be because the flowers themselves served as proximal cues or "beacons" at the spatial scale of our experiments, and these beacons encouraged bees to learn a sequence of motor patterns. Alternatively, perhaps the limited number of landmarks in the control conditions provided enough information for bees to trapline.

Why, then, did the bees decrease their travel speed when we added more landmarks? It is probable that the bees used the additional information for traplining, which required more time for retrieving stored snapshots and comparing them with the current view. Similarly, a human navigator in a giant metropolis might be slowed down by a plethora of landmarks, pausing for confirmation at every corner, when one or a few symbolic landmarks might suffice for adequate goal-finding at a faster speed. It is possible that

animals hold such apparently "redundant" information, partly because it assures some advantages in other situations. For example, landmark use may serve as a back-up system for bumble bees to minimize the risk of losing their routes in the field, where they would experience occasional displacements by the wind (Comba 1999) or interference competition from other organisms (Thomson 1989). The time cost will be larger when landmarks have similar visual features for the navigator. This may apply to our case, where the landmarks differed from one another in the combinations, but not in the repertoire, of color and shape (Fig. 1). In future studies it would be interesting to see whether there is such an "information load" or an "optimal number" of visual landmarks for animals to solve a certain spatial task, as well as whether greater distinctiveness among landmarks could improve performance.

Yet another possibility is that bees were actually distracted by the additional landmarks throughout the trials. In other words, bees in the treatment experiments failed to ignore the redundant information provided by the landmarks and suffered a heavier time cost of "paying attention" in return for no economic benefits. Generally, animals are able to dismiss redundant information in spatial or associative learning (reviewed by Shettleworth 1998). If the stimuli were perceptually strong, however, such "overshadowing" or "blocking" may not function properly. For example, Manning (1956) observed that bumble bees foraging in a dense stand of *Cynoglossum* plants spent more time in reacting to peripheral plants even if they seemed to have established their own traplines. Although we did not observe noticeable "exploration flights" during our experiments, it is conceivable that the bees' travel speed between flowers was depressed by their continuing curiosity regarding peripheral landmarks.

In contrast to the reduction in travel speed, the bees in the negative array made a quicker shift in their priority from traplining to fast traveling when the additional landmarks were available (Fig. 2). This result suggests that the additional landmarks may have helped the bees grasp some essential features of the array geometry, even while providing redundant (or distractive) information for route following. Because each landmark would indicate the distance to the array periphery or individual flowers, it is not improbable that the additional landmarks encouraged the bees to make quicker decisions in choosing between alternative tactics for a certain configuration of flowers. When sufficient options are available in a large plant population, such landmarks may further allow bees to select a set of plants or patches with a circular or oval arrangement,

in which traplining is less cognitively challenging (Ohashi et al. 2007).

Although it was not tested in this study, it should be noted that another condition of learning may also affect the point at which traplining and fast-traveling tactics balance out: conspicuousness of resource patches. In contrast to our conditions where the flowers were easily perceived from a distance, bees foraging on a plant population with low density, indistinctive displays, or visual obstacles would have to remember their exact locations to return faithfully (Manning 1956). In such conditions, accurate memories for locations and routes may provide bees with greater benefits as they gain experience, although those memories may be costly to acquire. For example, it may become more beneficial for bees to have more accurate spatial memories when plants are inconspicuous, because the detection of next plants incurs heavier time costs than the memory retrievals. Active memory use may also encourage bees to find more efficient route geometry, resulting in an additional improvement of their foraging performance, especially when plant density is low (Lihoreau et al. 2010, 2011, 2012). When these benefits of traplining cannot be equaled by faster foraging, then bees should give priority to traplining even if it entails heavy time and energy costs in early stages of their foraging career. It is thus possible that the observed differences in spatial-use patterns between the arrays would diminish as each flower became less salient (due to low density, inconspicuous displays, or complex landscape, etc.), especially when local landmarks significantly improve accuracy of place memory and route following.

Our results may also have some implications for the coevolution of plants and pollinators. The spatial-use patterns of pollinators will influence pollen dispersal among plants and, in turn, plant fitness. Our results suggest that landmarks may not function as a remedy for plant distributions that hamper pollinators from establishing their own traplines, but that they may help pollinators to select suitable configurations of plants for traplining. Therefore, plants growing in populations with rich visual landmarks may experience increased mating distance, mate diversity, and outcrossing rate (or reduced "iterogamy", i.e., self-pollination caused by immediate return visits) due to the increased proportion of trapliners (Ohashi and Thomson 2009), while they may suffer an increased geitonogamous self-pollination (Williams and Thomson 1998). Although we need to test it empirically, it is also possible that visual landmarks encourage pollinators to trapline in plant populations with low density, inconspicuous displays, or complex landscapes, thereby promoting pollen



flow among plants. We hope that our study serves as a motivation to consider how changes in environmental conditions could alter plant reproduction through their effects on the use of space by pollinators.

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**Table 1 — Effects of array type, landmark availability, and their interaction on route repeatability and travel speed between flowers for (a) the former and (b) the latter half, respectively (GLM with body size as the covariate)**

