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# Analysing plant-pollinator interactions with spatial

# 2 movement networks

- 3
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## 19 Abstract

20	1) Pollinators, such as bees, face the complex challenge of efficiently exploiting patchily distributed
21	floral resources across large landscapes.

- 22
- 23 2) Here we consider the utility of spatial network statistics for analysing the foraging patterns of bees
- 24 moving between feeding sites at various spatial and temporal scales.
- 25

3) First we explain how spatial movement networks can be derived theoretically and experimentally todescribe bee foraging decisions.

- 28
- 4) We then illustrate this approach by analysing six datasets of bumblebees and honeybees foraging
- 30 in arrays of artificial flowers, and showing how some specific network metrics vary predictably as
- 31 foragers gain experience with the spatial distribution of feeding sites.
- 32
- 5) We compare network analyses to more conventional statistics used to characterise bee foraging
- 34 movements and discuss the implications of this novel statistical and modelling approach for pollination35 ecology research.
- 36
- Keywords: bumblebees; foraging; honeybees; movement ecology; pollination; route optimization;
   spatial networks.
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## 44 Introduction

45 Bees play a key role in the reproduction of wild and cultured plants. Over recent years, their 46 widespread declines have raised considerable concern for food security and the sustainability of our ecosystems (Goulson et al., 2015; Klein et al., 2017). Central to understanding the impact of pollinator 47 48 loss on plant reproduction is the foraging behaviour of bees (Thomson, 1986; Waser, 1986). Most bees are central-place foragers, meaning that they collect food (nectar and pollen) to provision their 49 50 brood in a single nest (Michener, 2000). By exploiting plants and developing foraging routes to visit 51 them, individual bees may bias pollen flow and fashion the genetic structure of plant populations, 52 therefore calling for more research of bee spatial strategies at the individual and collective levels 53 (Ohashi & Thomson, 2009; Burkle & Alarcón, 2011; Mayer et al., 2011). 54 Historically, bees were assumed to use simple movement rules that would yield maximal 55 energy gains to exploit patchily distributed resources, such as moving between nearest unvisited flowers (Ohashi et al., 2007), making short trips after encountering highly rewarding flowers (Chittka et 56 al., 1997) or keeping constant heading directions between visiting flowers (Pyke & Cartar, 1992). 57 58 While these rules of thumb may hold true when bees forage at small spatial scales (within an 59 inflorescence or a flower patch) mounting evidence shows that this is not the case at larger spatial 60 scales, when bees move between distant locations (flower patches or plants). In these conditions, foragers of many bee species tend to develop stable foraging routes (sometimes called traplines in 61 analogy to the fixed circuits that trappers follow when examining a number of traps distributed widely 62 63 in space) that they follow for several hours or days (e.g. Euglossine bees: Janzen, 1971; bumblebees: Heinrich 1976; Thomson et al., 1997; honeybees: Buatois & Lihoreau, 2016). This routing behaviour is 64 65 based on the acquisition of spatial memories encoding the location of the different food resources, the 66 colony nest site and other prominent environmental features (e.g. visual landmarks) (Collett et al., 67 2013). With training, bumblebees and honeybees can learn to find the shortest path to visit a few 68 artificial flowers (equivalent to natural flower patches) once and return to the nest (Bombus impatiens: Ohashi et al., 2007; Bombus terrestris: Lihoreau et al., 2012a; Apis mellifera: Buatois & Lihoreau, 69 70 2016), an optimisation behaviour analogous to solving the Travelling Salesman Problem in graph theory (Cook, 2012). This mathematical problem is notoriously difficult (if not impossible) to solve for 71 72 large graphs, because the number of possible paths increases factorially with the number of nodes in 73 the graph (e.g. 6 paths for 3 nodes, > 3 million paths for 10 nodes), and finding efficient solutions often 74 requires complex algorithms and systematic approaches (Polyakovskiy et al., 2014; Dorigo & 75 Gambardella, 2016). 76 While there is evidence that bees exhibit routing behaviour in nature (Heinrich 1976; Janzen

- 1971), to what extent these observations in simplified experimental conditions can be extrapolated to
  the field, where individuals may interact to exploit numerous highly variable resources scattered
  across large landscapes, is an open question.
- Field data on such multi-destination routes among flower patches are even more complex and
  challenging to analyse, and conventional behavioural metrics do not suffice to capture detailed
  information about routing behaviour (Thomson *et al.*, 1997; Makino & Sakai 2004; Makino & Sakai
  2005; Makino 2013; Lihoreau *et al.*, 2016). We argue that network statistics derived from graph theory
  - 3

84 hold considerable promise to characterise these complex movement patterns at the individual and 85 collective levels, and to identify the decision rules underpinning spatial strategies. In developing routes between flowers, foragers form movement networks embedded in space (Barthélemy, 2011), where 86 87 'nodes' are feeding locations (flower patches or plants) and 'edges' are flight paths between them (see 88 examples in Figures 1A-E) (Thomson et al., 1997; Lihoreau et al., 2016). These spatial movement 89 networks are directed, meaning that individuals move from one particular location to another 90 (movement vectors). Networks are also weighted so that the thickness of edges is proportional to the 91 frequency of movements between nodes. Because most bee species are central-place foragers, their 92 spatial movement networks also include the nest site, a specific node at which every flower visitation 93 sequence starts and ends. Therefore, in principle, an optimal movement network for a bee connects all 94 flowers and the nest using the shortest possible path (optimal network in Figure 1E). Discrete temporal 95 network analysis can then be performed depending on the time intervals with which a visitation matrix 96 is built. For instance, matrices may be developed by considering flower visits made in a single foraging 97 bout (dynamic network) or by cumulating the flower visits of several foraging bouts (static network). A 98 major advantage of network statistics is that they allow for analyses of very large spatial datasets and 99 the derivation of new empirically testable hypotheses (e.g. Perna & Latty, 2014; Jacoby & Freeman, 2016). Several analytical packages (e.g. igraph, sna, tnet packages in R, graph-tool in Python, 100 101 UCINET) and both local metrics (e.g. measures describing the level of importance of a node in a 102 network) and global metrics (e.g. measures describing the general level of connectivity of the entire 103 network) can be readily calculated to characterize space use by pollinators from an individual-based 104 point of view to measure, compare and predict their behaviour across different temporal scales. 105 In a recent field survey, Dupont et al. (2014) applied an individual-based plant-pollinator 106 network analysis to flower visitation data of different bumblebee species. The study showed significant 107 modularity in space use by bees based on plant characteristics, so that foragers tended to visit 108 patches of aggregated plants with numerous flowers and use taller plants to move from one module to 109 another (Dupont et al., 2014). Although the analytical approach developed in this study is very 110 appealing, field surveys only provide partial information about the foraging experience of individual 111 bees, the location of their nest relative to different plant patches and the temporal dynamics of their 112 foraging patterns. All these parameters are critical in determining bee foraging behaviour (Chittka & 113 Thomson, 2001). Experimental advances on model bee species, such as bumblebees and 114 honeybees, using artificial flowers delivering controlled rates of food resources combined with automated movement tracking, now allow for collecting high resolution spatial and temporal data on 115 116 bee foraging patterns in complex, yet controlled, environments (e.g. motion detection cameras on 117 flowers: Lihoreau et al., 2016; Radio Frequency Identification (RFID): Ohashi et al., 2010; harmonic radars: Lihoreau et al., 2012b; QR tags: Crall et al. 2015; 3D video tracking: Ings & Chittka, 2008). 118 119 Extensive recordings of individual based data using these semi-field approaches provide an 120 interesting opportunity to start examining the cognitive processes underpinning the foraging patterns 121 of bees and how they change across time in ecologically relevant conditions. 122 Here we describe how spatial network statistics can be used to analyse the foraging patterns of

