

SHORT COMMUNICATION

Evidence of trapline foraging in honeybees

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ABSTRACT

Central-place foragers exploiting floral resources often use multi-destination routes (traplines) to maximise their foraging efficiency. Recent studies on bumblebees have showed how solitary foragers can learn traplines, minimising travel costs between multiple replenishing feeding locations. Here we demonstrate a similar routing strategy in the honeybee (*Apis mellifera*), a major pollinator known to recruit nestmates to discovered food resources. Individual honeybees trained to collect sucrose solution from four artificial flowers arranged within 10 m of the hive location developed repeatable visitation sequences both in the laboratory and in the field. A 10-fold increase of between-flower distances considerably intensified this routing behaviour, with bees establishing more stable and more efficient routes at larger spatial scales. In these advanced social insects, trapline foraging may complement cooperative foraging for exploiting food resources near the hive (where dance recruitment is not used) or when resources are not large enough to sustain multiple foragers at once.

KEY WORDS: *Apis mellifera*, Honey bee, Navigation, Spatial cognition, Route learning

INTRODUCTION

Pollinators such as bees face complex foraging problems as they exploit ephemeral floral resources that are scattered in space and vary in quality. Manipulative experiments in bumblebees foraging on artificial flowers show how individual foragers can learn stable, repeatable traplines, minimising travel distances between feeding locations (Lihoreau et al., 2010, 2012; Ohashi et al., 2007), an optimisation task akin to the well-known travelling salesman problem in network theory (Cramer and Gallistel, 1997). Mathematical models indicate that this routing behaviour is particularly efficient for foragers exploiting patchily distributed resources from a central place, thus suggesting that traplining is taxonomically widespread among pollinators (Ohashi and Thomson, 2005; Possingham, 1989). Better understanding of the complex spatial strategies of pollinators is crucial to assessing patterns of pollen flow and their consequences on plant populations and communities (Fortuna et al., 2008).

Despite intensive research on the honeybee, a key pollinator worldwide and a model species in insect navigation, this question has never been explored. In contrast to bumblebees, honeybee foragers communicate using a symbolic language (the waggle dance) that conveys information about the location of resources discovered more than ca. 100 m away from the hive (von Frisch,

1967; Riley et al., 2005). Therefore, most studies on honeybee navigation have focused on how foragers learn to fly back and forth between the hive and one (or two) distant feeding locations and how they communicate this information (Collett et al., 2013). However, little is known about how foragers move between different feeding locations. In nature, honeybees may visit hundreds of flowers per foraging trip (von Frisch, 1967), thereby creating ample opportunities for foragers to simultaneously exploit multiple flower patches or plants. Early field observations suggest that individual honeybees confine their foraging activities to relatively stable groups of plants over many successive days (Ribbands, 1949). More recent studies indicate that foragers can learn flight sequences between multiple visual landmarks to resolve mazes (Collett et al., 1993; Zhang et al., 1996) and discriminate the direction of different feeding locations from a single starting point or hub (Najera et al., 2012).

Here we examined the ability of honeybees to establish traplines. We observed individually marked foragers exploiting four artificial flowers over 30 consecutive foraging bouts. We compared their routing performances in the laboratory and in the field at different spatial scales to identify how environmental factors affect this behaviour.

MATERIALS AND METHODS

Study sites

All experiments were conducted in spring 2015. Laboratory observations were made in a 7×5 m flight room (Fig. 1A) equipped with 12 wide-spectrum LED lights (6500K). A poster uniquely characterised by a bicolored pattern was placed on each wall to provide 2D landmarks (Fig. S1). Field observations were made in a 300×150 m flat ploughed land free of natural flowers, on sunny days with clear sky (Fig. S2).

Bees and artificial flowers

We used a small colony of *Apis mellifera* (Linnaeus 1758) (ca. 2000 workers) in an observation hive. The hive entrance was equipped with a transparent tube fitted with shutters to control honeybee traffic. The colony was fed with *ad libitum* defrosted pollen directly into the hive. Workers collected sucrose solution (40% w/w) from artificial flowers made of a blue landing platform sitting on a transparent cylinder attached to a 50 cm stand (Fig. S3). For large-scale field observations, a wi-fi camera (D-Link) was positioned above each flower (Fig. S3C). Live images of all flowers were displayed on a single computer screen.

