

# Food Recruitment Information can Spatially Redirect Employed Stingless Bee Foragers

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#### Abstract

Within a rewarding floral patch, eusocial bee foragers frequently switch sites, going from one flower to another. However, site switching between patches tends to occur with low frequency while a given patch is still rewarding, thus reducing pollen dispersal and gene flow between patches. In principle, forager switching and gene flow between patches could be higher when close patches offer similar rewards. We investigated site switching during food recruitment in the stingless bee Scaptotrigona mexicana. Thus, we trained three groups of foragers to three feeders in different locations, one group per location. These groups did not interact each other during the training phase. Next, interaction among trained foragers was allowed. We found that roughly half of the foragers switched sites, the other half remaining faithful to its training feeder. Switching is influenced by the presence of recruitment information. In the absence of recruitment information (bees visiting and recruiting for feeders), employed foragers were site specific. Foragers only switched among feeders that were being visited and recruited to. Switching was not caused by learned aversion to experimental handling. Switching in response to recruitment could provide a fitness benefit to the colony by facilitating rapid switching among exploited patches and provide a benefit of increasing plant gene flow between patches.

### Introduction

The co-evolution of pollinators and angiosperms has contributed to the remarkable diversity and success of bees and bee-pollinated plants (Harder & Johnson 2008). Many pollinated plants provide nectar or pollen rewards that encourage foragers to be faithful to the same floral species (Gegear & Burns 2007). These rewards can also lead to site constancy, in which foragers visit the same foraging area, even when other rewarding areas are accessible (Osborne & Williams 2001).

Site constancy has been found in several bee groups and can enhance foraging efficiency in stable environments. For example, traplining (visiting food sources in a specific, repeatable sequence) is a form of site constancy and can enhance bumble bee food collection efficiency (Saleh et al. 2007). Osborne & Williams (2001) found 86–88% site constancy in the bumble bee, *Bombus lapidarus*, with most site switches occurring among adjacent patches. Employed honey bee foragers will also continue to visit a rewarding food patch with high quality food (Seeley et al. 1991). Stingless bees behave similarly. Employed *Melipona fasciata* foragers did not switch between feeders placed 200 m in opposite directions from the nest when both locations offered high quality food (Biesmeijer & Ermers 1999).

Site constancy can have a strong effect on pollen dispersal distances and gene flow.

Levin et al. (1971) modeled the effect of foraging directionality on pollen flow in bumble bees and

found that average dispersal distance can decrease by 29% when foragers move randomly among plants as opposed to straight line flight (corresponding to site constancy). Seed set can be lower following crosses between neighboring plants as compared with widely spaced plants (Levin 1981).

In social bees that can recruit nestmates to a specific spatial location, recruitment communication mechanisms have evolved to enhance colony exploitation of a rewarding location. However, within or nearby the rewarding patch, these mechanisms may facilitate a certain degree of switching, depending upon how precisely foragers use recruitment information. This is desirable because foragers must visit multiple flowers per trip to collect a full load of nectar or pollen, and natural food sources are not distributed as single point sources (Towne & Gould 1988). The honey bee waggle dance is a classic example in which foragers evidently tune the amount of directional error in their waggle dance according to the average patch sizes that they encounter in their environment (Weidenmuller & Seeley 1999), thereby spreading recruits out to exploit the patch (Towne & Gould 1988).

Recruitment information is often produced by foragers collecting rewarding food to recruit unemployed foragers who are not currently collecting food and are seeking rewarding patches. However, relatively little is know about how such recruitment information given at the patch (such as pheromones or visual cues provided by resident foragers) affects the foraging choices of employed nestmates on nearby, adjacent patches. Such information creates a potential for inter-patch switching if foragers encounter this information close to the patch they are currently exploiting. These field-based sources of recruitment information include odor-marking of rewarding food sources (which has evolved into odor-trail communication in some species, Nieh 2004), short-range attraction to the visual presence of conspecifics on food (visual local enhancement, Slaa et al. 2003), and following nestmates to a food source (piloting, Aguilar et al. 2005).

Thus, our goal was to explore whether such recruitment information could cause employed foragers to change foraging locations and begin foraging at a similarly profitable location occupied by nestmates near the colony. We chose the neotropical stingless bee *S. mexicana* for this study because it shows an efficient recruitment communication with relatively high spatial precision (Sánchez et al. 2004). Recently, we observed that employed *S. mexicana* foragers could switch to new locations when presented with feeders similar in appearance to those at which they were trained (Sánchez et al. 2007). We therefore used this species to determine if employed foragers could switch feeding locations based upon the recruitment information provided by nestmates in the field.