123 bees both at local and global levels. We illustrate the potential of this approach for comparative analyses

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- 124 by statistically comparing spatial optimisation in the movement patterns of bees of the same species or,
- 125 of different species across environments varying in spatial scales, number of flowers and flower
- 126 configurations using standard network metrics. We used published movement datasets of bumblebees
- 127 and honeybees of known age, foraging experience and colony origin, foraging in arrays of artificial
- 128 flowers in the lab and in the field. To validate the approach, we compared our results with analyses of
- 129 more conventional behavioural metrics used in previous studies, such as the number of re-visits to
- 130 flowers and overall travel efficiency (distance/number of flowers visited).
- 131

## 132 Materials and methods

#### 133 Experimental data

- 134 We analysed six datasets of bee flower visitation sequences. Three datasets were obtained on the
- 135 bumblebee Bombus terrestris (experiment 1: Lihoreau et al., 2012a; experiment 2: Lihoreau et al.,
- 136 2011; experiment 3: Lihoreau *et al.*, 2012b). The three other datasets were obtained on the honeybee
- 137 Apis mellifera (experiments 4-6: Buatois & Lihoreau, 2016).
- All the datasets were generated following the same general methodology and are thus
- 139 comparable. In all experiments bees were individually marked (coloured number tags or paint dots on
- 140 the thorax) and maintained in colony nest boxes (bumblebees) or hives (honeybees) equipped with a
- 141 transparent, colourless, entrance tube. The tube was fitted with a series of shutters to control all
- 142 departure and arrival of foragers at the colony. Workers collected sucrose solution (40% w/w) on
- 143 artificial flowers outside the colony. Flowers consisted of a blue plastic landing platform (diameter =
- 144 60mm) with a yellow feeding spot in the middle. Bees were initially pre-trained on a flower from which
- 145 they could collect *ad libitum* sucrose solution. Each individual was tested alone. A regular forager that
- 146 made at least five foraging bouts (foraging trips starting and ending at the nest colony box) in one hour
- 147 was selected. The crop capacity of this forager was estimated by averaging the total volume of
- sucrose solution collected from a training flower over another three foraging bouts. The forager was
- then tested with all test flowers placed in a specific spatial arrangement (see experimental arrays in
- Figure 2). During the test, each flower provided the same amount of sucrose solution, chosen so that the bee had to visit all flowers to fill its nectar crop to capacity before returning to the colony nest box
- 152 (e.g. 1/5<sup>th</sup> of the crop capacity available in each flower in an array of five flowers). Flowers were refilled
- 153 by the experimenter at the end of each foraging bout, meaning that any revisit to a flower within the
- same foraging bout was not rewarding. Bees were tested for 22 to 80 consecutive foraging bouts in
- 155 the same array of flowers. All flower visits (when a bee landed on a flower) were recorded and used to
- 156 reconstruct the complete foraging history of each bee.
- 157 Experiments were conducted in six different arrays, varying in their spatial scale, their number
- of flowers and the spatial configuration of flowers. Experiments 1, 2 and 4 (Figure 2A, B, D) were
- 159 completed in flight rooms at small spatial scales and with controlled illumination (Lihoreau *et al.*, 2011;
- Lihoreau *et al.*, 2012a; Buatois & Lihoreau, 2016). Experiments 3, 5 and 6 (Figure 2C, D, E) were
- 161 completed in outdoor open fields at small spatial scale for experiment 5 and large spatial scales for
- experiments 3 and 6 (Lihoreau *et al.*, 2012b; Buatois & Lihoreau, 2016). Details about the spatial

arrangement of flowers, the number of bees tested and the numbers of foraging bouts per bee are

164 given in Figure 2.

165

#### 166 Network analyses

We built spatial networks of bee foraging movements in which flowers were nodes and movements were edges (Figure 1). Edges weight corresponded to the frequency of movement between flowers. To describe foraging movements and compare them across experimental conditions and species, we calculated three local network metrics describing the role of each flower in the bee movement network and one global network metric to infer on the efficiency of the network structure.

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#### 173 Local network measures

174 At a local level, we calculated the "weighted clustering coefficient", which assesses the degree to

which nodes tend to cluster together (Barrat *et al.*, 2004). Here a high clustering value indicates that

neighbouring flowers of a given flower are themselves highly connected, i.e. frequently re-visited(Figure 1B).

We used the "Kleinberg's authority score" (Kleinberg, 1999) to measure the relative 178 importance of a node in a network (Figure 1C). Given A, an individual movement matrix across 179 180 flowers, the Kleinberg's authority score is defined as the principal eigenvector of the inverted matrix 181 t(A)\*A. This metric assigns large values to flowers that are most often used, while accounting for the 182 number of visits to adjacent flowers (i.e. flowers connected by at least one edge). Therefore a high Kleinberg's authority score indicates that a specific flower is more often visited than all its neighbour 183 184 flowers. This may be the case, for instance, at the early stages of a route development when bees 185 often return to a reference flower from which they explore and attempt to locate new flowers (Ohashi 186 et al., 2007; Lihoreau et al., 2010; 2016).

We calculated the "weighted betweenness centrality". This metric reflects the importance of a node as intermediary of the network, based on the number of shortest paths connecting all pairs of nodes that pass through the focal node (Opsahl 2009). In a bee movement network a high weighted betweenness centrality characterises a flower that is acting as a bridge among multiple other flowers (Figure 1D).

192 Because our aim was to study general trends of spatial optimization by bees across time at 193 the network level (route efficiency) and not at the node level (role played by individual flowers), for all 194 the local metrics we calculated mean values over all flowers at each foraging bout. Betweenness 195 scores were normalized following an algorithm that weights the betweenness value for the number of 196 flowers visited in the network (Freeman, 1979). Authority scores were scaled from 0 to 1. Clustering coefficients vary between 0 and 1 and need no normalization. In these conditions, an optimal network 197 198 (in which a bee would visit all flowers once and return to the nest by travelling the shortest distance to 199 visit all flowers) and a suboptimal network (in which a bee would travel longer distances for visiting the 200 same amount of flowers) would be characterised by the maximum average betweenness of 0.5, the 201 maximum average authority score of 1, and the minimum average clustering coefficient of 0 (Figure 202 1E).