Source	df	SS	F	P	$\beta$
a) Former half					
Route repeatability (R-squared = 0.52, $F_{4, 15} = 6.10$ , $P = 0.0041$ )					
Array type (independent -> negative)	1	0.026	9.77	0.0070	-0.53
Landmark availability(control -> treatment)	1	0.0010	0.37	0.55	-0.11
Body size	1	0.0032	1.16	0.30	0.21
(Array type) x (Landmark availability)	1	0.015	5.58	0.032	-0.41
Residuals	15				
Travel speed (R-squared = 0.43, $F_{4, 15} = 4.66$ , $P = 0.012$ )					
Array type (independent -> negative)	1	0.016	13.7	0.0021	0.68
Landmark availability(control -> treatment)	1	0.0057	4.96	0.042	-0.42
Body size	1	0.0040	3.45	0.083	0.39
(Array type) x (Landmark availability)	1	0.0046	3.96	0.065	0.37
Residuals	15				
b) Latter half					
Route repeatability (R-squared = 0.45, $F_{4, 15} = 4.93$ , $P = 0.009$ )					
Array type (independent -> negative)	1	0.067	8.18	0.012	-0.52
Landmark availability(control -> treatment)	1	0.00010	0.012	0.91	-0.020
Body size	1	0.034	4.19	0.059	0.43
(Array type) x (Landmark availability)	1	0.00091	0.11	0.74	0.062
Residuals	15				
Travel speed (R-squared = 0.59, $F_{4, 15} = 7.75$ , $P = 0.0014$ )					
Array type (independent -> negative)	1	0.012	19.1	0.00055	0.69
Landmark availability(control -> treatment)	1	0.0091	14.0	0.0020	-0.60
Body size	1	0.0014	2.11	0.17	0.26
(Array type) x (Landmark availability)	1	0.00019	0.30	0.59	0.087
Residuals	15				

Values of  $\beta$  represent standardized partial regression coefficient in GLMs, i.e., the change in response variable in standard deviation unit when all variables are standardized, which results from an increase of one standard deviation or a shift to the direction of the arrow in each explanatory variable

### Figure legends

Fig. 1 — The spatial arrangement of flowers and the additional landmarks used in the (a) treatment and (b) the control experiments. Yellow and purple landmarks are shown in light and dark gray, respectively.

Rectangle and arrow represents the cage screen and the gate of the colony, respectively.

Fig. 2 — Route repeatability (i.e., traplining accuracy) and travel speed between flowers in different learning conditions as to array type and landmark availability. Circles (open= treatment, closed = control) and error bars represent the estimated values of least-squares mean and SE for (a) the former and (b) the latter half, respectively.

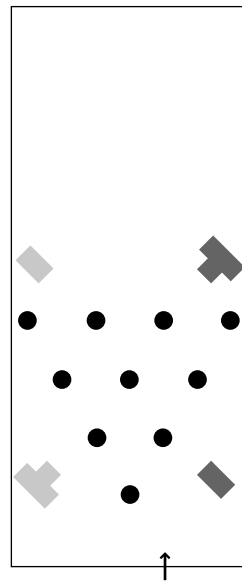
**Table 1 — Effects of array type, landmark availability, and their interaction on route repeatability and travel speed between flowers for (a) the former and (b) the latter half, respectively (GLM with body size as the covariate)**

Source	df	SS	F	P	$\beta$
a) Former half					
Route repeatability (R-squared = 0.52, $F_{4, 15} = 6.10$ , $P = 0.0041$ )					
Array type (independent -> negative)	1	0.026	9.77	0.0070	-0.53
Landmark availability(control -> treatment)	1	0.0010	0.37	0.55	-0.11
Body size	1	0.0032	1.16	0.30	0.21
(Array type) x (Landmark availability)	1	0.015	5.58	0.032	-0.41
Residuals	15				
Travel speed (R-squared = 0.43, $F_{4, 15} = 4.66$ , $P = 0.012$ )					
Array type (independent -> negative)	1	0.016	13.7	0.0021	0.68
Landmark availability(control -> treatment)	1	0.0057	4.96	0.042	-0.42
Body size	1	0.0040	3.45	0.083	0.39
(Array type) x (Landmark availability)	1	0.0046	3.96	0.065	0.37
Residuals	15				
b) Latter half					
Route repeatability (R-squared = 0.45, $F_{4, 15} = 4.93$ , $P = 0.009$ )					
Array type (independent -> negative)	1	0.067	8.18	0.012	-0.52
Landmark availability(control -> treatment)	1	0.00010	0.012	0.91	-0.020
Body size	1	0.034	4.19	0.059	0.43
(Array type) x (Landmark availability)	1	0.00091	0.11	0.74	0.062
Residuals	15				
Travel speed (R-squared = 0.59, $F_{4, 15} = 7.75$ , $P = 0.0014$ )					
Array type (independent -> negative)	1	0.012	19.1	0.00055	0.69
Landmark availability(control -> treatment)	1	0.0091	14.0	0.0020	-0.60
Body size	1	0.0014	2.11	0.17	0.26
(Array type) x (Landmark availability)	1	0.00019	0.30	0.59	0.087
Residuals	15				

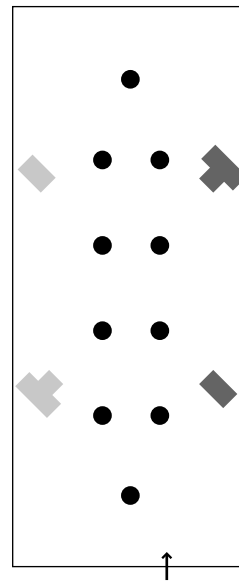
Values of  $\beta$  represent standardized partial regression coefficient in GLMs, i.e., the change in response variable in standard deviation unit when all variables are standardized, which results from an increase of one standard deviation or a shift to the direction of the arrow in each explanatory variable.