# Methods

# Study Site and Colonies

We sequentially used three colonies of the stingless bee *S. mexicana* housed in wooden boxes ( $25 \times 25 \times 50$  cm) from January 2005 to January 2006 at El Colegio de la Frontera Sur ( $14^{\circ}53'$ N,  $92^{\circ}17'$ W), in the city of Tapachula, Chiapas, Mexico. All trials were conducted between 8:00 and 14:00 hours. We conducted only one trial per day.

# Training

Colonies were brought from a meliponary located 30 km away to insure that all foragers had no prior experiences with feeders and no preferences for any specific flight direction at the test site. We trained foragers by injecting 5 ml of 1.0 M sucrose solution into the colony entrance and placing a 1.0 M sucrose feeder 2 cm from the entrance. Five foragers were randomly selected and individually marked with water-based paint on their thoraces. All other foragers that arrived during training were trapped in aspirators and released only at the end of the trial. Once foragers had learned to visit the feeder, we moved it to the experimental distance (15 m away) in short steps over 20 min.

# Expt 1: Recruitment Information and Site Constancy

To test the influence of recruitment information on *employed* foragers (bees currently visiting a food source), we trained three groups of foragers from the same colony to three different locations around the colony, each 15 m from the colony and thus equidistant from each other (Fig. 1). The shortest distance between each feeder was thus 26 m. At each site, a feeder supplied a rich 2.0 m sucrose solution *ad libitum*. To avoid any exchange of foragers among the feeders during the training phase, we trained the foragers sequentially, to each site. We trained the first group to site 1, the second group to site 2 and finally the third group to site 3 (Fig. 1).

We caged foragers at their training site when we trained other groups of foragers to different sites



**Fig. 1:** Setup of the feeders in this study. The central square represents the stingless bees' colony, and each of the three circles is a feeder site, placed at equidistant and equiangular positions relative to the colony.

(1 mm nylon mesh cylinder: 10 cm diameter and 15 cm height). We confined foragers only after they had fed for 10 min to allow them to deposit odor marks (Sánchez et al. 2008) and recruit. During these 10 min of free foraging, we captured all recruits with aspirators. Foragers recruited and thus deposited recruitment information for all three feeders in all trials. On average (mean  $\pm$  SD), foragers were confined  $45 \pm 10$  min for the first group and  $25 \pm 7$  min for the second group. We never confined group three. Replicate trials consisted of training a new group of five foragers from the focal colony and shifting the location to which the first, second and third groups were trained in successive trials to eliminate potential site training bias (rotating training 60° counterclockwise for each successive trial). We conducted 15 trials with 225 different foragers.

After we trained foragers to the third site, we released foragers confined at the other two sites. All three groups could then feed and recruit for 10 min. Foraging conditions were such that recruitment for these relatively close feeders (15 m from the nest) began as soon as foragers were allowed to return to the nest. After this recruitment phase, we trapped all foragers with an aspirator at each respective feeder. After 10–20 min, all trained foragers were caught. We then examined forager paint marks to determine if trained foragers remained faithful to the site they were trained to or had switched to a different site. Thus, each trial began with foragers. At the end of

each trial, we painted any unmarked bees that had been captured (to avoid using them in future trials) and then released all bees. To avoid pseudoreplication, we used and analyzed the choices of a new group of 15 bees in each trial. We performed nine trials with colony A and six trials with colony B. In this experiment, we observed a high proportion of foragers switching between feeders (see Results section). We tested reasons for such switching with colony C (expts 2–4).

#### Expt 2: Effect of Short Distance

Fifteen meters (Fig. 1) is a relatively short distance for stingless bee foragers to fly from the nest to a food source (Roubik & Aluja 1983; Roubik 1989). Thus, the proximity of the feeders to each other could result in low site fidelity because of random switching that is not influenced by recruitment information. To test the effect of short distances alone, we trained five foragers to site 1. We then placed feeders at sites 2 and 3, without training foragers to them. There was consequently no recruitment information at or for these sites. We allowed the individually marked, trained foragers to forage freely for 30 min and recorded their choices. If foragers switch sites in the absence of recruitment information, then some site 1 foragers should switch to the other two sites. We conducted four trials with a total of 20 different foragers.

#### Expt 3: Effect of Confinement

Confinement could have negatively influenced trained foragers, causing them to reject their confinement site and thus move to a new rewarding site (even in the absence of recruitment information). We tested this possibility in two different ways. First, we examined data from expt 1. In expt 1, foragers trained to the site 3 were never confined. If confinement increases the probability of switching, then site 3 foragers should exhibit a significantly lower rate of switching than foragers trained to sites 1 and 2, which were confined (see general methods). We conducted five trials with a total of 25 different foragers.