#### 203 Global network measures

204 At the global level, we examined the triadic structures of the network - i.e. motifs (Milo et al., 2002), which represent triadic patterns of connection between nodes in a directed network. Network motifs 205 can be representative of various biological processes such as information flow (Nandi et al., 2014), 206 207 resource exchange (Quevillon et al., 2015) or disease spread (Waters & Fewell, 2012). In a bee 208 movement network the analyses of network motifs might help identifying behavioural rules 209 underpinning trapline formation (Figure 1F) while allowing for unbiased comparison across different 210 datasets (Shizuja & McDonald, 2015). Whereas a detailed temporal network analysis of motifs might be used to better understand the mechanisms of network functionality (Kovanen et al., 2011), here we 211 212 used a discrete approach by counting all the 16 possible triadic motifs to connect three flowers (including the nest) observed at each foraging bout and thus not strictly related to the exact temporal 213 214 sequence of visits on flowers (Figure 1F). For each experiment, we compared the triadic motifs of the 215 observed bee foraging networks at each foraging bout to those of the theoretical optimal network 216 connecting all flowers and the nest using the shortest possible path. Since only two out of the 16 217 possible triadic motifs (Figure 3A-F) can be observed in the optimal movement network (Figure 1E), these global measures inform us about the overall efficiency of the routes developed by bees. 218 219

#### 220 Data analyses

221

#### 222 Local network measures

All analyses were conducted in the statistical environment R (i.e. version 3.2.3). For each foraging 223 224 bout of each bee we extracted weighted clustering coefficient values, authority scores and weighted 225 betweenness centrality values of each flower, using the functions "clustering local w" and "betweenness\_w" in the tnet package (Opsahl, 2009) and the function "authority.scores" in the igraph 226 227 package (Csardi & Nepusz, 2006). We ran three different regression models for weighted 228 betweenness, authority and weighted clustering coefficient values using the sequential number of 229 foraging bouts, type of array (i.e. small or large spatial scale), species (i.e. bumblebee or honeybee) and all the interactions among these predictors as fixed effects. We used individual identity nested in 230 231 experimental array as a random effect in all models. We carried out model selection for the three 232 different parameters ranking candidate models according to their Akaike Information Criterion (Akaike, 233 1985). We used beta regression for the three averaged local network measures (clustering coefficient, weighted betweenness centrality and authority scores) because their values were constrained 234 235 between 0 and 1. We also applied a zero inflation method using the Beta Inflated (BEINF) family function from the gamIss package (Rigby & Stasinopoulos 2005). Model selection of the Beta 236 regression mixed models are shown in the supplementary materials (Tables S1, S2 and S3). Because 237 network metrics are correlated, we applied a Bonferroni correction by setting the alpha level of 238 239 significance at 0.017 (Tylianakis et al., 2007). 240

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#### 243 Global network measures

- 244 Motifs were calculated using the "triad.census" function in the "igraph package" (Csardi & Nepusz,
- 245 2006). Only two out of the 16 possible triadic motifs (motif 2 and 6: Figure 3A-F) are representative of
- the optimal movement network (Figure 1E). Depending on the number of flowers in the array, motif 1
- 247 (triadic structure where the three nodes A, B, C have no connection among them, i.e. the empty
- graph A, B, C) can also occur and being represented for a maximum of 7 times in a network with 7
- flowers. Motif 2 (triadic structure with a single connection between the three nodes, i.e. A>B, C) and
- 250 motif 6 (triadic structure where A>B>C are all connected by two directed lines) of the optimal
- 251 movement network can also occur at different frequencies depending on the network size (i.e. for 7
- flowers: 21 and 7; for 6 flowers: 12, 6; for 5 flowers: 5, 5; as indicated by red horizontal lines in Figure
- 3). We analysed the tendency of bees to modify their motifs frequency with time by applying a
- 254 generalized linear mixed effect model for count data (i.e. GLMM with Zero Inflated Poisson distribution
- error) using the observed frequency for each motif and for each dataset as response variable, the
- number of foraging bouts as predictor, and individual identity as random effect.
- 257

#### 258 Other measures

- 259 To illustrate the benefits of using the network approach relative to more conventional analyses, we also calculated non-network measures used in previous studies for assessing the ability of bees to 260 261 develop efficient routes (Lihoreau et al., 2011; Lihoreau et al., 2012a; Lihoreau et al., 2012b; Buatois & 262 Lihoreau, 2016). For each foraging bout of each bee we calculated the number of revisits to flowers 263 and the distance travelled (assuming straight lines between flowers) divided by the number of flowers visited. Both measures of route efficiency are expected to decrease with increasing network efficiency, 264 265 and reach a minimum in an optimal movement network. We applied a GLMM for count data to study the impact of experience (foraging bout) on the number of revisits to flowers and a linear mixed effect 266 267 model (LMM) for the travelled distance divided by the number of flowers visited. Both models were run 268 for each experiment using individual identity as random effect.
- 269

### 270 **Results**

#### 271 Local network measures

272 The average weighted betweenness centrality increased as bees accumulated foraging experience in 273 the six experiments (estimate<sub>bout</sub> = 0.066, standard error (se) = 0.004, t = 17.11, P < 0.001), indicating 274 that individuals tended to visit all flowers at a similar frequency by the end of training (Figure 4). This tendency was stronger in large spatial scale arrays (estimate<sub>small array</sub> = -0.067, se = 0.005, t = -13.55, 275 *P* < 0.001). Interestingly, in small spatial scale arrays bumblebees showed higher average weighted 276 betweenness centrality (estimatehoneybees = -1.172, se = 0.164, t = -7.15, P < 0.001) and a tendency to 277 develop optimal networks faster (estimate<sub>honevbees</sub> = -0.020, se = 0.008, t = -2.533, P = 0.011) than 278 279 honeybees (Figure 4).

The average Kleinberg's authority scores also increased as bees accumulated experience in the six experiments (estimate<sub>bout</sub> = 0.083, se = 0.03, t = 2.781, P = 0.004), meaning that all flowers became equally important in the network. For both bee species, the average authority scores were

- lower in small spatial scale arrays than in large spatial scale arrays (estimate<sub>small\_arrays</sub> = -0.446, se =
- 0.103, t = -4.334, P < 0.001). However, honeybees had larger average authority scores than
- bumblebees in the small spatial scale arrays (estimate<sub>small\_arrays\_honeybees</sub> = 0.582, se = 0.111, *t* = 5.229,
- P < 0.001) meaning that they tended to use all possible connections between flowers equally whereas
- 287 bumblebees only used a few.
- 288 The average clustering coefficient tended to decrease with time, as bees accumulated 289 foraging experience (Figure 6). Specifically, bumblebees showed a significant decrease in average 290 clustering coefficient while honeybees maintained stable values throughout the experiments 291 (estimate<sub>bout honeybees</sub> = 0.018, se = 0.004, t = 4.185, P < 0.001). Honeybees showed completely 292 different trend at small spatial scales, by increasing their average clustering coefficient scores with experience (estimatehoneybees small arrays = 0.407, se = 0.148, t = 2.752, P = 0.006). This again illustrates 293 the much reduced route optimisation efficiency of honeybees in comparison to bumblebees at small 294 295 spatial scales (Figure 6).
- 296 Overall, these changes in all three local network measures were more pronounced at larger 297 spatial scales, where flowers were distant from each other and from the colony nest, both for 298 bumblebees and honeybees (Figures 4, 5 and 6).
- 299

#### 300 Global network measures

While bees initially used the 16 possible motifs to link flowers, they gradually reduced the number of motifs to only use two of them by the end of training (motifs 2 and 6), a behaviour that is characteristic of route optimisation (Figure 3). This tendency was less pronounced for honeybees at small spatial scales (Figures 3D and 3E). Analyses of the frequency usage of each motif confirmed that honeybees at small spatial scales often presented opposite tendencies than honeybees at large spatial scales or bumblebees at all spatial scales (Figure 3D: motifs 3, 7, 8 and 15; Figure 3E: motifs 4, 5, 10 and 15) (for detailed motifs analysis see Table S4-S9).