Honeybees were tested in a four-flower array (24 possible sequences to visit all flowers once, starting and ending at the hive). The spatial configuration of flowers maximised the discrepancy between the two optimal sequences minimising path length to visit all flowers and the sequence linking unvisited nearest-neighbour flowers (Fig. 1). The same configuration was used at a small spatial scale (distance between flowers: 1.48–6.27 m) and at a larger spatial scale (distance between flowers: 14.8–62.7 m). In both cases, flowers were located less than 100 m away from the hive, thus

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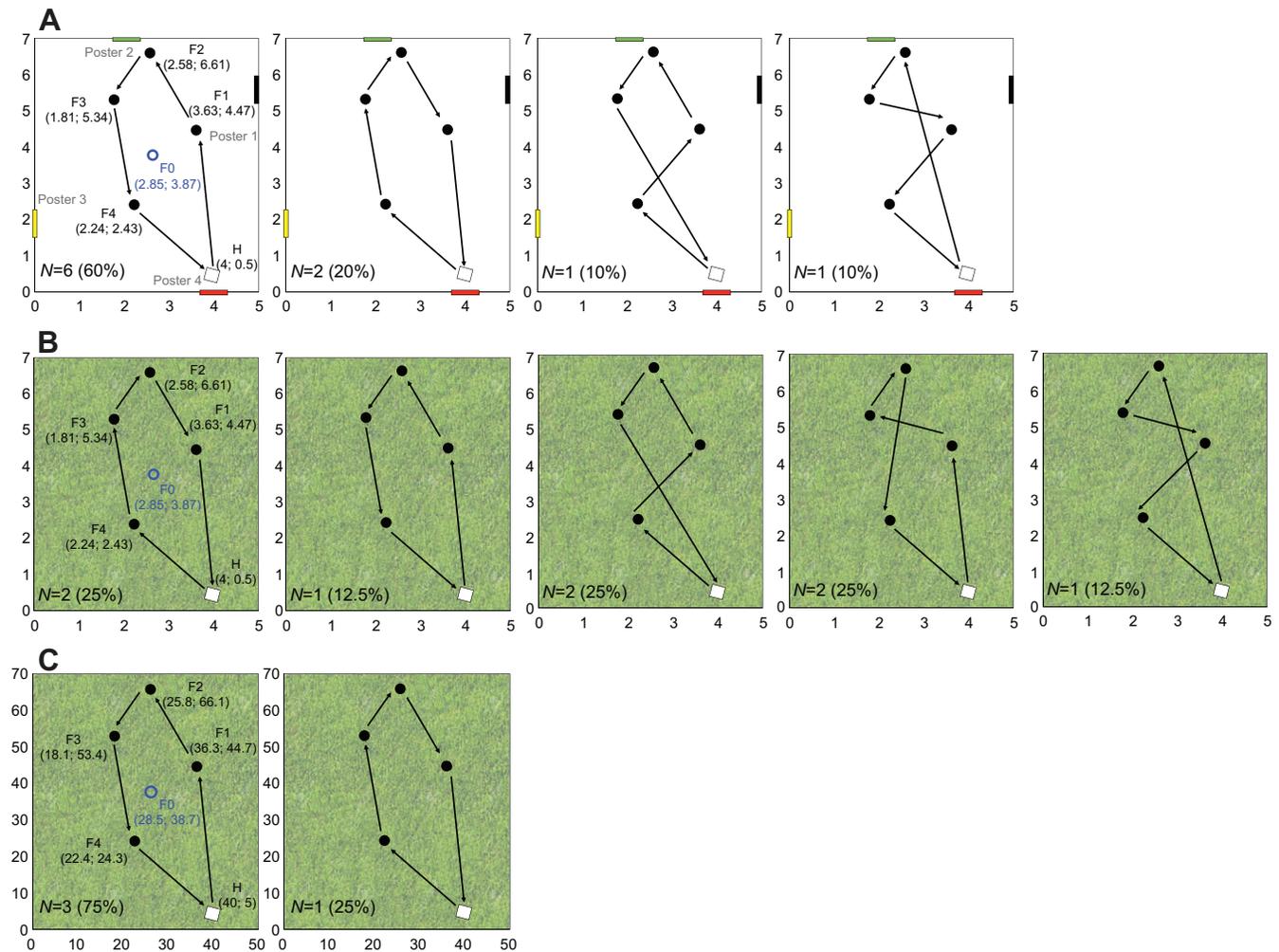