a) Treatment



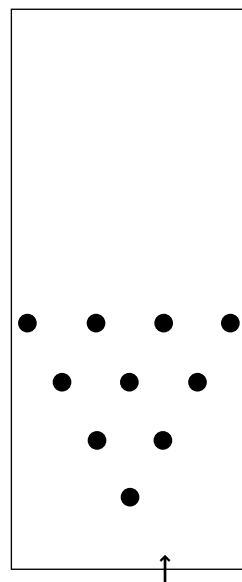
Independent



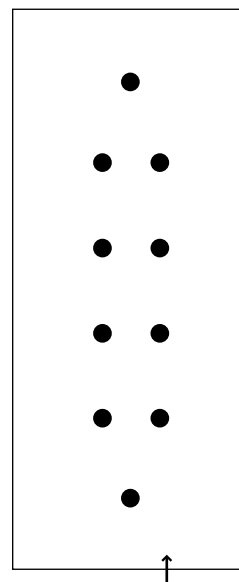
Negative

1 m

b) Control



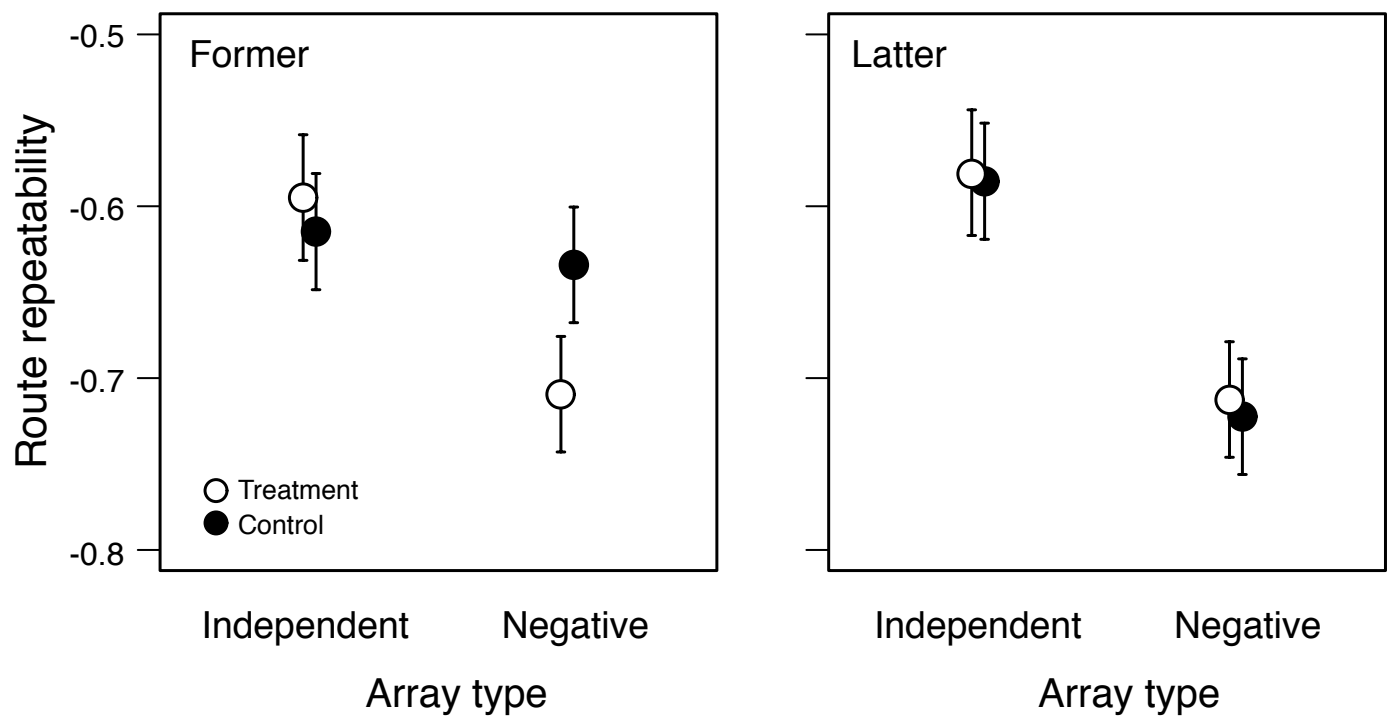
Independent



Negative

Fig. 1

### a) Route repeatability



### b) Travel speed between flowers

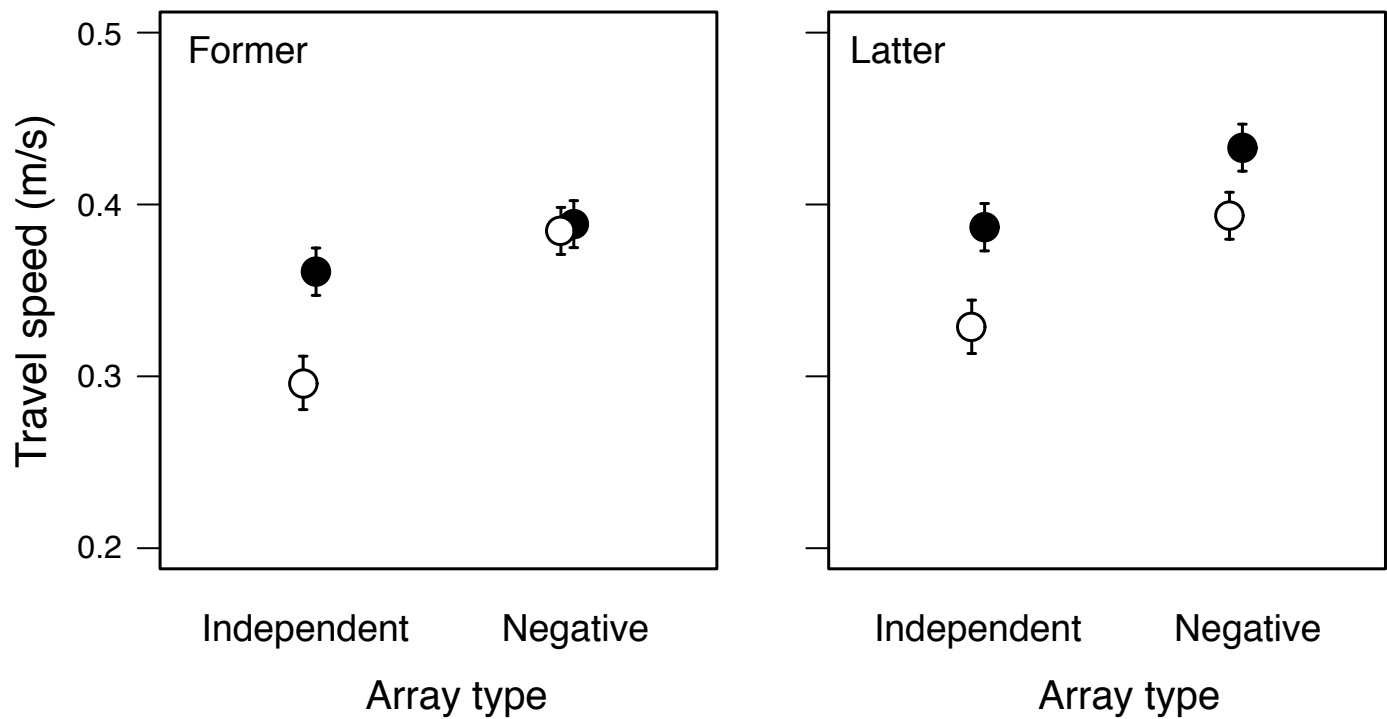
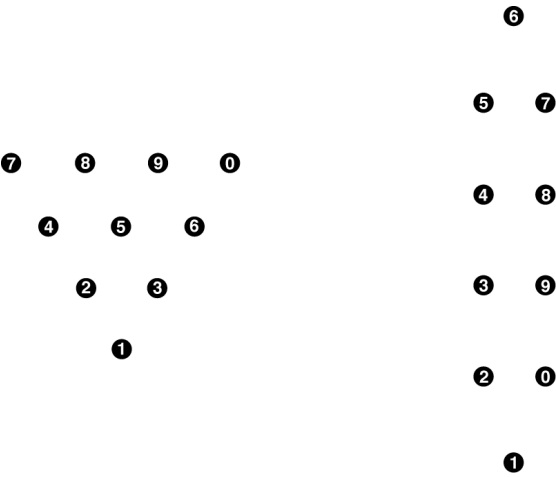


Fig. 2

Table S1— Flower visitation sequences sorted in chronological order (trip 1-30: former phase; trip 30 <: latter phase). Numbers 0-9 in each row refer to the spatial location of each flower in the experimental array (right; flower 1 is the closest to the cage entrance): e.g., bee moving clockwise round the negative array in order starting with a visit to flower 1 would be recorded as "1234567890123..."  
(a) Treatment - Independent, (b) Treatment - Negative, (c) Control - Independent, and (d) Control - Negative (see also Fig. 1).



a) Treatment - Independent

	Trip #	Bee ID 1	Bee ID 2	Bee ID 3	Bee ID 4	Bee ID 5
Former	1	3609874270612598471	353598751	369847	36425890	3575960312
	2	32147890632590631	3694797128036	305421257842	1368409456130742314536	39024784281
	3	125960312598	36987947425124208324213	0643	324789	12478984296
	4	12596421358960	3609875474316098	6068421	1235903609	2478942136
	5	32984531247247	3698731247160987421360987421	360857	609874789031	1360953124
	6	896031257	3690874721360984742136098721	30954	1365898794	25984742136
	7	453147423131396	369874213609874721698713698213	1354	568713247498	159852147
	8	5960474312	36987906321908721247421369098	13587	6589789063	124785903
	9	325960659847	3698790631248018790872136	135874	90631587031345398	12478903124
	10	3590743124721315960	36987890631247890874213698	135879	3987478906	1359094787213