308

309 Other measures

- 310 Conventional statistics for bee movement analyses showed trends towards a general increase in
- 311 movement efficiency with experience. In all experiments bees decreased the number of revisits to
- 312 flowers as they accumulated foraging bouts (Table S10). Bees also tended to decrease their travelled
- 313 distance divided by the number of visited flowers, except in the case of honeybees foraging in small
- 314 spatial scale arrays (Table S10).
- 315

#### 316 **Discussion**

317 Network analyses are increasingly used in behavioural and ecological research, providing a whole

- 318 new range of metrics to describe and model interactions between individuals and their environment
- 319 (Croft et al., 2008; Jeanson, 2012; Pinter-Wollman et al., 2013). In pollination ecology, this approach
- 320 has proved particularly powerful to describe interactions between plant and pollinator species, for
- instance using undirected bipartite networks based on field surveys of pollinator abundance (e.g.
- 322 (Fontaine et al., 2006; Bascompte & Jordano, 2007; Campbell et al., 2011; Burkle et al., 2013; Coux et

*al.*, 2016). Here we show how spatial network analyses can be developed to study the movement patterns of individual bees exploiting multiple feeding locations at various spatial and temporal scales in simplified experimental conditions. We argue that this approach may prove particularly helpful to analyse pollinator movements in more complex and ecologically realistic experimental designs and to generate new empirically testable hypotheses for pollination ecology research.

328 As illustrated above, bee movement patterns can be described in terms of local and global 329 network metrics that change predictably as individuals accumulate foraging experience. For instance, 330 in a simple situation where only one bee exploits a stable array of flowers refilled between each 331 foraging bout, both average betweenness values and average authority scores increased with time. By contrast the average clustering of flowers decreased with time as bees started to develop optimal or 332 suboptimal stable movement networks. This tendency for optimisation of spatial movement networks 333 334 was also reflected in the dynamics of motif usage, resulting in bees increasing their usage of the only 335 two motifs representative of an optimal foraging route. Interestingly, and in accordance with previous studies (e.g. Saleh & Chittka, 2007; Lihoreau et al., 2012a; Buatois & Lihoreau 2016), we found that 336 337 bumblebees and honeybees rarely use optimal spatial networks at small spatial scales, where the cost 338 of using a longer (suboptimal) path may be negligible. By contrast, foragers bees always used optimal spatial networks at large spatial scales, suggesting that they use more complex optimisation 339 340 movement rules in more costly conditions. These results were confirmed with more conventional statistical approaches (e.g. flower re-visits, travel efficiency), thereby validating our approach. 341 342 Importantly, the global network approach, based on motif analyses, brought new insights into the spatial behaviour of bees. For instance the foraging patterns of honeybees were characterised by 343 344 frequent back and forth movements between flowers (Figure 6D - i.e. motifs 7 & 8) and

disproportionate usage of specific flowers or local hubs (Figure 6D – i.e. motif 4).

The aim of this exploratory study was to introduce spatial network analyses for characterising 346 347 bee movement patterns using relatively standard metrics. Further developments of this approach will 348 provide a powerful, complementary, analytical tool to conventional behavioural metrics in order to 349 inform researchers about spatial processes that are not captured by other measures. This approach 350 should focus more on global measures of path optimality (e.g. network path length, geodesic distance 351 "Wasserman & Faust, 1994") to discriminate these different scenarios. For instance, network triads 352 give new information about specific movement routines that may be repeated within a route but that are hardly detectable with current measures of sequence repeatability (Thomson et al., 1997; Ayers et 353 354 al., 2015). Ultimately, a major challenge for future studies will be to consider the high levels of 355 heterogeneity among flower resources that bees may face in nature, taking into account variation in 356 resource reward quantity and quality, signals, and competition among foragers in addition to spatial constraints of resource locations, in order to extend our approach to field conditions. Experimentally, 357 358 bumblebees foraging in arrays of artificial flowers providing different nectar rewards face a trade off 359 between maximising their nectar intake rate and minimizing travel distances when developing traplines 360 (Lihoreau et al., 2011). Analyses on non-averaged local metrics could be used to capture the effect of resource diversity in network formation, and bring new insights into how bees integrate memories of 361 362 multiple individual flowers in their spatial memory. The Kleinberg's authority score likely informs us

- 363 about how bees use flowers as reference points relative to neighbouring flowers, perhaps to locate
- new flowers at the beginning of route formation. The weighted clustering coefficient is a mean to
- 365 determine the level of connections between sub-groups flowers, a measure that should greatly vary
- during the process of route optimisation. Other network measures, not used here, may also help
- understand how bees change their foraging area with experience or in the face of competition (e.g.

368 modularity in Dupont *et al.*, 2014).

369 While some of the predictions tested here may seem rather intuitive, our analysis of bumblebee and

honeybee movement patterns in relatively simple foraging conditions aims at illustrating how network
 statistics could serve future research in field and semi-field conditions. Motif network analyses offer the

372 possibility to statistically compare networks to each other, either for the same individuals at different

373 stages of route formation, or between different individuals, and between different species.

- 374 Characterising the spatial foraging strategies of a wider range of pollinators, including wild and
- 375 managed species is a key challenge of pollination ecology in order to identify and compare the real
- 376 impact of these species on pollination services (Garibaldi *et al.*, 2013). For instance, our preliminary
- 377 analysis suggests that at small spatial scales bumblebees display more efficient spatial movements
- than honeybees. Bumblebees tended to reach a frequency of each triadic structure that would lead to
- an optimal foraging network, whereas honeybees often showed the opposite behaviour. A possible
- 380 explanation is the difference of social life style between these two pollinator species. Honeybees, in
- contrast to bumblebees, have evolved a unique food recruitment system (the waggle dance) by which
- 382 successful foragers communicate locational information about food resources to their nestmates upon
- their return to the hive (Von Frisch, 1967; Dornhaus *et al.*, 2006). These insects may thus invest less
- in individual sampling and efficient route learning than species lacking the means to communicate
- foraging locations, such as bumblebees (Buatois & Lihoreau, 2016). Another possibility is the
- difference of typical foraging range between the two species. While bumblebees rarely cover more
- than three kilometres to exploit floral resources (Osborne *et al.*, 2008), honeybees can travel more
- than ten kilometres within a single foraging trip (Pahl *et al.*, 2011), suggesting that they are better
- 389 adapted to long flights and could start exhibiting optimisation movement patterns at larger spatial
- 390 scales than bumblebees. Systematic comparisons of both species across a wider range of spatial
- 391 scales will be needed to test these hypotheses.

392 Another key advantage of network analyses is that they allow for working on complete (raw) 393 datasets and thus reduce the risks of arbitrarily discarding important information. In the case of 394 pollinators, such approach may allow identification of specific movement patterns that occur at the 395 early stage of route learning, for instance exploration flights to locate flowers and store them in spatial 396 memory, or exploitation flights to return to familiar locations (Woodgate et al., 2016). Further development of pollinator movement networks may also include detailed dynamic temporal analyses 397 398 of flower visitation sequences, which might reveal differential effect of the individual experience on the 399 probability to optimize the foraging route. Stochastic agent-based methods (Snijders et al., 2010) 400 recently applied to animal social networks (Boucherie et al., 2016; Pasquaretta et al., 2016), may also prove useful to integrate rate of change of flower visitation sequences. New metrics could be 401 402 developed to estimate network efficiency in order to account for the specificity of the structure of bee