Fig. 1. Arrays of flowers and geometry of favourite sequences. Data are shown for (A) experiment 1, small array of flowers in the laboratory; (B) experiment 2, small array of flowers in the field, and (C) experiment 3, large array of flowers in the field. H is the hive, F0 the pre-training flower, F1–F4 the experimental flowers, and posters 1–4 the landmarks. Numbers in parentheses are Cartesian coordinates (m). Arrows indicate the direction in which the honeybee moved. *N* is the number of honeybees that have selected the sequence. A bee moving between nearest-neighbour flowers (F4–F1–F2–F3) would fly 11.6% longer than a bee using an optimal sequence (F1–F2–F3–F4 or F4–F3–F2–F1).

preventing dance communication (von Frisch, 1967). Because *A. mellifera* workers detect visual targets from a background subtending a visual angle of ~ 5 deg (Giurfa et al., 1996), we assume that honeybees could see our 50 cm flowers at a maximum distance of 5.7 m. Thus honeybees could detect all flowers from any flower location in the small array but not in the large array.

Procedure

Honeybees were pre-trained on a flower providing *ad libitum* sucrose solution (Fig. S3A) and paint-marked for individual identification (von Frisch, 1967). Once a honeybee made regular foraging bouts (foraging trip starting and ending at the hive), its nectar crop capacity was measured (range: 32–54 μ l, $N=22$). The honeybee was then tested for 30 successive bouts (ca. 6 h) with all experimental flowers in their final position (Fig. 1). At each foraging bout, each flower provided one-fourth of the honeybee's crop capacity. Sequences of flower visits were recorded, detailing the time of arrival to and departure from each flower. Between testing honeybees, flowers were cleaned with ethanol (70% w/w) to remove chemical cues that could influence the next foragers (Giurfa and Núñez, 1992). The same procedure was used in the small flower

array in the laboratory (experiment 1, $N=10$) and in the field (experiment 2, $N=8$), and in the large array in the field (experiment 3, $N=4$). The lower sample size used in experiment 3 reflects the increased difficulty of pre-training bees and keeping them motivated to forage on artificial flowers at greater distances from the hive.

Sequence analyses

Foraging performances were analysed using generalised linear mixed models (GLMM) on bins of 10 foraging bouts (random factor: bouts within individual) in SPSS. Route repeatability was examined using determinism (DET), a metric for detecting repeating sequences in traplining data (Ayers et al., 2015). DET varies between 0 (the honeybee never repeats the same sequence) and 1 (the honeybee always repeats the same sequence). For each honeybee, a DET was calculated for bins of 10 bouts on sequences of four-flower visits. Observed DET were compared with 1500 simulated DET of randomly generated sequences, either including or excluding revisits (the R code to generate random sequences is available on request from the corresponding author). For analyses of route frequency, four-flower sequences (excluding revisits) were used (Fig. S4). Observed frequencies were compared with random

using binomial test with a probability of 1/24 (Lihoreau et al., 2010). Sequences repeated at least four times over the 30 bouts were used more often than expected by chance (binomial test, $P < 0.05$).

