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A comparative analysis of foraging route development by bumblebees and honey bees

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Abstract

Many pollinators, such as bees, hummingbirds and bats, use multi-destination routes (traplines) to exploit familiar plant resources. However, it is not clear to what extent the mechanisms underpinning trapline development and optimisation are comparable across species. Here we compared route formation, repeatability and efficiency by foragers of two social bee species, the solo foraging bumblebee *Bombus terrestris* and the mass foraging honey bee *Apis mellifera*, in the same laboratory conditions. In a simple routing task (with four artificial flowers), all bumblebees and honey bees developed a route, although honey bees were slower to do so. In a more complex routing task (with six flowers), however, only bumblebees developed a route between the 6 flowers. Honey bees took a longer time to discover all flowers and developed routes between fewer flowers. Comparing bumblebees and honey bees using the same experimental paradigm thus revealed key behavioural differences likely resulting from their contrasting collective foraging strategies.

Significance statement

Pollinators face some of the most complex foraging challenge among animals: collecting small amounts of nectar from large numbers of flowers in a minimum of time. Previous studies report bees, birds and bats rely on spatial memory to develop routes optimising foraging efficiency. Here we compared this routing behaviour in two major pollinators, the buff-tailed bumblebee and the Western honey bee, in the same experimental conditions. We found that bumblebees were much faster and more efficient at visiting flowers following repeatable sequences than honey bees. This behavioural difference suggests bee cognition is shaped by their social ecology and illuminates about the known complementarity of different bee species for pollination.

Keywords Honeybees · Bumble bees · Trapline foraging · Navigation · Comparative cognition

Introduction

Pollinators often used repeatable multi-destination routes (often called "traplines") to exploit patchily distributed resources from their nest or resting area (Thomson 1996).

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Trapline foraging is particularly widespread in nectar feeding insects (orchid bees: Janzen 1971; butterflies: Gilbert and Singer 1975; bumblebees: Thomson et al. 1997; honey bees: Buatois and Lihoreau 2016) but also in birds (hummingbirds: Tello-Ramos et al. 2015) and mammals (bats: Lemke 1984; opossums: Wooller et al. 1999). This behaviour is thought to optimize the exploitation of plant resources, by enabling foragers to adjust the timing of flower visits to nectar production, while deterring competitors through exploitation (Possingham 1989; Ohashi and Thomson 2005).

The formation of traplines has been studied in detail by observing bumblebees foraging in arrays of artificial flowers (i.e., feeders) (Ohashi and Thomson 2009; Lihoreau et al. 2013). Individual bumblebees given access to a small number of these flowers for several consecutive hours often find the shortest path to visit each flower once and return to their

nest, and reuse it over time (Ohashi et al. 2007; Lihoreau et al. 2010, 2012a, b; Woodgate et al. 2017). This behaviour has been modelled using an iterative improvement heuristic replicating trial-and-error learning by bees (Lihoreau et al. 2012b; Reynolds et al. 2013). In this approach, the simulated bee computes the net length of all the route segments (i.e. straight movements between two flowers or between a flower and the colony nest) from the route it has just experienced, and increases its probability to reuse these segments in future foraging bouts if this new route is shorter (or of the same length) than the shortest route experienced before.

Recently, honey bees were also reported to be capable of developing traplines between four artificial flowers (Buatois and Lihoreau 2016). The indirect comparison of the foraging patterns of bumblebees and honey bees, using data obtained in studies with different designs, suggests honey bees develop less stable routes than bumblebees (Pasquaretta et al. 2017). This may be due to major differences in the social ecology of these pollinators. Bumblebees, as solo foragers, primarily rely on individual information (i.e., learning and memory) to locate and exploit plant resources (Dornhaus and Chittka 1999). By contrast, honey bees, as mass foragers, use social information (i.e., the waggle dance) to collectively exploit large food resources located more than 100 m away from the nest (von Frisch 1967). Honey bees may therefore invest less in individual sampling and spatial learning than bumblebees, ultimately developing less efficient traplines (Buatois and Lihoreau 2016).

Clarifying these behavioural differences across bee species could thus illuminate the impact of social interactions on the evolution of animal cognition (Lihoreau et al. 2012c; Farris 2016; Traniello et al. 2019; Poissonnier et al. 2023). Such knowledge on two widespread pollinators used for crop pollination could also have considerable implications for improving agricultural practices in the context of a looming pollination crisis (Ghazoul 2005). For instance, a detailed understanding of bee spatial movements could help identify assemblages of pollinator species that could best increase crop yield based on their complementary behaviour. Critically, such comparative approach requires to study the animals in identical experimental setups and make quantitative comparisons of their individual performances (Chittka et al. 2012).

Here, we tested the hypothesis that honey bees have poorer abilities to develop efficient traplines than bumblebees because they rely less on individual learning when foraging. We compared the spatial foraging patterns of honey bees (*Apis mellifera*) and bumblebees (*Bombus terrestris*) in the same arrays of artificial flowers under controlled conditions in an indoor flight room. We analysed the quality and the repeatability of the routes used by each individual. Since the number of possible sequences to visit each flower once

and return to the nest increases factorially with the numbers of flowers to visit (Lihoreau et al. 2013), we explored the influence of task complexity by testing bees of both species in arrays of four flowers (24 possible flower visitation sequences) and six flowers (720 possible flower visitation sequences).