- 403 spatial movement based on individual experience. For instance, the direct integration of probability 404 values based on the spatial distances between flowers will allow for a finer calculation of local network 405 metrics which could be used to characterize the individual learning process and compare the likelihood 406 to obtain an optimal foraging route depending on the early spatial experience of the bee. Explicit 407 consideration of the nest as a specific node in the network, different from flowers, may also bring 408 useful information about bee network dynamics and efficiency.
- 409 For all these reasons, we believe that pollinator movement networks constitutes a highly 410 promising conceptual framework for studying plant-pollinator systems from a mechanistic point of view 411 in complement to more conventional behavioural measures. Ultimately, a comprehensive 412 understanding of bee movement patterns between plants may provide new fundamental insights into pollination processes and the genetic structuralism of plant populations. The development of optimal 413 414 routes by individual bees between particular plants can have important and predictable effects on 415 plant reproduction and inbreeding (Ohashi & Thomson, 2009). Advances in DNA pollen analyses (see Clare et al., 2013; and metabarcoding; Pornon et al., 2016) now allow identification of flower species 416 417 visited by individual bees during a given foraging trip. One can readily downscale the approach at an 418 intraspecific level by using pollen DNA and more variable genetic markers (e.g. microsatellite; Arif et 419 al., 2010) to identify individual plants visited by pollinators and infer patterns of pollen flow within a 420 plant population that can then be verified by paternity analyses using plant progeny genotypes for 421 these markers (Bernasconi, 2003). Coupling these approaches with existing models of bee 422 movements (Lihoreau et al., 2012b; Reynolds et al., 2013; Becher et al., 2016) will provide critical information about how the foraging strategies of bees directly influence pollen transfer and plant 423 424 mating patterns across landscapes, and therefore a better assessment of consequences of bee 425 declines on pollination.
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#### 428 **Contribution of the authors**

429 ML designed and conducted the experiments; CP built and analysed the networks; CP, RJ, CA, LC

- and ML wrote the manuscript. All authors gave final approval for publication. Authors declare nocompeting interests.
- 432

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### 440 **References**

- 441 Akaike H. (1985) Prediction and Entropy. In: Atkinson A.C., Fienberg S.E. (eds) A Celebration of
- 442 Statistics. Springer, New York, NY. pp. 387–410.

- 443 Arif, I.A., Khan, H.A., Shobrak, M., Al Homaidan, A.A., Al Sadoon, M., Al Farhan, A.H., *et al.* (2010)
- 444 Interpretation of electrophoretograms of seven microsatellite loci to determine the genetic diversity of
- the Arabian Oryx. *Genetics and Molecular Research*, **9**, 259–265.
- 446 Ayers, C.A., Armsworth, P. R., & Brosi, B. J. (2015). Determinism as a statistical metric for ecologically
- important recurrent behaviors with trapline foraging as a case study. *Behavioral ecology and sociobiology*, **69**, 1395-1404.
- 449 Barrat, A., Barthelemy, M., Pastor-Satorras, R. & Vespignani, A. (2004) The architecture of complex
- 450 weighted networks. *Proceedings of the National Academy of Sciences of the United States of*
- 451 *America*, **101**, 3747–3752.
- 452 Barthélemy, M. (2011) Spatial networks. *Physics Reports*, **499**, 1–101.
- 453 Bascompte, J. & Jordano, P. (2007) Plant-animal mutualistic networks: The architecture of
- biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 567–593.
- 455 Becher, M.A., Grimm, V., Knapp, J., Horn, J., Twiston-Davies, G. & Osborne, J.L. (2016) BEESCOUT:
- A model of bee scouting behaviour and a software tool for characterizing nectar/pollen landscapes for
   BEEHAVE. *Ecological Modelling*, **340**, 126–133.
- 458 Bernasconi, G. (2003) Seed paternity in flowering plants: an evolutionary perspective. *Perspectives in* 459 *Plant Ecology, Evolution and Systematics*, **6**, 149–158.
- 460 Boucherie, P.H., Sosa, S., Pasquaretta, C. & Dufour, V. (2016) A longitudinal network analysis of
- social dynamics in rooks *corvus frugilegus*: repeated group modifications do not affect social network
   in captive rooks. *Current zoology*, zow083. doi: 10.1093/cz/zow083
- Buatois, A. & Lihoreau, M. (2016) Evidence of trapline foraging in honeybees. *The Journal of Experimental Biology*, **219**, 2426–9.
- Burkle, L.A. & Alarcón, R. (2011) The future of plant-pollinator diversity: Understanding interaction
  networks acrosss time, space, and global change. *American Journal of Botany*, **98**, 528–538.
- 467 Burkle, L.A., Marlin, J.C. & Knight, T.M. (2013) Plant-pollinator interactions over 120 years: Loss of 468 species, co-occurrence, and function. *Science*, **339**, 1611–1615.
- Campbell, C., Yang, S., Albert, R. & Shea, K. (2011) A network model for plant-pollinator community
  assembly. *Proceedings of the National Academy of Sciences of the United States of America*, **108**,
  197–202.
- Chittka, L., Gumbert, A. & Kunze, J. (1997) Foraging dynamics of bumble bees: correlates of
  movements within and between plant species. *Behavioral Ecology*, **8**, 239–249.
- 474 Chittka, L. & Thomson, J.D. (2001) Cognitive ecology of pollination: animal behaviour and floral
  475 evolution. Cambridge University Press. New York. NY
- 476 Clare, E.L., Schiestl, F.P., Leitch, A. R., & Chittka, L. (2013) The promise of genomics in the study of 477 plant-pollinator interactions. *Genome Biology*, **14**, 207.
- Collett, M., Chittka, L. & Collett, T.S. (2013) Spatial memory in insect navigation. *Current Biology*, 23,
  R789–R800.
- 480 Cook, W. (2012) *In pursuit of the traveling salesman: mathematics at the limits of computation.*
- 481 Princeton University Press. Princeton. New Jersey
- 482 Coux, C., Rader, R., Bartomeus, I. & Tylianakis, J.M. (2016) Linking species functional roles to their
   483 network roles. *Ecology Letters*, **19**, 762–770.