RESULTS AND DISCUSSION

First we tested the influence of environmental cues on route learning by comparing the foraging sequences of honeybees in the small array of flowers in the laboratory and in the field (experiment 1 versus experiment 2). In both settings, honeybees improved foraging performance as they gained experience. Honeybees made shorter foraging bouts (Gaussian GLMM, bout: $F_{1,48} = 7.08$, $P = 0.011$; experiment: $F_{2,48} = 2.27$, $P = 0.114$; bout \times experiment: $F_{2,48} = 1.87$, $P = 0.165$), visited more flowers per bout (gamma GLMM, bout: $F_{2,48} = 13.11$, $P < 0.001$; experiment: $F_{1,48} = 0.63$, $P = 0.431$; bouts \times experiment: $F_{2,32} = 2.01$, $P = 0.145$) and decreased their frequency of immediate revisits to flowers (gamma GLMM, bout: $F_{2,48} = 42.59$, $P < 0.001$; experiment: $F_{1,48} = 9.31$, $P = 0.004$; bouts \times experiment: $F_{2,48} = 1.07$, $P = 0.351$; Fig. 2A) as the number of foraging bouts completed increased. Honeybees also used increasingly repeatable flower visitation sequences through time, reaching statistically indistinguishable, non-random DET in the last 10 bouts in the laboratory and in the field (Fig. 2B). All individuals used a favourite sequence (the most common four-flower visitation sequence excluding revisits) in $31.3 \pm 6.2\%$ (mean $\pm 95\%$ CI, $N = 18$ honeybees) of their foraging bouts. This sequence was increasingly used over time (Fig. 2C), and the majority of honeybees (61%) selected an optimal sequence (Fig. 1). Only four individuals (22%) had stabilised the sequence (used more than five times in a row) by the end of the 30 bouts (Fig. S4), indicating that route fidelity is imperfect when foraging in close feeding locations, a situation replicating within-patch foraging. The similarity of the foraging performances of honeybees in the laboratory and in the field indicates that natural visual cues (sun, polarised light) are not essential for the establishment of a route in these insects.

Next we tested the influence of spatial scale on this routing behaviour by comparing the foraging sequences of honeybees in the small and large arrays of flowers in the field (experiment 2 versus experiment 3). When tested at larger spatial scales, honeybees also considerably improved foraging performance with experience by reducing the duration of their foraging bouts (Gaussian GLMM, bout: $F_{2,30} = 15.51$, $P < 0.001$; experiment: $F_{1,30} = 1.4$, $P = 0.263$; experiment \times bout: $F_{2,20} = 0.12$, $P = 0.89$), visiting more flowers per bout (gamma GLMM, bout: $F_{2,30} = 17.09$, $P < 0.001$; experiment: $F_{1,30} = 2.25$, $P = 0.144$; experiment \times bout: $F_{2,30} = 6.13$, $P = 0.006$) and making less immediate revisits to flowers (gamma GLMM,

bout: $F_{2,30} = 13.92$, $P < 0.001$; experiment: $F_{1,30} = 2.53$, $P = 0.123$; experiment \times bout: $F_{2,30} = 0.24$, $P = 0.787$; Fig. 2A). However, route following was considerably more pronounced in the large array than in the small array. The DET was two times higher in the last 10 foraging bouts (Fig. 2B), honeybees used their favourite sequence twice as often (Fig. 2B) and each individual selected an optimal favourite sequence that they stabilised (Fig. 2D, Fig. S4). Therefore, honeybees show much higher levels of route fidelity between distant feeding locations, a situation replicating between-patch foraging.

Historically, research on honeybee navigation has focused on the ability of foragers to learn routes between a few important locations, such as the hive and a feeder (Collett et al., 2013; von Frisch, 1967). Using arrays of feeders, we show that honeybees can learn more complex foraging circuits integrating at least five different locations. Although we used relatively low sample sizes, all foragers tested behaved in a similar way, indicating that this routing behaviour is not specific to only one individual.

Route following by honeybees meets several key features of trapline foraging previously described in bumblebees and some other nectar-feeding insects, birds and mammals (Janzen, 1971; Lihoreau et al., 2010, 2012; Ohashi et al., 2007; Tello-Ramos et al., 2015): (1) honeybees used flower visitation sequences that became increasingly similar with training, ultimately stabilising into a single route (Lihoreau et al., 2012); (2) route establishment was accompanied by a reduction of revisits to empty flowers (Ohashi et al., 2007) and overall travel distances (Lihoreau et al., 2010); and (3) route optimisation was more pronounced at larger spatial scales (Lihoreau et al., 2012). Presumably, the energetic costs of flying long (suboptimal) routes in the large-scale array increased the investment of foragers in route learning. However, it is also possible that route learning is facilitated when foragers navigate between discrete locations further apart. Future experiments manipulating the travel cost of visiting all flowers while keeping the distance between neighbour flowers constant are needed to disentangle these two hypotheses.