Materials and methods

Bees

We used a small honey bee colony (*A. mellifera*, Buckfast, a queen and ca. 2000 workers) raised at our experimental apiary (University Paul Sabatier – Toulouse III, France) and a regular bumblebee colony (*B. terrestris*, a queen and ca. 200 workers) obtained from a commercial supplier (Biobest, Belgium). The two colonies were maintained in hives equipped with a transparent entrance tube fitted with gates to control the traffic of foragers. Colonies were provided *ad libitum* defrosted honey bee collected pollen directly into the hives. Workers collected sucrose solution (40% w/w in water) from artificial flowers in the experimental flight room (see below). The good health status of the colonies (parasites, brood, food stores, mortality, etc.) was checked every week by our beekeeper.

Flight room

We conducted all the experiments in an indoor flight room (length: 7 m, width: 5 m, height: 3 m; Fig. S1A). The size of the room was sufficiently large for the bees to develop traplines (Lihoreau et al. 2010, 2011, 2012b) and small enough to preclude dance recruitment by honey bees (von Frisch 1967). Working indoor ensured bees of the two species were tested in the same conditions, thereby removing variation of potential confounding factors associated to field work between testing individuals. Room temperature was controlled and maintained at 25 °C for the whole experiment. Illumination was provided by 12 wide spectrum LED lights (6500 K, Phillips, The Netherlands) replicating natural sunlight and daily photocycle (15 h Light/ 9 h Dark). Four A0 posters, each characterized by a different bicolored pattern, were presented vertically on the room walls to provide 2D visual landmarks facilitating bee orientation (Fig. S2).

Artificial flowers

We built six identical artificial flowers with a vertical design (Fig. S1B, Buatois and Lihoreau 2016). Each flower consisted of a 6 cm diameter blue plastic landing platform sitting on a 10 cm high transparent plastic cylinder. The

cylinder was attached to a metallic clamp stand and hung 50 cm above ground. A yellow mark in the middle of the landing platform indicated the location of a controlled volume of sucrose solution (range: min 20 μl – max 140 μl depending on the nectar crop capacity of tested bee, see below) dispensed by the experimenter using an electronic micropipette (Brand Handystep 705,000, Germany).

Flower arrays

We tested bees in one of two different arrays of flowers presented horizontally on the ground surface of the experimental room (see arrays with flower coordinates in Fig. S1A). The two arrays provided routing tasks of increasing complexity. In the four-flowers array, there were 24 possible sequences to visit all flowers once starting and ending at the colony nest entrance (including the two optimal sequences minimising travel distance: F1-F2-F3-F4 or F4-F3-F2-F1; see Fig. 1 and S1A). In the six-flowers array, there were 720 possible flower visitation sequences (including the two optimal sequences minimising travel distance: F1-F2-F3-F4-F5-F6 or F6-F5-F4-F3-F2-F1; see Fig. 2 and S1B). We generated these flower arrays using a computer program (R code available in Text S1) designed to maximize the discrepancy between the two optimal flower visitation sequences minimizing travel distance to visit all flowers once (e.g. four-flowers array: F1-F2-F3-F4 or F4-F3-F2-F1) and the flower visitation sequence linking all unvisited nearest neighbour flowers (e.g. four-flowers array: F1-F4-F5-F2) given the dimensions of the flight room (Lihoreau et al. 2012a) (see details in Figs. 1 and 2). Since bee workers detect visual targets from a background subtending a minimum visual angle between ca. 3° (*B. terrestris;* Dyer et al. 2008) *and* 5° (*A. mellifera;* Giurfa et al. 1996), we assumed foragers were able to detect a 6 cm diameter flower at a maximum distance of 68.7 and 114 cm respectively (Buatois and Lihoreau 2016). We used flower arrays alternately (i.e. we tested one bee in the four-flowers array, and then another bee in the six-flowers array, and so on) to make sure we tested foragers from colonies at the same developmental stages in both arrays.

Experiments

We ran the experiments sequentially, first with honey bees and then with bumblebees. For each species, we began by pre-training the bees collectively on an artificial flower delivering *ad libitum* sucrose solution (i.e. 3.5 cm petri dish full of sucrose solution on top of blue landing platform). We then marked all the bees that landed on the flower with acrylic paint on the thorax using a colour code for individual identification. Once a bee made regular foraging bouts

(i.e. foraging trips starting and ending at the colony hive entrance), we measured its nectar crop capacity by estimating the average total amount of sucrose solution collected by that bee over three foraging bouts (average ± SE; bumblebees: 120 ± 20 μL, N = 20; honey bees: 42.3 ± 10 μL, N = 19) (Lihoreau et al. 2010).

We then tested the bee either for 30 consecutive foraging bouts in the four-flowers array or 50 consecutive foraging bouts in the six-flowers array. We observed the bees making different amounts of foraging bouts in the two arrays based on previous observations showing that bees require more time to develop routes between six flowers than between four flowers (Lihoreau et al. 2010, 2012a). Each flower provided either 1/4th (four-flowers array) or 1/6th (six-flowers array) of the bee's nectar crop capacity and was refilled by the experimenter with sucrose solution between each foraging bout. Between testing different bees, we cleaned the flowers with ethanol (70% w/w) to remove chemical cues that could influence the next foragers (Giurfa and Núñez 1992; Pearce et al. 2017). We tested 10 bumblebees and 10 honey bees in the four-flowers array, and another 10 bumblebees and 9 honey bees in the six-flowers array.