- 484 Crall, J.D., Gravish, N., Mountcastle, A.M. & Combes, S.A. (2015) BEEtag: a low-cost, image-based
   485 tracking system for the study of animal behavior and locomotion. *PloS ONE*, **10**, e0136487.
- 486 Croft, D.P., James, R. & Krause, J. (2008) *Exploring animal social networks*. Princeton University
   487 Press. Princeton. New Jersey
- 488 Csardi, G. & Nepusz, T. (2006) The igraph software package for complex network research.
   489 *InterJournal, Complex Systems*, **1695**, 1–9.
- 490 Dorigo, M. & Gambardella, L.M. (2016) Ant-Q: A reinforcement learning approach to the traveling
- 491 salesman problem. In *Proceedings of ML-95, Twelfth International Conference on Machine Learning*.
  492 Eds Morgan Kaufmann. pp. 252–260.
- 493 Dornhaus, A., Klügl, F., Oechslein, C., Puppe, F. & Chittka, L. (2006) Benefits of recruitment in honey
  494 bees: effects of ecology and colony size in an individual-based model. *Behavioral Ecology*, **17**, 336–
  495 344.
- 496 Dupont, Y.L., Trøjelsgaard, K., Hagen, M., Henriksen, M. V., Olesen, J.M., Pedersen, N.M.E., *et al.*497 (2014) Spatial structure of an individual-based plant-pollinator network. *Oikos*, **123**, 1301–1310.
- Fontaine, C., Dajoz, I., Meriguet, J. & Loreau, M. (2006) Functional diversity of plant-pollinator interaction webs enhances the persistence of plant communities. *PLoS Biology*, **4**, 0129–0135.
- 500 Freeman, L. (1979) Centrality in social networks conceptual clarification. Social Networks, 1, 215–239.
- Frisch, K. Von. (1967) *The dance language and orientation of bees*. Harvard University press.
   Cambridge
- 503 Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A., et al.
- 504 (2013) Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science*, **339**, 505 1608–1611.
- 506 Goulson, D., Nicholls, E., Botías, C. & Rotheray, E.L. (2015) Bee declines driven by combined stress 507 from parasites, pesticides, and lack of flowers. *Science*, **347**, 1255957.
- Heinrich, B., (1976) the foraging specializations of individual bumble-bees. *Ecological Monographs*,
  46, 105-128.
- Ings, T.C. & Chittka, L. (2008) Speed-accuracy tradeoffs and false alarms in bee responses to cryptic
   predators. *Current Biology*, **18**, 1520–1524.
- Jacoby, D.M.P. & Freeman, R. (2016) Emerging network-based tools in movement ecology. *Trends in Ecology & Evolution*, **31**, 301-314.
- 514 Janzen, D.H. (1971) Euglossine bees as long-distance pollinators of tropical plants. *Science*, **171**, 515 203–205.
- 516 Jeanson, R. (2012) Long-term dynamics in proximity networks in ants. *Animal Behaviour*, **83**, 915– 517 923.
- 518 Klein, S., Cabirol A., Devaud, J.M., Barron, A.B. & Lihoreau, M. (2017) Why bees are so vulnerable to 519 environmental stressors. *Trends in Ecology & Evolution*, **32**, 268-278.
- Kleinberg, J. (1999) Authoritative sources in a hyperlinked environment. *Journal of the ACM (JACM)*,
  46, 604–632.
- 522 Kovanen, L., Karsai, M., Kaski, K., Kertész, J. & Saramäki, J. (2011) Temporal motifs in time-
- 523 dependent networks. Journal of Statistical Mechanics: Theory and Experiment, 11, P11005.
- 524 https://doi.org/10.1088/1742-5468/2011/11/P11005

- 525
- 526 Lihoreau, M., Chittka, L. & Raine, N.E. (2010) Travel optimization by foraging bumblebees through
- readjustments of traplines after discovery of new feeding locations. *The American Naturalist*, **176**,
   744–757.
- Lihoreau, M., Chittka, L. & Raine, N.E. (2011) Trade-off between travel distance and prioritization of high-reward sites in traplining bumblebees. *Functional Ecology*, **25**, 1284–1292.
- Lihoreau, M., Chittka, L., Le Comber, S.C. & Raine, N.E. (2012a) Bees do not use nearest-neighbour rules for optimization of multi-location routes. *Biology letters*, **8**, 13–6.
- Lihoreau, M., Raine, N.E., Reynolds, A.M., Stelzer, R.J., Lim, K.S., Smith, A.D., et al. (2012b) Radar
- tracking and motion-sensitive cameras on flowers reveal the development of pollinator multidestination routes over large spatial scales. *PLoS Biology*, **10**, 19–21.
- Lihoreau, M., Chittka, L. & Raine, N.E. (2016) Monitoring flower visitation networks and interactions between pairs of bumble bees in a large outdoor flight cage. *PloS ONE*, **11**, e0150844.
- 538 Makino, T.T. & Sakai, S. (2004) Findings on spatial foraging patterns of bumblebees (Bombus ignitus) 539 from a bee-tracking experiment in a net cage. *Behavioral Ecology and Sociobiology*, **56**, 155-163.
- 540 Makino, T.T. & Sakai, S. (2005) Does interaction between bumblebees (Bombus ignitus) reduce their
- foraging area?: bee-removal experiments in a net cage. *Behavioral Ecology and Sociobiology*, **57**,
  617-622.
- 543 Makino, T.T. (2013) Longer visits on familiar plants?: testing a regular visitor's tendency to probe more 544 flowers than occasional visitors. *Naturwissenschaften*, **100**, 659-666.
- 545 Mayer, C., Adler, L., Armbruster, W., Dafni, A., Eardley, C., Huang, S., *et al.* (2011) Pollination ecology 546 in the 21st Century: key questions for future research. *Journal of Pollination Ecology*, **3**, 8–23.
- 547 Michener, C.D. (2000) The Bees of the World. JHU Press. Baltimore
- Milo, R., Shen-Orr, S., Itzkovitz, S., Kashtan, N., Chklovskii, D. & Alon, U. (2002) Network motifs:
  Simple building blocks of complex networks. *Science*, **298**, 824–827.
- 550 Nandi, A.K., Sumana, A. & Bhattacharya, K. (2014) Social insect colony as a biological regulatory
- system: modelling information flow in dominance networks. *Journal of The Royal Society Interface*, **11**,
   20140951
- 553 Ohashi, K., Thomson, J.D. & D'Souza, D. (2007) Trapline foraging by bumble bees: IV. Optimization of 554 route geometry in the absence of competition. *Behavioral Ecology*, **18**, 1–11.
- 555 Ohashi, K. & Thomson, J.D. (2009) Trapline foraging by pollinators: Its ontogeny, economics and 556 possible consequences for plants. *Annals of Botany*, **103**, 1365–1378.
- 557 Ohashi, K., D'Souza, D. & Thomson, J.D. (2010) An automated system for tracking and identifying 558 individual nectar foragers at multiple feeders. *Behavioral Ecology and Sociobiology*, **64**, 891–897.
- 559 Opsahl, T. (2009) Structure and evolution of weighted networks. University of London (Queen Mary
- 560 College), London, UK, pp. 104-122. Available at
- 561 https://toreopsahl.files.wordpress.com/2009/05/thesis\_print-version\_withoutappc.pdf
- 562 Osborne, J.L., Martin, A.P., Carreck, N.L., Swain, J.L., Knight, M.E., Goulson, D., et al. (2008)
- 563 Bumblebee flight distances in relation to the forage landscape. *Journal of Animal Ecology*, **77**, 406– 564 415.