Second, we conducted a new experiment in which we manipulated confinement duration. If confinement increases the probability of switching, then increased confinement duration should result in increased switching. Thus, we trained five marked foragers to the site 1 and confined them for either 45 min (the average time site 1 foragers were confined, three replicate trials) or 25 min (the time site 2 foragers were confined, two replicate trials). During confinement, we placed identical control feeders at sites 2 and 3. After confinement, we released the foragers and monitored all sites.

## Expt 4: Delayed Effects of Confinement

If foragers experience confinement as aversive, they could learn to avoid olfactory and visual cues associated with the training feeder and switch to new sites. In this scenario, foragers could prefer unmarked or differently marked food sources to food at the confinement location. To test this possibility, we trained one group of foragers to site 1 (confined 45 min) and another group to site 2 (confined 25 min). During confinement, we placed a control feeder at site 3. Confined foragers were then simultaneously released and their choices recorded. If confinement causes foragers to form an aversive association with recruitment information (olfactory and visual cues on the feeder), a significant proportion of foragers should then switch to site 3 rather than return to sites 1 and 2. We conducted three trials and used 30 foragers in total.

#### Statistics

We used ANOVA and chi-squared tests to analyze our data. All data analyzed with ANOVA conformed to parametric assumptions according to residual analysis (Shapiro–Wilk normality statistics). We used ANOVA with REML analysis and Wald's Z statistics to determine if there is a significant effect of colony. For expt 1, we tested for a potential effect of site with chi-squared tests. Our hypothesis for this experiment is that employed foragers will redirect themselves equally at all three feeder sites. For expts 2–4, we used chi-squared tests to test the hypothesis that the observed distribution of the employed foragers does not fit the null expectation of a uniform distribution (neither confinement nor distance affected

the forager site choices). Analyses were carried out with spss v13 software (Chicago, IL).

## Results

### Expt 1: Recruitment Information and Site Constancy

Slightly more than half of foragers switched from their training site (52%, n = 225) to one of the other sites (Fig. 2). Data conformed to the normal distribution (W = 0.924, df = 45, p = 0.391). There is no significant effect of training site (fixed effect:  $F_{2,41} = 1.276$ , p = 0.290) or colony (random effect:  $\sigma^2 = 0.123 \pm 0.28$ , Wald's Z = 0.439, p = 0.661) on the number of switching foragers. We therefore pooled the data from both colonies and all three training sites and found no significant difference between the distribution of foragers that were faithful to their training site and distribution of foragers that switched sites ( $\chi^2 = 0.36$ , df = 1, p = 0.55).

#### Expt 2: Effect of Short Distance

In this experiment, only one forager switched sites (5% switching). Out of 20 foragers trained, 19 came to site 1 and the remaining foragers came to site 2. This observed distribution departs significantly from  $(\chi^2 = 33.39,$ uniform distribution df = 2,а p < 0.001). Although the feeders were quite close to the colony (15 m), trained foragers had high site fidelity and did not visit feeders that were not marked with recruitment information. Thus, in the absence of recruitment information. close feeder spacing within a patch did not lead employed foragers to switch locations (Fig. 3a).

# Expt 3: Effect of Confinement

In this experiment, *none* of the foragers confined for 25 min switched from their training site (10 foragers



Fig. 2: Results of expt 1. The fraction of foragers that remained on their training feeder (open circles) or switched to another feeder (filled circles) in each of the different training positions (a–c).



**Fig. 3:** Results of expts 2–4. The fraction of foragers that remained on their training feeder (open circles) or switched to another feeder (filled circles) in the control experiments. (a) Results of short distance (expt 2); (b, c) results after confinements of 25 and 45 min (expt 3), respectively. (d and e) results of expt 4 testing potential negative association at sites 1 and 2, , respectively.

trained: 10 recorded at site 1, Fig. 3b). This significantly deviates from a uniform distribution  $(\chi^2 = 20.01, df = 2, p < 0.001)$ . Similarly, nearly all foragers confined for 45 min came back to their training site, and only one (6.7%) switched to another site (15 foragers trained, 14 recorded at site 1, one at site 2, Fig. 3c). The proportions of both 25 and 45 min treatments are not significantly different each other ( $\chi^2 = 0.694$ , df = 1, p = 0.600). Thus, there is no significant relationship between confinement duration and the probability of switching.

Confinement also did not affect the results of expt 1: 20% and 27% of foragers trained to a site with no confinement (site 3) switched to the sites 1 and 2, respectively (n = 75, Fig. 2c). Thus, 53% of the trained foragers remained faithful to their original training site. This proportion of switching is not significantly different from the proportion of switching for foragers trained to and confined at sites 1 and 2 ( $\chi^2$  = 4.023, df = 4, p = 0.403). Thus, confinement did not affect forager switching behavior. These results suggest that confinement did not provide an aversive stimulus.