During the observations, the experimenter sat on the right of the colony hive to manually open the gates of the entrance tube and allow the focal bee to forage (Fig. S1). The experimenter did not move during the observations, thereby acting as a visual landmark for the bees. For each foraging bout of each bee, the experimenter recorded the time when the bee left the hive, each time it landed on a flower, took off, and the time when it returned to the nest, using the software Ethom (Shih and Mok 2000).

Data analyses

We analysed the data in R (R Core Team 2021) and ran model simulations using Python.

Behavioural data

A subset of the behavioural data (10 honey bees tested on four flowers) has been used in a previous study to test whether honey bees could develop routes between multiple artificial flowers (see Fig. 1A in Buatois and Lihoreau 2016). Here we re-analysed these data together with data of three new experiments ran at the same time (Spring 2015) in order to address the new question of whether bumblebees and honey bees show comparable efficiencies for trapline formation across different experimental conditions. All the analyses presented in this article are original.

From the raw data collected on Ethom (i.e. times of landing and taking off at each flower), we extracted sequences of flower visits. For each bee, we calculated the number of
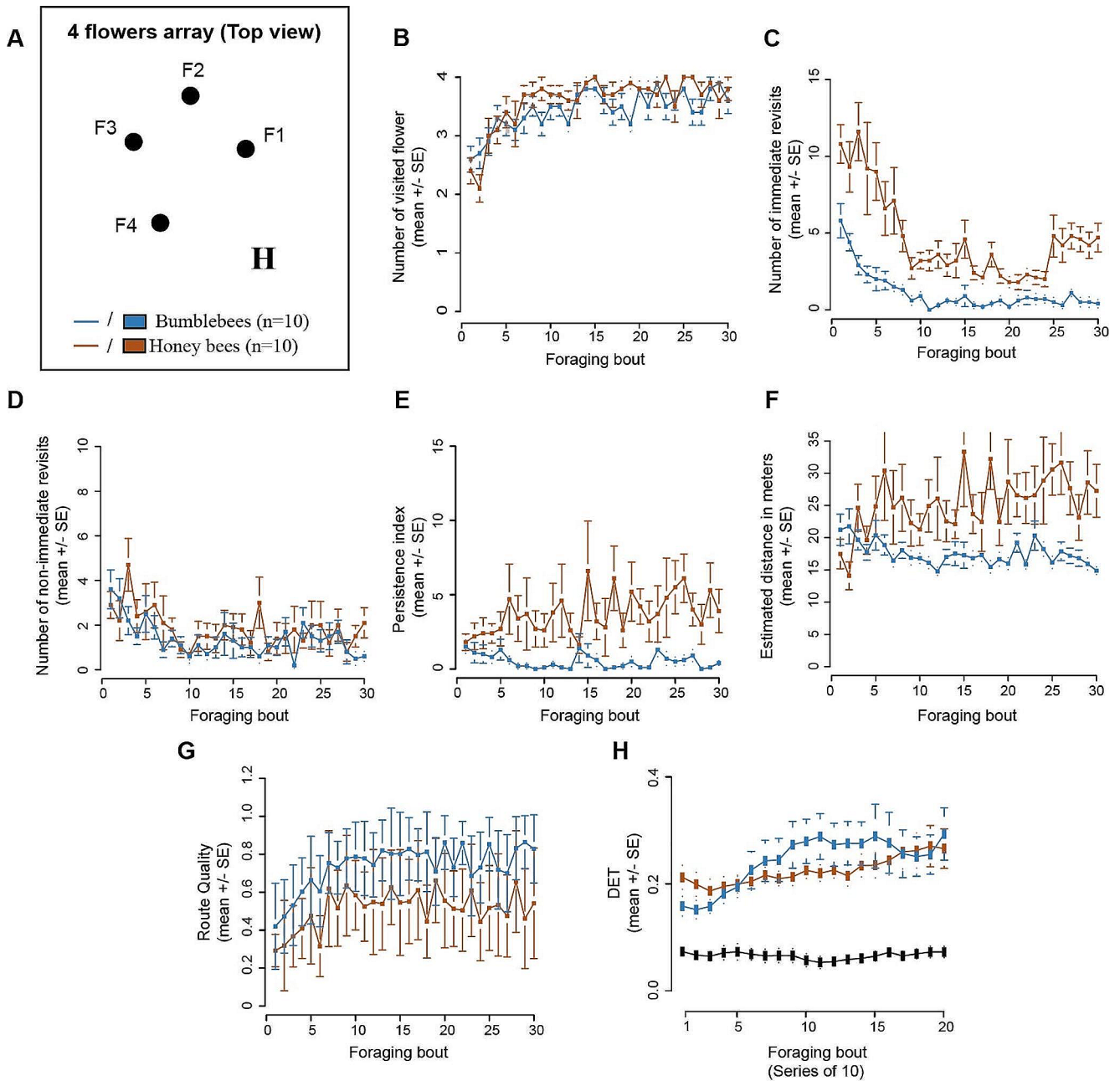
Fig. 1 Route formation in the four-flowers array. **(A)** Schematic representation of the distribution of flowers on the floor of the experimental room (see details in Fig. S1A). Flowers are labelled F1-F4. H is the colony hive location. The distance between neighbour flowers ranged from 1.48 to 4.19 m. The minimum travel distance to visit all flowers once was 13.41 m and the maximum distance was 20.27 m. **(B)** Number of flowers visited per foraging bout. **(C)** Number of immediate revisits to flowers per foraging bout. **(D)** Number of non-immediate different flowers visited per foraging bout, the number of immediate revisits to the same flowers per foraging bout (i.e., no other flower was visited in between revisits) and the number of non-immediate revisits to the same flowers per foraging bout (i.e., other flowers were visited in between revisits to flowers per foraging bout. **(E)** Persistence index **(F)** Travel distance per foraging bout. **(G)** Route quality per foraging bout. **(H)** DET per bins of 10 foraging bouts (i.e. DET for 1 was calculated from foraging bout 1 to 10, DET for 2 was calculated from foraging bout 2 to 11, etc.), calculated on a bases of four flowers' occurrence. The black line corresponds to the DET calculated from simulations of random foragers (n = 10)