	11	9874231359608	369874213698790632169847872136	136	3698790631	247906
	12	325960874216098731258	136987908063121369878901	136987	69084780312	1247909843127
	13	325984721360984782	1369874	13601359	84789603132	89098547
	14	359084742135963160	369875	136098	369085	2590842136
	15	35908474231369	369874	3594	3984790631	247874906
	16	9098484213606312598	36987421	3587	25984780	5908521361
	17	958424713598785606312	98742136987421697890	32	3842136087	596095479
	18	259063258742136098	908706313125	3596	906312478903247	4985987063
	19	984742125489563	398903124742136987063	36087	98470632474213136098	478906312
	20	359847852315980	9087421369875	608789064	903548742	459847906
	21	98487421359875316	69874213698787806312	52	520324870312584	24789063
	22	847531360	5324787906313690314	9870	824789603124	536952478
	23	6325486098	690987474213606310979324721	359	5890631247478906	24890631
	24	9879631606310	698478703124714741421369	35874	21903124787603131608	4789063124
	25	847212459	987421369878906	58742	087421312474890	890631609524
	26	60954874213	369842413698706	3906	48908478531	890631247
	27	6984213609872136	087421360987421360984	23609824	93248909842136	89084531247
	28	847489606321312590	90687421369803125	321	878421247890314742136	80695312
	29	609548907421360	84241360871609603125	42487421	063136098784	8906521365247
	30	98474213609848785631	085321247890	9087421	5906314	74213690312
	31	6095214896084721369	698503125987421	874247063	721608789096	42598478903
	32	9842790632136031063125	69842136903125874	097421	584213690	89056312478
	33	4872124785313609	6874213609874890631	60985	4789065213128	90695476031
	34	48721360954872136	1254759872136980	087421	584247890631247478909	906547806324