#### Expt 4: Delayed Effects of Confinement

In this experiment, none of the foragers trained to sites 1 and 2 switched to site 3, where no forager was trained and which did not have recruitment information. Thus, foragers did not avoid sites at which they were confined, and there is no evidence that they formed an aversion to recruitment information provided at these sites. However, foragers did switch *between* sites that contained recruitment information. At sites 1 and 2, 43% and 23% of foragers were faithful to their respective training sites (Fig. 3d, e). This distribution is significantly different from a uniform distribution ( $\chi^2 = 22.17$ , df = 2, p < 0.001). Such switching may have been due to avoidance of each site, but this should also have led foragers to switch to site 3, and this did not occur. Thus, foragers evidently switched between sites 1 and 2 because these sites were marked with recruitment information.

### Discussion

Studies have shown that employed stingless bee foragers develop a high fidelity to the site at which they collect food (Biesmeijer & Slaa 2004). We demonstrate that such site fidelity does not always occur and can be influenced by the presence of recruitment information to switch to a nearby location. We found that roughly half of employed foragers within a 15 m radius of the colony (706.5 m<sup>2</sup>) switched to new location even when their former location continued to offer equally rewarding food. However, foragers only switched when alternative locations were also visited by nestmates and thus were marked with olfactory recruitment information and the visual presence of other foragers (visual local enhancement). This phenomenon would allow employed foragers to explore their surroundings and could facilitate more rapid switching among exploited patches based upon fieldbased information. In contrast, intranidal labor reallocation requires foragers from an exhausted patch to return to the nest and wait for intranidal recruitment information before being reallocated to forage at rewarding locations. Increased reallocation speed would provide a fitness benefit by allowing the colony to more quickly adjust to changes in food availability.

Studies of meliponine food recruitment have generally found a high degree of site fidelity. For example, Scaptotrigona depilis foragers were almost 100% faithful to their training feeder when another feeder was presented at angles of  $2-20^{\circ}$  from the training feeder (all feeders located 50 m from the nest. Schmidt et al. (2003). Contrera & Nieh (2007) also found high stingless bee site fidelity: 72% of Partamona peckolti foragers chose the training feeder out of an array of five identical feeders, each separated by 20 cm and all located 75 m from the nest. Similarly, 87% of Melipona panamica remained faithful to training feeder in a five-feeder array (feeders separated by 20 cm and located 25 m from the nest). In both cases, eliminating forager-deposited odor marks decreased site fidelity (Contrera & Nieh 2007), although this experiment provided feeders that were very closely spaced and acted more as inflorescences within a small patch.

In contrast, under a different set of experimental conditions, we found a substantial proportion of foragers (55%) switching among three foraging sites (expt 1). In this experiment, all feeders were visited and attracted recruits, offered the same rich 2.0 м sucrose solution ad libitum, and were placed at short distances from the nest and each other. This design permitted us to simulate a situation of mass flowering around the colony and communication of feeding sites within such a large patch around the colony. Site-switching in this study probably arose because of recruitment communication. Recruitment information evidently influenced employed foragers to switch because such switches only occurred for visited feeders (expt 3). This influential recruitment information is most likely field based (food odormarking, odor trails, local enhancement and piloting-type mechanisms, Nieh 2004; Slaa et al. 2003). Recently we found that odor marks and visual cues (presence of nestmates) could attract S. mexicana newcomers to a food source (Sánchez et al. 2008). However, it is possible that information transfer within the nest could have played a role.

Further experiments will be required to determine what recruitment information is most important for employed forager switching. However, bumble bees (Macuda et al. 2001) and honey bees (Srinivasan & Lehrer 1988) have quite limited visual acuity and are not able to see the presence of foragers on feeders 15 m away. We consider orientation to olfactory odor trail or odor mark information on the feeder the most likely source of information influencing switching because stingless bees can detect odor marks 1-12 m away (Nieh & Roubik 1998; Hrncir et al. 2004), depending upon species, pheromone concentration, wind direction, wind speed, temperature and humidity. Thus, an employed forager leaving the nest could be attracted to olfactory information deposited to mark a feeder 15 m away (Fig. 1).

Future experiments could examine the effect of individual experience and, possibly, genetic makeup in the propensity of employed foragers to switch sites. Testing the effect of forager age, status (recruit, scout, reactivated forager, etc.), and previous experience would be informative. Furthermore, it would be valuable to determine which communication mechanisms (odor marks, visual presence of other foragers, etc.) are involved in the switching of employed foragers visiting rewarding food.

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