revisits). We estimated a persistence index indicating the tendency for a bee to search for more flowers before returning to the colony nest. Persistence was computed using number of flower revisits (i.e. immediate and non-immediate revisits to empty flowers) made by a bee after the last
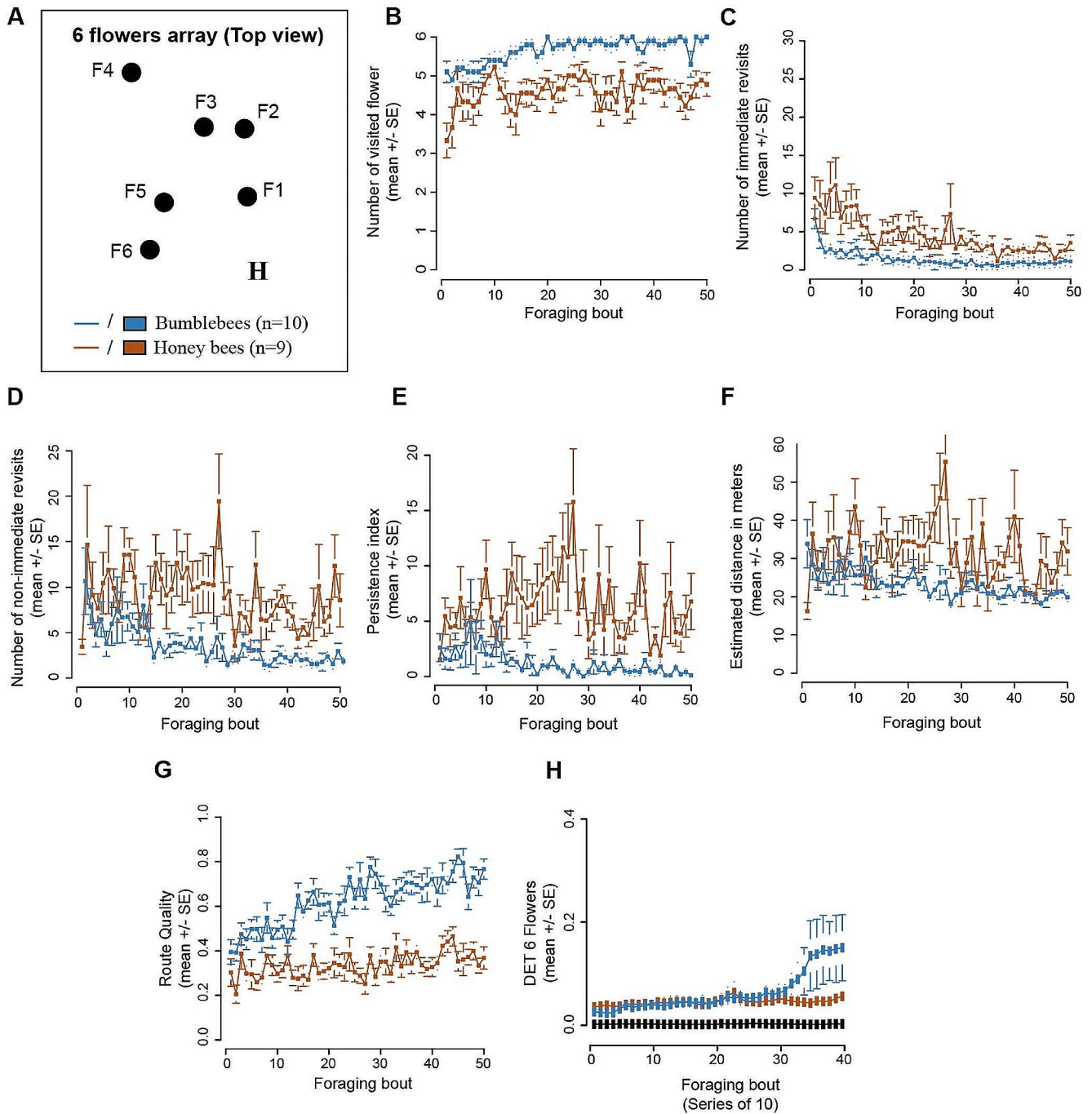
Fig. 2 Route formation in the six-flowers array. **(A)** Schematic representation of the distribution of flowers on the floor of the experimental room (see details in Fig. S1A). Flowers are labelled F1-F6. H is the colony hive location. The distance between neighbour flowers ranged from 1.13 to 4.22 m. The minimum travel distance to visit all flowers once was 14.76 m and the maximum distance was 26.51 m. **(B)** Number of flowers visited per foraging bouts. **(C)** Number of immediate revisits to flowers per foraging bout. **(D)** Number of non-immediate revisits to flowers per foraging bout. **(E)** Persistence index. **(F)** Travel distance per foraging bout. **(G)** Route quality per foraging bout. **(H)** DET per bins of 10 foraging bouts (i.e. DET for 1 was calculated from foraging bout 1 to 10, DET for 2 was calculated from foraging the bout 2 to 11, etc.), calculated on a bases of 6 flowers' occurrence. The black line corresponds to the DET calculated from simulations of random foragers (n = 10)