Latter	35	598421360984213609847	09547863125313506474236031	2874213	747906313247890	8524789087531
	36	984879632487853160	3690847906312472136903125	60985	590631247890631478906312	802147908
	37	084721360987421360	8784213258785609	30654	987421369087890312	4789063
	38	974213603125847421360	5847424213698790631	609842	4213609874213609874	479063247421
	39	84721259631258	69584213609847	58742	9707247213603124748906	0603121598
	40	98747421360952487	2348906312547850	60954	474213609847478906312	78906
	41	7214712436584	90874231478903124870631	6587421	5	89095247
	42	609548742	5909842147890631247	54747890	853698903136087	890632587
	43	9084213	5879031258475313609847	3084247906	90631247890	90631254
	44	52136987	35479063136098742421312587	54879063	136984742136098	7890631
	45	987853609847421	5908785213247879063	5478903	8475321474790	89063124
	46	487213609875360321316098	9842	09548742	598703124742136	90631475
	47	87215609874063258421363124	4569879063125	063	1360987421478903	478906312
	48	3695248596324748590632587	358421358790631609847	09854872	874753130874	45906317
	49	2131248906584213603254	59842413698790631247890631360987	063	03184725906312	8908470631
	50	98475213609548	5909847253603124	095487	789063124874721360	85984789063
	51	98421474859536	548906312584789063	1	9074214789063124890	45985216947
	52	847459609879632	24213690631924789032	063	5312590874721	85247906
	53	509853247213	598479063121247890312	21	042160631247890631	48960985213
	54	3254721360632123609	53690987421359063124	0948	9874785313609874	908747213
	55	59632847	698790631247213590315909847	0958	087421360987421	906313247
	56	60954721360632	5984753609878906312487906	874	0321698742136	789063169
	57	84721360	742135906312478	3609747	85312487896	478906312472130
	58	596324	9068421359063247	5879063	0316984789031	84213631095
	59	9548763063248	539069878721531247	087421	79021507136074215479638548748	9063124721301478
	60	56095328421369	590847890631212478	07470763	0647160159085247890	47890631
	61	69847213690	5424790631360987472135	359	98790312	0954742136
	62	98795321360987136	598590536074213590631247	3587	6317	7896084
	63	8747213609842	5847906312478721359063124	587890	539854890	789053601
	64	987853213609	589060984213536987	09532	0874210312	21369547
	65	36984796321360	-	09542	90631248767	90631247
	66	9874213690324872131246098	-	0974248	-	8908472136
	67	98789609879063242131360987	-	35874	-	7890631
	68	609532458789	-	09547478747421	-	8474213609
	69	9848532489632136095	-	096	-	095213
	70	98479631258	-	35478978906	-	78960
	71	-	-	095479047421	-	-
	72	-	-	09587421	-	-

## b) Treatment - Negative

	Trip #	Bee ID 6	Bee ID 7	Bee ID 8	Bee ID 9	Bee ID 10
Former	1	0129	93093847	123485	3902	0202390
	2	093	0294756742093	0256753790236	0298457	1298567029875658763204
	3	02198	0987576212398475	0934876531047675	0239845765765758321	02934
	4	0129348	0934857684893029847	0938756540184	857675657584	1293845
	5	0293485029856	9348757656754393021	093875456567490987601	2038475	02348
	6	03947567	0934875487568932	098476021	202321209	0238456
	7	9348574834849387568457654	023848934757564393201	029849384756	1021393485	02393457
	8	95639848938568476432	1021939847565493920	109843476784390109875	1020384757	023948475
	9	093934576549348	1234875654932021093484	098756890210902129875	10203485756	93487564856543901
	10	84578476487345654938487576	102093484754749320193875	8567601098754576543	102093	2023984754756540
	11	9487584764534584757684575658457563	09323093875764932021	7567543202109	1029348576	48756456432
	12	320	1032018487567549321	690201213845	1023457576	39845756564320
	13	9393475754843	0239384875784932109321	75654390210938	293485765493457	0239845764320
	14	03293948475756845843	87575630210938475	765439020121348756	023487576543	234876754390
	15	93848756584564568756548939493476	102939848756493201293875	8756543902101984765	1024857	02348756492
	16	9393875756584847565847432	0393847576	6439021347543576843902	02348575765	02398475
	17	475674549320	0293238487575493201	75684390213847565439	123934	020298475
	18	43021	02393847575432021	5690201987549320123847	102348576	39487565643
	19	394765758	938475849201210938475	690198756018756	023475749201	0348756549
	20	934843201	98475478932021984754920	5654390213487549356	0239485757649	756493420
	21	54393487578439386	293848756	75654390201384756543902	10348757659	3498475643421
	22	939212029348432019847	04847576	68439021098765654902134	9348757565483902	934875463210
	23	4932012938434576	10239845	75654539021348756543902	2345765643902	43201093487
	24	4876564847656432032010	1093984875675893021	684390219876543475	03984857576	3484756
	25	4209321094847	0284757649392012	57654390212132348756	234857654902	4756549023902
	26	87657658493920	2398475675493201	75765439023214757565439	847576564932	76743902
	27	8765848456758393476875762120109347	8475756543209321098475	756543902109845	04756583201	8475654932
	28	48767548493202019876	0239849875765678493201	4390213487565439012	456590234	023976543902490
	29	654843932012019	8756748932012193875	64320123487565492	48457565839	2934875657489
	30	76584393487658493	38754875632012102984757	3902123847575434	93487576490	3475656321
	31	4845649843932021	2393843487564930120975	3902198756	934857564390	87576320
	32	875654845656439320	239384757689390212	6543902123487	485756564902	4756543920
	33	489756432120	847564932021098475	65439010212398	294857	9348756
	34	849392012397546	8475767893901210238475	656490213843213487	234857576	894756490
	35	476574393201098475	75693201239847562	657565439021098	2485765643	87565392
	36	7654843932012094875	9843756493202109384	7543902109848756	203948792	87549340
	37	934875643920198	56021098475654939021	43902123847574392109348	2021	021
	38	398476543920948	620123984875493201	675483902109875	237575756564	875769
	39	765648392012029	7564320212098475692	7576564390121345432012348	23487576549	75658439
	40	398475432102939875	9848938475684902010934	656484575765490201	29348576564923	875654930
	41	757648432021	675648939012010938475	43902109847	754390	48934756564392
	42	8439320920934756393203	7564932021210938487569	43902123875	234856493021	2398487564
	43	8439475645463201	87567432021093847565493	7562010948	8457576	87565392
	44	8765684875648	7568490212019398475	754390213984	7567589021	48754932
	45	876548439320	75761384375692	67568439021098975	4902393457	7565490
	46	987643934845657564301	8756754930212	60187621875630	893201	475654390
	47	987543209348	1023938475	490212384875	5756584	875654390
	48	984320129348756	8487564932	64890213984347576	8457649021	7565432
	49	8475756439	56493201	543902198756	39348457	56320