- Pahl, M., Zhu, H., Tautz, J. & Zhang, S. (2011) Large scale homing in honeybees. *PLoS ONE*, 6,
  e19669.
- 567 Pasquaretta, C., Klenschi, E., Pansanel, J., Battesti, M., Mery, F. & Sueur, C. (2016) Understanding
- 568 dynamics of information transmission in *Drosophila melanogaster* using a statistical modeling
- 569 framework for longitudinal network data (the RSiena package). *Frontiers in psychology*, **7**, 539.
- Perna, A. & Latty, T. (2014) Animal transportation networks. *Journal of the Royal Society, Interface / the Royal Society*, **11**, 20140334.
- 572 Pinter-Wollman, N., Hobson, E.A., Smith, J.E., Edelman, A.J., Shizuka, D., de Silva, S., et al. (2013)
- 573 The dynamics of animal social networks: Analytical, conceptual, and theoretical advances. *Behavioral* 574 *Ecology* **25**, 242-255.
- 575 Polyakovskiy, S., Bonyadi, M.R., Wagner, M., Michalewicz, Z. & Neumann, F. (2014) A
- 576 comprehensive benchmark set and heuristics for the traveling thief problem. In Proceedings of the
- 577 2014 Annual Conference on Genetic and Evolutionary Computation. Eds. Christian Igel. ACM, New
- 578 York. NY pp. 477–484.
- 579 Pornon, A., Escaravage, N., Burrus, M., Holota, H., Khimoun, A., Mariette, J., *et al.* (2016) Using 580 metabarcoding to reveal and quantify plant-pollinator interactions. *Scientific Reports*, **6**, 27282.
- 581 Pyke, G.H. & Cartar, R. V. (1992) The flight directionality of bumblebees: Do they remember where 582 they came from? *Oikos*, **65**, 321–327.
- 583 Quevillon, L.E., Hanks, E.M., Bansal, S. & Hughes, D.P. (2015) Social, spatial, and temporal 584 organization in a complex insect society. *Scientific reports*, **5**, 13393
- 585 Reynolds, A.M., Lihoreau, M. & Chittka, L. (2013) A simple iterative model accurately captures
- complex trapline formation by bumblebees across spatial scales and flower arrangements. *PLoS Computational Biology*, 9, e1002938.
- Rigby, R.A. & Stasinopoulos, D.M. (2005) Generalized additive models for location, scale and shape.
   *Journal of the Royal Statistical Society: Series C (Applied Statistics)*, **54**, 507-554.
- 590 Saleh, N. & Chittka, L. (2007) Traplining in bumblebees (Bombus impatiens): a foraging strategy's
- ontogeny and the importance of spatial reference memory in short-range foraging. *Oecologia*, **151**,719-730.
- 593 Shizuka, D. & McDonald, D.B. (2015). The network motif architecture of dominance hierarchies. 594 *Journal of The Royal Society Interface*, **12**, 20150080.
- 595 Snijders, T.a.B., van de Bunt, G.G. & Steglich, C.E.G. (2010) Introduction to stochastic actor-based
- 596 models for network dynamics. *Social Networks*, **32**, 44–60.Thomson, J.D. (1986) Pollen transport and 597 deposition by bumble bees in *Erythronium*: influences of floral nectar and bee grooming. *Journal of*
- 598 Ecology, **74**, 329-341.
- 599 Thomson, J.D., Slatkin, M. & Thomson, B.A. (1997) Trapline foraging by bumble bees: II. Definition 600 and detection from sequence data. *Behavioral Ecology*, **8**, 199–210.
- Tylianakis, J.M., Tscharntke, T. & Lewis, O.T. (2007) Habitat modification alters the structure of tropical host–parasitoid food webs. *Nature*, **445**, 202-205.
- Waser, N.M. (1986). Flower constancy: definition, cause, and measurement. *The American Naturalist*,
   **127**, 593-603.
- Wasserman, S. & Faust, K. (1994) Social network analysis: Methods and applications. Cambridge
   University Press. New York. NY

- Waters, J.S. & Fewell, J.H. (2012) Information processing in social insect networks. *PLoS ONE*, 7, e40337.
- Woodgate, J.L., Makinson, J.C., Lim, K.S., Reynolds, A.M. & Chittka, L. (2016) Life-long radar tracking
   of bumblebees. *PloS ONE*, **11**, e0160333.

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## **Figure legends**



Examples of local and global metrics calculated on a bee spatial movement network. Nodes of the network (white circles) represent flowers (F1-F6) and the colony nest (black square). Edge directions indicate individual movements between flowers and the nest. Edge thickness is proportional to the frequency of bee movements from one flower to another (i.e. edge weights). In this hypothetical network, from A to E, the forager tends to increase the number of visited flowers with experience (t0, t0 + 1, t0 + 2, t0 + n) while reducing both the number of revisits to flowers and the time needed to visit all (i.e. network optimization). Examples of local network measures are shown (black arrows): 1) High clustering coefficient calculates the degree to which neighbours of a given node are themselves highly connected; 2) Authority score indicates the existence of highly visited nodes; 3) High betweenness centrality value counts the number of shortest paths that pass through a focal node. (F) Hypothetical network illustrating two common network motifs (red arrows) in bee movement data (motifs 3 and 6, see Figure 3).



Spatial arrangements of the artificial flowers (F1-F6) and the colony nest (black square) in the six experiments under investigation (scale is in meters). Number of bees (n) and foraging bouts (fb) are shown for each experiment. A. Experiment 1: bumblebees in the lab (Lihoreau *et al.*, 2012a). B. Experiment 2: bumblebees in the lab (Lihoreau *et al.*, 2012b). D. Experiment 4: honeybees in the lab (Buatois & Lihoreau 2016). E. Experiment 5: honeybees in the field (Buatois & Lihoreau 2016). F. Experiment 6: honeybees in the field (Buatois & Lihoreau 2016). Spatial scales are provided for each array (i.e. SMALL or LARGE).



Distribution of all possible network triadic motifs across foraging bouts. For each motif, the x-axis represents the temporally ordered foraging bouts. Red horizontal lines indicate the frequency of each motif expected in the optimal network. Best fitted lines obtained from generalized linear models using foraging bouts as predictor and frequency of motif as response variable are shown for each motif along with their standard errors (blue line and shaded grey area). Significant effects of time on the frequency of each motif are highlighted with asterisks. GLMM estimates, Z-values and P-values for each motif in each experiment are available in Tables S4-S9.

Relationship going in the opposite direction of the optimal network are numbered in red. Alpha level is set at 0.05. Spatial scales are provided for each graph (i.e. SMALL – a,b,d,e - or LARGE – c,f -, see also Figure 2).



Average weighted betweenness centrality values for each individual bee at each foraging bout. Black lines and grey shaded areas represent respectively the best fitted lines and their standard errors obtained from zeroinflated mixed effect models built using foraging bouts as fixed effect and individual identity as random (see details in the methods). Spatial scales are provided for each graph (i.e. SMALL – a,b,d,e - or LARGE – c,f -; see also Figure 2).



Average authority score values for each individual bee at each foraging bout. Black lines and grey shaded areas represent respectively the best fitted lines and their standard errors obtained from zeroinflated mixed effect models built using foraging bouts as fixed effect and individual identity as random (see details in the methods). Spatial scales are provided for each graph (i.e. SMALL – a,b,d,e - or LARGE – c,f -; see also Figure 2).



Average clustering coefficient values for each individual bee at each foraging bout. Black lines and grey shaded areas represent respectively the best fitted lines and their standard errors obtained from zeroinflated mixed effect models built using foraging bouts as fixed effect and individual identity as random (see details in the methods). Spatial scales are provided for each graph (i.e. SMALL – a,b,d,e - or LARGE – c,f -; see also Figure 2).

## Supplementary material legends:

### Supplementary materials

**Table S1:** Betweenness centrality model selection using the Generalised Akaike information criterion (GAIC).The three ranked best models with both FULL and NULL models are shown.

Beta regression	df	GAIC	
~Bout*Type*Bee (FULL)	48.86721	-2235.839	
~Bout*Type + Bee	46.53556	-2229.735	
~Bout*Type + Bee*Type	46.53556	-2228.518	
~Bout*Type + Bee*Bout	47.58002	-2228.460	
~ 1 (NULL)	39.68915	-1897.678	

**Table S2:** Authority model selection using the Generalised Akaike information criterion (GAIC). The three ranked best models with both FULL and NULL models are shown.

GLMM Proportional model	df	GAIC	
~Bout*Type + Bee	25.17792	524.4065	
~Bout*Type + Bee*Bout	24.60263	525.0094	
~Bout*Type + Bee*Type	25.71766	527.1393	
~Bout*Type*Bee (FULL)	28.58996	530.3337	
~ 1 (NULL)	31.60761	538.5515	

**Table S3:** Clustering coefficient model selection using the Generalised Akaike information criterion (GAIC). The three ranked best models with both FULL and NULL models are shown.