visit of that bee to a flower containing a sucrose reward during a foraging bout. The higher the persistence index, the more the bee searched for food and failed to do so before returning home. For instance, in the four-flowers array a bee using the flower visitation sequence F1-F2-F3 would have a persistence index of 0. By contrast, a bee using the flower visitation sequence F1-F2-F3-F2-F1-F2-F3-F1-F2 would have a persistence index of 6. We also estimated travel distance for each bee during each foraging bout based on the sequence of flowers visited (excluding immediate revisits). We could only estimate distances as straight lines between flowers, since we did not record actual flight trajectories between visiting flowers.

We characterised the routing behaviour of bees using two indices:

(1) Route quality:

$$Quality = \frac{\frac{F^2}{d}}{Quality_{opt}}$$

Where F represents the number of different flowers visited during a foraging bout, d the total distance travelled between the flowers (assuming straight movements between flowers, or between flowers and the hive). This index was divided by the quality of the optimal route minimising travel distance $Quality_{opt}$. Route quality thus varied between 0 (a route of very poor quality) and 1 (the optimal route).

(2) Route repeatability

We applied the determinism index (DET, see details in Ayers et al. 2015) on flower visitation sequences excluding immediate revisits. This index is set between 0 (no repeated patterns in a flower visitation sequence) and 1 (full traplining). We computed the DET for bins of 10 successive foraging bouts, using a minimum length of recurrence of 3 and 4 in the four flowers arrays and 3, 4, 5 and 6 in the six flowers arrays and without considering perpendicular diagonals.

We compared the experimental data to simulated data from a random model generated using Python 3.8.5 and the package Numpy. For each array condition (4 vs. 6 flowers), we simulated flower visitation sequences by 10 random foragers. The number of foraging bouts completed by each random forager matched the number of foraging bouts of completed by real bees (30 vs. 50 bouts). Each foraging bout started and ended at the colony nest. The flowers visited in each foraging bout were drawn successively, following a random uniform distribution (i.e., all flowers are equally likely to be drawn), until all flowers had been visited at least once. Immediate revisits to flowers were forbidden,

which means that the same flower could not be visited several times consecutively. The resulting dataset was used to compute the DET of random foragers.

We compared the behavioural performance (i.e., number of flowers visited, number of immediate revisits, number of non-immediate revisits, persistence index, travel distance) of bumblebees and honey bees using generalized linear mixed models (GLMMs) with foraging bouts as a fixed factor and bee identity as a random factor, using the lme4 package (Bates et al. 2015). Both behavioural indices (Route quality and DET) were compared using a generalized estimated equation (GEE) with foraging bouts as a fixed factor and bee identity as a random factor using the geepack package (Højsgaard et al. 2005). Whenever necessary, a post-hoc Tukey pairwise comparison was done to examine in detail the differences observed.

Results

Four-flowers array

We first compared the flower visitation sequences of bumblebees and honey bees in an array of four flowers (Fig. 1A). Foragers of both species similarly increased the number of different flowers visited per foraging bout as they gained experience with the array (Fig. 1B; Table 1). With time, bumblebees and honey bees decreased their number of revisits to flowers. Both species reduced non-immediate revisits at a similar rate (Fig. 1D; Table 1, Average number of revisits $\pm$ SE during last foraging bout: Honey bees: 2.1 ± 0.7, Bumblebees: 0.6 ± 0.2). However, honey bees made more immediate revisits than bumblebees (Fig. 1C; Table 1, Average number of revisits $\pm$ SE during last foraging bout: Honey bees: 4.7 ± 0.9, Bumblebees: 0.4 ± 0.3). Honey bees showed higher persistence to find new flowers before returning to the colony nest than bumblebees (Fig. 1E; Table 1, Average persistence index $\pm$ SE during last foraging bout: Honey bees: 3.9 ± 1.5, Bumblebees: 0.4 ± 0.2). As a direct consequence, honeybees increased their travel distance through time while bumblebees decreased it (Fig. 1F; Table 1, Average travel distance $\pm$ SE during last foraging bout: Honey bees: 27.3 ± 4.1 m, Bumblebees: 14.9 ± 0.6 m).