Latter	50	8757484393201	847564932	4902139843487576	938757658902	2398475
	51	875684576432	02938487547649021	6548902198487576543	239487576490	7567432091
	52	84756543934576301	398487564920219321	68439021765490	1	756439201
	53	75643201	938756493201098756321	2015653907678901	4857565490	3984756
	54	98439320321	756743201213984756930	47575643	84576576490	475654302
	55	8475643202	10938475649392012984	75690210984	847578320	5654393201
	56	6392120984	87564390201239847563202	67575439021	2023984385	8754302
	57	757648439320	7567493201209384756	176702109875	1	4756432
	58	87657548454393432021	029384756493201	67839021098	2023847	875656
	59	6575643201	8487564932012398475	198475	91	8457674902
	60	7576439320202198	75649320123984756302	678439021	0239	756439201
	61	98756543201098	12098439875764932091	675489321	120239394	757643201
	62	875643201987	47563201213847564939201	54902109848756	876	756543201
	63	7654875439321219	023984756493201984756	6789021876901	102390201	84756942
	64	9847654320	8439320129847576932010	0164902	8487	23984320
	65	87654393201	847563201293984756502	65748	85	6754390
	66	2019843209875921	93284756432021239847	675765483	845756	8754902
	67	483984302010294876	48932023984756493201	8754390	202139	9875643
	68	8765439320	02398487543201238756021	8487567543902	9387576	39847565490
	69	7657439320109	93209847567490219384	75439021	57656490	48756432
	70	758439320921	845643202109848756	75765439021	4857564	875674392
	71	-	939848756849320213875	-	-	023984
	72	-	93847564932098487563201	-	-	2393485756
	73	-	754392019848756201384	-	-	8757654320

c) Control - Independent

	Trip #	Bee ID 11	Bee ID 12	Bee ID 13	Bee ID 14	Bee ID 15
Former	1	1617032809563	359842472	4853587906312	313589	1248987537963
	2	259086576314742313690	5087161247217	236987417148790631690856312	3248565890124	28749758607821063
	3	36954789631	6018453132487	12598479063159063132124	4790631247631	3589607
	4	369847213198	48743169060605	15478963135690	2587414154897	12589047609632360312
	5	169098548741	609547423690639063241	248596369847	632485	248908747063219085871698597
	6	369878952316	58906324790	24789063147523136	3547423609	487606313590632
	7	6987472169063	0956107452132471	24874903147908	612487416908103160312475	135874590987963
	8	356987896313021	25890542471	324789098742174721325	17065235631	13695485963698479063160987360
	9	90654785236	690487063	247896315890	6312487	1258963609874
	10	36909874231908747	61247596084241	354785358909847906	6325874	13690971369521363124
	11	632474158747214789	6972147896084721489	4790987472316	95324	548496098748789631
	12	6909745312478901	974187405247823	587853690632474231	6523548741	609847063136953216908
	13	6312478963131690609874742	0785369	479080632474216906312	125879870	247859890631690547213596310987
	14	69706312478906	3847890631248479	48423690631369031690987	806312587	1247896312460987963213608790631
	15	690631259087	09853124892474906	14785316909874	95213606312487	587963163101698609874
	16	632136984785906	7012912369854236906521	27171423124890632	52136984789061	247487854213
	17	598742790654742136	90842358421369	890631485963987	952487421369606312	65489609879631248742124728
	18	69874721698706	742124874590631	2596984789690	487959606879063160	1258963124856987
	19	69087421369878	69032174560313158	7890631259879063	317856063136987	548909878524721369098
	20	96310847421316987474231	68742369031	0187236984236906	745213609	587960896312
	21	069874532	356948784790	2569874239098721	65212598789063124879	25879863136098796063124
	22	6906872421690987	653248742319	36906324897421312548	742124906321369031697	258963248597
	23	547213169874213	0713690631247890657	652596098478569063	17987584	52470316098796312
	24	098742312698749216098742	0925472365487906531	695212459847896	1785606316012	97960313690824790796031248
	25	6987474213160908785	1248906312489	2569524890874231	21369065214	2485360987
	26	632482136987	10654786321	48906098753124721474713259098	170984531	5690987421
	27	5489063	906324874787906	984879639098747806	36065248745631	89609874
	28	609874213698	9098474569063	56906524898709747474231	063252489063125	13609879631
	29	06324783169874213	12590847890631	856906324874231	0952132456098	609847852
	30	80324874213603125	08742369063212487	25690848742321348	632487890631	125963063124
Latter	31	987421316980	321458908524874231	4874231690631609847	1369874789063124879	0987489609098721
	32	6879614713698487	08479609842369063254	569654874232487	4236987890631245	25924785631360
	33	94874231609874721	1032136952487479085	2569098487231259	063216031248790631	59631256098
	34	60976084231259	47906312487423690	9063248747423124713690	213609874213698	587452
	35	068474213631258	0987423145	89087063247423690931	69874	2487456098763
	36	47213254874213248726980	095232148906	0652348963248742	9560	5896312587
	37	095487474531	545963187590590632484216902	56987298789098752481	132	1253609874213
	38	6316098421369	4879063124890985243	3547906312	9632458	253689630987475
	39	687453125489	59842314890652487	53690984874213	0959248	96031325369847
	40	96542131698903	068459631248745231	548963269098742487	095212487	89609874213609879063124871609870
	41	42312874213698	632487459631248749	259063458785235	948789063	427896312524789063
	42	62135487472131	6321352487890	5609874231248	9524787906	2536095247
	43	09845906421325487	312487426906324874236	90653248909847	0952135878906	485360987421
	44	69874721369063	2590952124587	636909874535690980	247890987421325	54785360987421
	45	987421316906874563	31248742369065	48963258487474231247136	6316087421	6548796098790312479631
	46	93879045606312	473169063154789063248	523169098487906587063	63162487421369	1259631248909876
	47	9087453213548	2478596523124742	796354906316098790	8731324	485213609824878960312
	48	6984742136906328	6987421369875	7136098487890652	214796063124	856098742136090631254
	49	79060874213124890	6325478906314	606587478531	8952487	28789631360987963124789096312
	50	8478906087423125	8563124789084790	5606909584896587	890624742136	12587421
	51	1690987421	0632454874531	56879065236	2132487421369	58960952479631
	52	9876090421354590	423698748906321	5606952458906548	52178560	9095879063136095489721
	53	98742136	2484789063198747890631325987	489063248909847	2148798790	369087908742136090631603124