The development and validation of an experimental approach for studying trapline foraging by honeybees holds considerable promise for exploring the full complexity of spatial cognition in bees and addressing the major unresolved question of how features of the environment are memorised in their miniature brains (Collett et al., 2013; Degen et al., 2015). Simulation models already provide some empirically testable predictions. For instance, it has been suggested that trapline development can emerge using a simple route-based guidance system (a suite of vector flights joining different locations) supported by path integration and visual memories of landmarks,

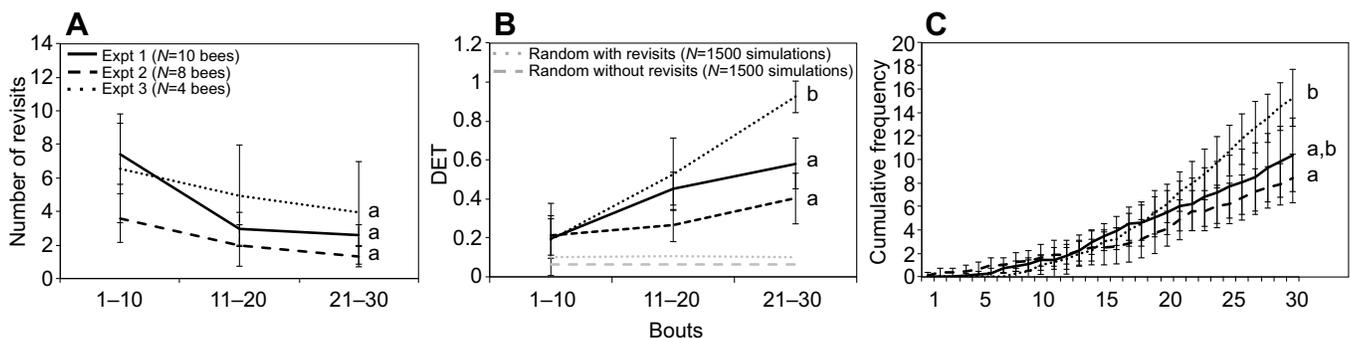


Fig. 2. Foraging performance. (A) Mean frequency of immediate revisits to flowers, (B) mean determinism (DET) value (sequence repeatability) and (C) mean cumulative frequency of favourite sequence (the most common four-flower visitation sequence, excluding revisits, used by each bee). Means are given with 95% CI. N is the number of replicates. Different lowercase letters next to curves indicate significant pairwise differences between experiments (two-tailed pairwise Wilcoxon tests with Bonferroni correction, $P < 0.017$) for the last bin of 10 bouts (A,B) or the last bout (C).

without the necessity of learning metric relationships between all main locations (Lihoreau et al., 2012; Reynolds et al., 2013). Learning of route segments and gradual rearrangement of their utilisation order allows for a dynamic optimisation of flight paths and finding of novel solutions in responses to environmental perturbations, such as the addition or removal of resources (Lihoreau et al., 2010).

Growing evidence shows that honeybees flexibly use private and social information in a context-dependent manner when foraging (Grüter and Leadbeater, 2014). Our study indicates that private information is sufficient to support complex spatial strategies in these insects. Trapline foraging may efficiently complement cooperative foraging to exploit resources in the vicinity of the hive (where dance recruitment does not occur) or in environments in which resources are less clumped or not large enough to sustain multiple foragers at once (early or late in the season). In contrast to dance communication (Dornhaus and Chittka, 1999), the ability to rely on individual memory to search and exploit foods is observed in a large diversity of bee species (Janzen, 1971; Lihoreau et al., 2010, 2012; Ohashi et al., 2007), including the most socially advanced, suggesting that trapline foraging is ancestrally shared in this insect group.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

M.L. designed the experiments. A.B. collected the data. A.B. and M.L. analysed the data. A.B. and M.L. wrote the manuscript.

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Supplementary information

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