Although both species increased route quality with experience, bumblebees showed better overall route quality (Fig. 1G; Table 1, Average route quality $\pm$ SE during last foraging bout: Honey bees: 0.54 ± 0.1, Bumblebees: 0.82 ± 0.1). For both species, route repeatability (DET based on 4 flowers' occurrence) was significantly higher than chance, as assessed with model simulations (Fig. 1H; Table 1). Repeatability increased in a comparable manner for both species, but only reached a moderate average score

Table 1 Summary of the statistics for the four-flowers array. Significant effects are highlighted in bold. ($P < 0.05$). BB: bumblebee; HB: honey bee; Random: simulated bee

Figure	Type of model	Model description	Parameter estimated	Stat value	P value
Figure 1B	Poisson GLMM	Number of flowers ~ species + foraging bout + species*foraging bout	Species	Est = 0.017	0.85
			Foraging bout	**Est = 0.008**	**0.03**
			Species*foraging bout	Est = 0.002	0.75
Figure 1C	Poisson GLMM	Number of immediate revisits ~ species + foraging bout + species*foraging bout	**Species**	**Est = 1.26**	**< 0.0001**
			Foraging bout	**Est=-0.1**	**< 0.0001**
			Species*foraging bout	**Est = 0.05**	**< 0.0001**
Figure 1D	Poisson GLMM	Number of non-immediate revisits ~ species + foraging bout + species*foraging bout	Species	Est = 0.15	0.21
			Foraging bout	**Est=-0.03**	**< 0.0001**
			Species*foraging bout	Est = 0.01	0.14
Figure 1E	Poisson GLMM	Persistence index ~ species + foraging bout + species*foraging bout	**Species**	**Est = 1.17**	**< 0.0001**
			Foraging bout	**Est=-0.03**	**< 0.0001**
			Species*foraging bout	**Est = 0.05**	**< 0.0001**
Figure 1F	Poisson GLMM	Travel distance ~ species + foraging bout + species*foraging bout	Species	Est = 0.10	0.23
			Foraging bout	**Est=-0.01**	**< 0.001**
			Species*foraging bout	**Est = 0.02**	**< 0.0001**
Figure 1G	GEE	Route quality ~ species + foraging bout + species*foraging bout	**Species**	**$X^2 = 74.003$**	**< 0.0001**
			Foraging bout	**$X^2 = 9.77$**	**< 0.01**
			Species*foraging bout	**$X^2 = 20.5$**	**< 0.0001**
Figure 1H	GEE	DET ~ species + foraging bout + species*foraging bout	**Species**	**$X^2 = 176.5$**	**< 0.0001**
			Foraging bout	**$X^2 = 29.9$**	**< 0.0001**
			Species*foraging bout	**$X^2 = 8.4$**	**0.01**
Figure 1H	Tukey multiple comparison	DET ~ species + foraging bout + species*foraging bout	**BB vs. HB**	**Est = 1.52**	**< 0.0001**
			BB vs. Random	**Est = 1.52**	**< 0.0001**
			HB vs. Random	Est = 0.1	0.22

of 0.26 ± 0.04 for honey bees and 0.29 ± 0.05 for bumblebees during the 10 last foraging bouts (Fig. 1H; Table 1).

Therefore, in the four-flowers array, all bumblebees and honeybees improved their foraging efficiency and used repeated flower visitation sequences with experience. However, honey bees were slower to reduce their visits to empty flowers, thereby flying longer distances and reaching lower route quality than bumblebees.

Six-flowers array

To test whether the complexity of the spatial problem had an influence on the routing behaviour of bees, we compared the flower visitation sequences of bumblebees and honey bees in an array of six flowers (Fig. 2A). Here again, foragers of both species increased the number of different flowers visited per foraging bout as they gained experience with the array (Fig. 2B; Table 2). However, honey bees visited less flowers per foraging bout than bumblebees (Fig. 2B; Table 2, Average number of flowers visited $\pm$ SE during last foraging bout: Honey bees: 4.8 ± 0.3, Bumblebees: 6 ± 0). Bumblebees and honey bees similarly decreased their number of immediate revisits to flowers (Fig. 2C; Table 2, Average number of revisits $\pm$ SE during last foraging bout: Honey bees: 3.6 ± 1, Bumblebees: 1.1 ± 0.7). Honey bees made more non-immediate revisits than bumblebees and this number did not decrease over time (Fig. 2D; Table 2, Average number of revisits $\pm$ SE during last foraging bout:

Honey bees: 8.5 ± 2.9, Bumblebees: 1.5 ± 0.3). In addition, honeybees showed higher persistence to find new flowers than bumblebees (Fig. 2E; Table 2, Average persistence index $\pm$ SE during last foraging bout: Honey bees: 10.2 ± 3.9, Bumblebees: 0.4 ± 0.3). Consequently, honeybees flew longer distances than bumblebees (Fig. 2F; Table 2, Average estimated flight distance $\pm$ SE during last foraging bout: Honey bees: 31.8 ± 6.2 m, Bumblebees: 19.9 ± 1.2 m).

Although foragers of both species increased their route quality through time, honey bees showed lower overall quality than bumblebees towards the end of the experiment (Fig. 2G. Table 2, Average route quality $\pm$ SE during last foraging bout: Honey bees: 0.32 ± 0.04, Bumblebees: 0.72 ± 0.03). Route repeatability (DET based on 6 flowers' occurrence) was for both species significantly different from random (Fig. 2H; Table 2) and increased through time (Fig. 2H; Table 2). DETs of both species were not significantly different when all foraging bout was considered (Fig. 2H; Table 2). However, considering only the end of the last foraging bouts (i.e., last 5 bins of 10 foraging bouts), the DET was significantly higher for bumblebees than for honey bees (Fig. 2H; Table 2, Average DET $\pm$ SE during last bin of 10 foraging bouts: Honey bees: 0.04 ± 0.01, Bumblebees: 0.13 ± 0.06). Interestingly, route repeatability based on DET computed with fewer flowers' occurrence showed that performance was similar between both species (Fig. S4, Table S1),