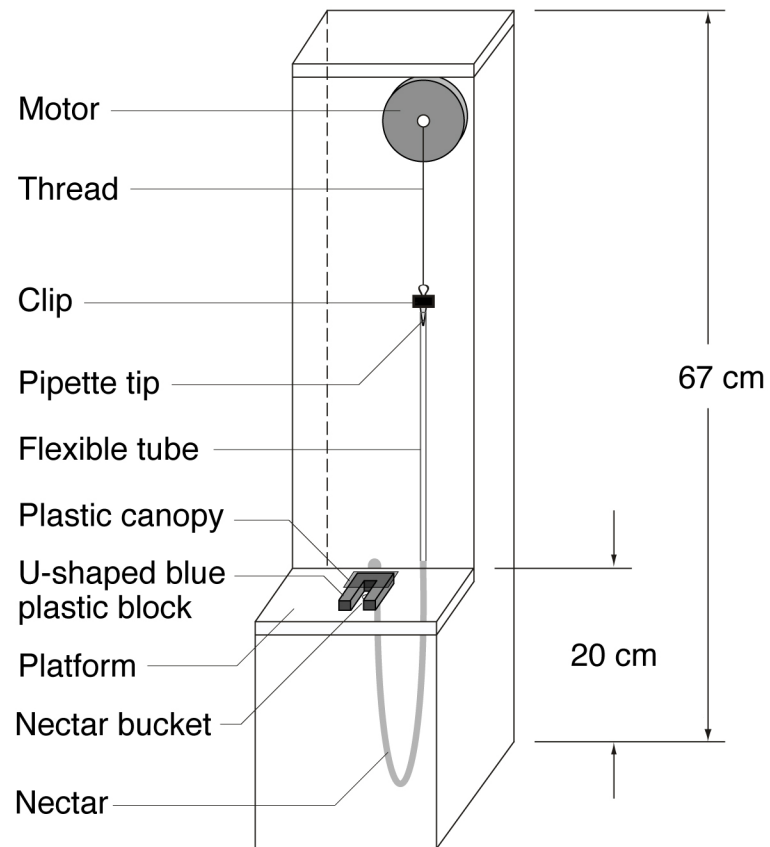
	54	654531369098742	5265487459063248745	7065231690316906098487	9232560	796312478960631248963
	55	0879063125874216509	5248790879531	8534785690	132487856	69547896312
	56	863130987472131	256908423	16987456324721606136098	84721359631	253169879606312487960
	57	6312569874213590874	06324874596	052706303645690652361248742	906521248	5296098796316098706313603134
	58	36098742785213	324789098562	352474231412474534190653	690487324	8987896321360987
	59	3248742312487269870	459065312489063	0648487896989095873	5609531	85248531360
	60	3169842136906310987	312489063215478906	96523	95609874	79652136095247
	61	079459612542890	98423698478906301	906524847890	953132	5609879687213606312478963123
	62	69087425632458745	2456906548987423	0654870985236306	9054842356363125	2879632489063124789098706953124879
	63	16906531487	35478906321	98474531	1458789	06521360631248789631
	64	8456314874231289	98423698745321	89098524890654	74216063125	60953609524
	65	630985487452312489	4716098742436984890635	569065985231	1360987	25848578963
	66	09874521359874706532	13214536987890479098706	69087453609824	062587421356	8796313606313095
	67	30313690631698745	5489084231	890652487421360987906	56063125487	489609874213696095
	68	10954789063198	247890632489074521	965324	3587458909536	79063160313953124896098
	69	0987432135	085474213698478906	5653906548	95248742136	609874723698796321348742136098706312
	70	16987423698706312589	47241365478906	9065248909548906	016063125	253602478536987072136980932
	71	-	-	-	06316095367	8795213247896312478963125
	72	-	-	-	-	5842369095472123695248