Table 2 Summary of the statistics for the six-flowers array. Significant effects are highlighted in bold ($P < 0.05$). BB: bumblebees; HB: honeybees; Random: simulated bees

Figure	Type of model	Model description	Parameter estimated	Stat value	P value
Figure 2B	Poisson GLMM	Number of flowers ~ species + foraging bout + species*foraging bout	**Species** **Foraging bout** Species*foraging bout	**Est=-0.18** **Est = 0.003** Est=-0.001	**0.003** **0.03** 0.6
Figure 2C	Poisson GLMM	Number of immediate revisits ~ species + foraging bout + species*foraging bout	**Species** **Foraging bout** Species*foraging bout	**Est = 1.02** **Est=-0.04** Est = 0.006	**< 0.0001** **< 0.0001** 0.06
Figure 2D	Poisson GLMM	Number of non-immediate revisits ~ species + foraging bout + species*foraging bout	**Species** **Foraging bout** **Species*foraging bout**	**Est = 0.35** **Est=-0.04** **Est = 0.03**	**< 0.0001** **< 0.0001** **< 0.0001**
Figure 2E	Poisson GLMM	Persistence index ~ species + foraging bout + species*foraging bout	**Species** **Foraging bout** **Species*foraging bout**	**Est=-0.67** **Est=-0.05** **Est = 0.04**	**< 0.0001** **< 0.0001** **< 0.0001**
Figure 2F	Poisson GLMM	Travel distance ~ species + foraging bout + species*foraging bout	**Species** **Foraging bout** **Species*foraging bout**	**Est = 0.14** **Est=-0.01** **Est = 0.006**	**< 0.0001** **< 0.0001** **< 0.0001**
Figure 2G	GEE	Route quality ~ species + foraging bout + species*foraging bout	**Species** **Foraging bout** **Species*foraging bout**	**X^2 = 154.8** **X^2 = 35.6** **X^2 = 18.43**	**< 0.0001** **< 0.0001** **< 0.0001**
Figure 2H	GEE	DET ~ species + foraging bout + species*foraging bout	**Species** **Foraging bout** **Species*foraging bout**	**X^2 = 186.9** **X^2 = 7.35** **X^2 = 6.9**	**< 0.0001** **< 0.01** **0.03**
Figure 2H	GEE + Tukey multiple comparison	DET ~ species + foraging bout + species*foraging bout	BB vs. HB **BB vs. Random** **HB vs. Random**	Est = 0.17 **Est = 3.86** **Est = 3.69**	0.7 **< 0.0001** **< 0.0001**
Figure 2H (Last 5 bins)	GEE + Tukey multiple comparison	DET ~ species + foraging bout + species*foraging bout	**BB vs. HB** **BB vs. Random** **HB vs. Random**	**Est = 1.5** **Est = 6.2** **Est = 4.7**	**0.02** **< 0.001** **< 0.001**

Thus, overall, bumblebees reached a higher foraging efficiency than honey bees on a more complex array of flowers. Interestingly, this is explained by the fact that honeybees rarely found all flowers during a foraging bout, resulting in alternative strategies based on the use of multiple short route sequences between fewer flowers before returning to the nest (Fig. S3). The fact that honey bees were persistent in searching for flowers after having performed these short routes indicates that their performance was primarily limited by a difficulty to relocate all flowers.

Discussion

Previous studies on trapline foraging have been designed to test whether animals can develop optimal routes to visit multiple feeding sites (see reviews by Ohashi and Thomson 2009; Lihoreau et al. 2013). Comparing trapline formation across individuals and species is a gateway to understand the mechanisms of route development and the evolution of spatial cognition (Klein et al. 2017). Here we compared the routing behaviour of two naturally co-occurring generalist pollinators with similar foraging ranges (Beekman and Ratnieks 2000; Osborne et al. 2008), in the same arrays of artificial flowers. While both species developed comparable routes between four flowers, only bumblebees developed routes between six flowers. In these conditions, honey bees used shorter routes between less flowers and made many revisits to empty flowers before returning to their nest, indicating that they were searching for more flowers to visit. This suggests honey bees had difficulties to relocate flowers between foraging bouts. Our results are therefore consistent with the conclusion that honey bees have poorest abilities to develop traplines than bumblebees, drawn from indirect comparisons of semi field data collected in different experimental designs (Pasquaretta et al. 2017).

Why did honey bees and bumblebees not behave the same? Despite these social pollinators occupy very similar ecological niches, they differ in social organization. Honey bees use the waggle dance to recruit foragers to remote feeding sites (von Frisch 1967) and may thus invest less in individual sampling and learning than bumblebees that do not exhibit such dance (Dornhaus and Chittka 1999). The sizes of colonies are also dramatically different in the two pollinators. While a honey bee colony can contain thousands of workers (Seeley 2009), a bumblebee colony only contains hundreds (Goulson 2010). The foraging workforce of honey bees is therefore greater, resulting in a higher

potential for distributed exploration and discovery of new resources that can support efficient collective exploitation of large resources (Seeley 2010). By contrast, the reduced potential for exploration in bumblebee colonies may favour higher investment in individual learning for monopolisation of smaller resources. Several studies indicate that individual honey bee foragers are less accurate than bumblebee foragers in many aspects of foraging, for instance when selecting pollen of different qualities (Leonhardt and Blüthgen 2012) or searching for a target in an arena (Morawetz and Spaethe 2012). The reduced ability of honey bees to discover all the artificial flowers or to reduce their tendency for immediate revisits to empty flowers in our experiments is consistent with these observations.

These behavioural differences may also result from differences in the bio-energetics of bees. Honey bees and bumblebees have very different body sizes and nectar crop capacities (ca. 60 µL for a (Nunez 1966), ca. 100–200 µL for a bumblebee (Lihoreau et al. 2010, 2012a, b). Additionally, bumblebees often return home with a full crop (Lihoreau et al. 2010), whereas honey bees tend to abandon non-depleted food sources with a partially filled crop (Schmid-Hempel et al. 1985). This behaviour of honey bees does not maximize the net rate of energy extraction from the food sources but instead appears to maximize energetic efficiency (net energetic gain/unit energy expenditure) (Schmid-Hempel et al. 1985). However, this is unlikely to explain the fact that honey bees developed routes between less than six flowers in our experiments since the bees were consistently observed making many revisits to empty flowers before returning to the nest, suggesting that they were searching for additional food. This behavioural difference is also unlikely due to differences in energy costs of foraging since bumblebees and honey bees flew similar amounts of times and their flight metabolic rates are comparable (around 300 $Cal.gm^{-1}.h^{-1}$) (Kammer and Heinrich 1974; Heinrich 1975a,b, Schaffer et al. 1979, Harrison and Fewell 2002, Darveau et al. 2014). Instead, it is again possible that the difference regarding the crop filling decision is triggered by differences in social organisation. In the small bumblebee colonies, where each forager plays a crucial role for colony provisioning, complete filling of nectar crops by each forager ensures a maximal food collection for the colony. By contrast, in the large honey bee colonies where efficient communication and cooperation are essential, the foraging efficiency of each individual can be much variable without compromising colony efficiency (Tenczar et al. 2014; Klein et al. 2019). Such behavioural variability in large insect colonies can even be beneficial to face changes in environmental conditions (Dyer et al. 2014; Finke et al. 2023).

It is also possible that the apparent difficulty of honey bees to develop long traplines between six flowers is caused

by a lower visual acuity than that of bumblebees, limiting their ability for flower relocation. Morphological differences between honey bees and bumblebees involve considerable variations in the size of their sensory organs that may limit the routing capacities of honey bees. For instance, the bumblebee eye surface is about two times larger (Streinzer et al. 2013; Streinzer and Spaethe 2014) and its resolution 25% higher than that of the honey bee eye (Macuda et al. 2001). Consequently, bumblebees are more likely to notice small objects at a long distance than honey bees. This difference in visual acuity may explain why bumblebees were more efficient at finding all flowers in our experiments.

How generalisable are our observations? We focused on trapline development by bees at small spatial scales, within a flower patch in an outdoor flight room, in order to provide identical experimental conditions for foragers of the two species. Although this might not totally reflect their naturalistic foraging behaviours (bees navigate using sun compass (Cheeseman et al. 2014) and can fly up to several kilometers (Goulson and Stout 2001; Pahl et al. 2011), direct comparisons between indoor and outdoor foraging patterns at the same spatial scales failed so far to show differences in trapline development and foraging performances indoor and outdoor (Buatois and Lihoreau 2016). Our results are thus particularly relevant for crop pollination in greenhouses for which bumblebees (and to a lesser extent honey bees) are commonly used (Ahmad et al. 2015). Since previous studies also reported route optimization is faster and bees are more faithful to these routes at larger spatial scales, when feeding sites are visually isolated from each other (Lihoreau et al. 2012b; Buatois and Lihoreau 2016; Woodgate et al. 2017), future experiments should explore whether the differences we observed between bumblebees and honey bees are maintained in a similar array of flowers at larger spatial scales, within a few kilometers from the hive. In these conditions, the higher energetic costs associated with revisits to empty flowers or flying long suboptimal routes may lead honey bees to invest more in route optimization between distant feeding sites. The long distances between flowers may also facilitate spatial learning since the visual scenes associated to each flower are likely more different than in a flight room. Another critical point to test in the future is the level of intra-specific differences in the foraging behaviour of bumblebees and honey bees, for instance using more colonies of each species. This approach will be essential for more quantitative comparisons of routing behaviour across pollinators. Although we only used one colony of each species, both colonies were maintained in the same conditions and in good health throughout the experiment. The fact that our results are coherent with previous indirect comparisons between bumblebee and honey bees based on different colonies (Pasquaretta et al. 2017) strongly suggests

intra-specific variance in trapline formation is lower than the inter-specific variance we report here.

Ultimately, a more systematic analysis and comparisons of the spatial behaviours of key pollinators across spatial scales is needed to clarify their impact on plant reproduction through predictive patterns pollen transfer (Ohashi and Thomson 2009) and their potential complementarity or redundancy for crop yield (Garibaldi et al. 2016). We believe that such empirical knowledge would considerably benefit the development of predictive models of bee behaviour (Becher et al. 2014; Becher et al. 2016 Le Moël et al. 2019; Dubois et al. 2021) that could be used for conservation and agriculture in a context of a looming pollination crisis (Ghazoul 2005).

Declarations

Conflict of interest The authors declare no conflict of interest.

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