d) Control - Negative

	Trip #	Bee ID 16	Bee ID 17	Bee ID 18	Bee ID 19	Bee ID 20	Bee ID 21	Bee ID 22	Bee ID 23
Former	1	01202	10210202393	034320203	48475	09391	01219348752383	20	10938
	2	02984	294849321239401397	0209845485687	102	02194	01934756	01934578761	1098432
	3	57574394	120239457	38901239	129843901093485768784905	9384574301	109845720193456754	1209843765	09384576
	4	657658302934839845765430121	2039475765893	12934875	09	084576583201	102398487675489023213768430	098576527831345765690	0239845767532
	5	6548493985757543874321	3010212393948457	3902948	12029	08756758432	0984756564321098790198757678475432198	8756543902	093487
	6	7584348320139	23984576320123985	4845	102984	84576493457321	1098487565432090128576758432102987689021219875489021	20984757675432123487568457321	984576
	7	757848575439010212012932021	293934875756	203484845	1023	875649023485	098487576576549320219875756548430248757575654	09875764902101098487564	09201
	8	84857457432084832010932029848	39756	87575876	10987	8576754902134	0984875649201948756565482021984576754202	847565439012	02984576
	9	84392021238432134239837	02398561	93	109875	03765432012348754356	09848757654920284756542024875659321	0987575768430932123984	38756783
	10	84393201212398475	248394756	203	109876	875658432012348756549	987567548920298756420298756564921948756	9875765439021239348756431	34845764932
	11	84320213847548576	02398576	13	1209875	845764213487545601348	098475656542021876564298757545767548420982198756	02098487576543209321098457	5764201
	12	45783202198430	210212375	2034	1098754	87565758320190210298	98757565484298757675842198757656542094875	09029484757656439021	090348457
	13	8475743292013984375456	0239875678932	0234845	1209876	9756584932010239348	9847565748920	984875765439021239348457658320	345764393201
	14	84320121293576	1256430101395768	8439384345	109876	09845765490210198457	0	0987654390213484757643021	23756575843
	15	7543932109387	102398765643921	0202948457	1098756843	8457656849302101	02984	9875643902193847576543902	09348762021
	16	57548438202121029	1921430219475687567843	85757345	1298765	98576548320139019348	1	0984756543902102398457	1212984
	17	8432109329384907	32390212015757656543	2029348	1298765493	84576843932017568490	10984875765843	098487565430213487564321	56320213487
	18	47576548430109	23987565765430	9348457	109875654321245649210856	754392012398457654302148	1984757565483	875764302134564321934875	7652101948
	19	45674832012193	02398385765457654831	9848432435	987564	7576784902017567892018457	1098475765483	098756543901984756439021	02934845
	20	8475732013487562102987540	2175654302	325756	1298756753	85764832019845	09475643	8764390210984765439021098475691	3487565432
	21	457568432019321098	239848567832	756	1285675489	75490201987568492012198	19847543902	87564890212093847576543021	02934845
	22	832102102384845754654320	0213987561	029	1298575675483	84576549020123487546	2984756548932093	87654390219847565439021	84756392021
	23	8432198432021209487546	0292012398457576	84857	12987576543	8490201293487565430	984756543902347564321	8765843902109847565	38457684392
	24	73201984756	1239845757639021	8457576	129845765498902319857	757654390212398457	9847564393201	0219398487565439021	8487565483202021
	25	457584320193098476	213848321984857678743921	2023	1298457654876758490	75432019857675493201	9348756548321	8765843902109348756543902	576484392
	26	5483932010984	94875678312102129321284	84848575635	1284576543	7548321348756490219847568	0984375639202198475764320234875656543209	75489021939847565439021	320213485
	27	843201984756	3021930320184576	9345756578	285754932857646754	675489019845768902187567890193	984756489343202120938457684320198475654893201	87564390212384765432010939847	475658432021
	28	7584320120239875	056784321	5484392	123	45765490201576543201398	1984576565432020212984576432012343457654393213	87656439021098475643201	9384756439320
	29	457586393210984567	2984832021398487	845760	234893	9845765849019843	984756839320197575654393201987563202091	93487565439021093487569021	90210293487568
	30	76484320212984321984756	56754830	4576902301	2387576575754	37564932019348	9848757654320932010984576543201984756543932	9875648390212093845756021	9345764320120934
	31	19432847576543201	18329321087	1	2345765849	756843201984576	9848756439320234875765648393219398475	847565439021093848756	02129323945765484392
	32	75784320198756732016	2102302185756	3021	238575493	7564320212398434576	0987565483932129387576584393457654392342134875	847564390210938475802	093487576543920
	33	675432021239847565638475	232984756	1201210257	208575767584	689010201984875432	2984875765432987576643920356328475654325312934875432	6543902109398475683902	12945765432012
	34	02019875487565320298432	87439321021	675756576	239845756	3984757684901234	9875743932029384875654839320129348754356543932	87564390210938475639	02193487576
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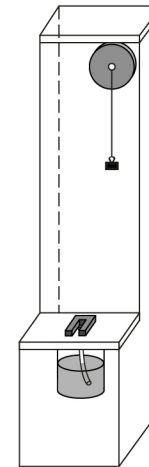
a)



b)



c)



d)

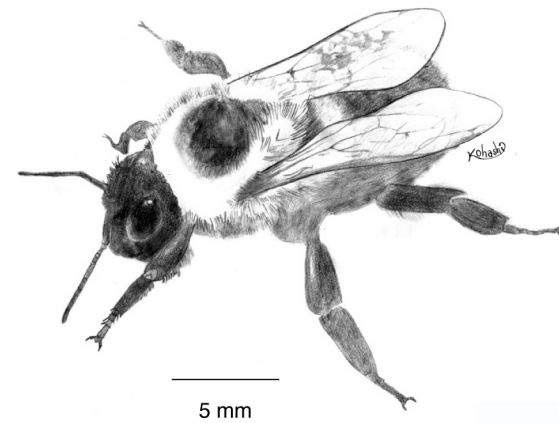


Figure S1 — Views of the (a) artificial flower, (b) training flowers (left: vial type; right: Petri-dish type), (c) vial-type training flower in use, and (d) worker of *Bombus impatiens*.