Beta regression	df	GAIC	
~bout*bee+bee*type	38.44953	1829.870	
~bout*type+bee*bout	39.11552	1830.968	
~bout*type+bee*type+ bout*bee	39.48056	1831.826	
~Bout*Type*Bee (FULL)	40.46720	1833.819	
~1 (NULL)	35.43024	1838.964	

	Estimate (SE)	t-values	P-values
Motif 1	0.035 (0.042)	0.84	0.403
Motif 2	0.113 (0.021)	5.44	<0.001
Motif 3	-0.008 (0.048)	-0.16	0.872
Motif 4	-0.702 (0.166)	-4.24	<0.001
Motif 5	-0.636 (0.149)	-4.26	<0.001
Motif 6	0.094 (0.047)	2.00	0.046
Motif 7	-0.235 (0.078)	-3.03	0.002
Motif 8	-0.235 (0.078)	-3.02	0.003
Motif 9	-1.732 (0.474)	-3.66	0.001
Motif 10	-0.574 (0.193)	-2.98	0.003
Motif 11	-0.387 (0.151)	-2.56	0.011
Motif 12	-1.791 (0.781)	-2.92	0.022
Motif 13	-0.604 (0.399)	-1.51	0.13
Motif 14	-1.523 (0.232)	-6.57	<0.001
Motif 15	-0.966 (0.194)	-4.98	<0.001
Motif 16	-3.006 (1.015)	-2.96	0.003

## **Table S4:** Experiment 1 - GLMMs frequency of each network motifs and foraging bouts.

## **Table S5:** Experiment 2 - GLMMs frequency of each network motifs and foraging bouts.

Estimate (SE)	t-values	P-values

Motif 1	-0.389 (0.057)	-6.79	<0.001
Motif 2	0.176 (0.043)	4.05	<0.001
Motif 3	-0.164 (0.083)	-1.98	0.048
Motif 4	-0.310 (0.270)	-1.15	0.252
Motif 5	-0.199 (0.279)	-0.71	0.475
Motif 6	0.220 (0.049)	4.44	<0.001
Motif 7	-0.219 (0.141)	-1.59	0.122
Motif 8	-0.304 (0.154)	-1.98	0.048
Motif 9	-1.823 (0.821)	-2.18	0.029
Motif 10	-0.520 (0.246)	-2.11	0.035
Motif 11	-0.445 (0.179)	-2.48	0.013
Motif 12	-0.021 (0.001)	-9.70	<0.001
Motif 13	0.528 (1.603)	0.33	0.742
Motif 14	-0.758 (0.343)	-2.21	0.028
Motif 15	-1.008 (0.470)	-2.148	0.032
Motif 16	-1.102 (0.953)	-1.157	0.248

**Table S6:** Experiment 3 - GLMMs frequency of each network motifs and foraging bouts.

	Estimate (SE)	t-values	P-values
Motif 1	-0.883 (0.057)	-15.58	<0.001
Motif 2	0.235 (0.075)	3.14	0.002

Motif 3	-0.500 (0.165)	-3.02	0.003
Motif 4	0.295 (0.572)	0.45	0.651
Motif 5	0.299 (0.515)	0.58	0.562
Motif 6	0.324 (0.168)	1.98	0.048
Motif 7	0.053 (0.311)	0.17	0.863
Motif 8	0.116 (0.306)	0.38	0.704
Motif 9	0.610 (0.688)	0.89	0.377
Motif 10	-0.402 (0.320)	-1.26	0.210
Motif 11	-0.949 (0.461)	-1.93	0.050
Motif 12	0.090 (1.238)	0.07	0.942
Motif 13	0.161 (0.991)	0.163	0.871
Motif 14	-0.681 (0.763)	-0.89	0.373
Motif 15	-0.301 (0.806)	-0.37	0.709
Motif 16	-0.335 (2.070)	-0.16	0.871

**Table S7:** Experiment 4 - GLMMs frequency of each network motifs and foraging bouts.

	Estimate (SE)	t-values	P-values
Motif 1	-0.414 (0.125)	-3.31	0.002
Motif 2	-0.007 (0.098)	-0.07	0.947
Motif 3	-0.326 (0.144)	-2.27	0.024
Motif 4	0.762 (0.266)	2.86	0.004

Motif 5	0.464 (0.356)	1.30	0.194
Motif 6	-0.074 (0.122)	-0.61	0.545
Motif 7	-0.048 (0.190)	-0.25	0.800
Motif 8	-0.186 (0.191)	-0.97	0.331
Motif 9	-0.618 (0.889)	-0.69	0.488
Motif 10	0.264 (0.183)	1.45	0.149
Motif 11	-0.022 (0.239)	-0.09	0.927
Motif 12	0.225 (2.994)	0.07	0.940
Motif 13	0.771 (0.947)	0.81	0.416
Motif 14	0.236 (0.317)	0.74	0.458
Motif 15	1.037 (0.526)	1.97	0.049
Motif 16	2.306 (2.748)	0.84	0.401

 Table S8: Experiment 5 - GLMMs frequency of each network motifs and foraging bouts.

	Estimate (SE)	t-values	P-values
Motif 1	-0.539 (0.078)	-6.92	<0.001
Motif 2	-0.215 (0.083)	-2.59	0.009
Motif 3	-0.114 (0.094)	-1.21	0.225
Motif 4	0.277 (0.357)	0.77	0.439
Motif 5	0.045 (0.327)	0.14	0.889
Motif 6	0.112 (0.108)	1.04	0.299

Motif 7	0.374 (0.150)	2.49	0.013
Motif 8	0.218 (0.155)	1.39	0.163
Motif 9	0.891 (0.568)	1.57	0.118
Motif 10	0.180 (0.220)	0.82	0.413
Motif 11	-0.024 (0.166)	-0.15	0.883
Motif 12	-0.024 (0.166)	-0.15	0.883
Motif 13	1.195 (0.620)	1.95	0.051
Motif 14	-0.265 (0.186)	-1.42	0.156
Motif 15	0.472 (0.268)	1.76	0.079
Motif 16	0.339 (0.677)	0.50	0.617
1			

	Estimate (SE)	t-values	P-values
Motif 1	-1.359 (0.239)	-5.74	<0.001
Motif 2	0.060 (0.171)	0.35	0.726
Motif 3	-0.479 (0.274)	-1.74	0.084
Motif 4	1.286 (1.179)	1.09	0.279
Motif 5	1.181 (1.164)	1.02	0.310
Motif 6	0.136 (0.259)	0.52	0.601
Motif 7	-0.107 (0.489)	-0.22	0.827
Motif 8	-0.160 (0.469)	-0.34	0.734
Motif 9	NC	NC	NC
Motif 10	-0.216 (0.432)	-0.50	0.618
Motif 11	-0.040 (0.739)	-0.05	0.957
Motif 12	NC	NC	NC
Motif 13	NC	NC	NC
Motif 14	0.029 (0.830)	0.03	0.972
Motif 15	-1.755 (1.852)	-0.95	0.345
Motif 16	NC	NC	NC

## **Table S9:** Experiment 6 - GLMMs frequency of each network motifs and foraging bouts.

**Table S10:** Conventional statistics. Number of re-visits to flowers and travelled distance divided by the number of visited flowers for bumblebees (experiments 1,2 and 3) and honeybees (experiments 4, 5 and 6) in large (experiments 3 and 6) and small (experiments 1,2,4 and 5) spatial scale arrays. Two types of statistical modelling approaches were used: a generalized linear mixed effect model for count data (GLMM) to study the impact of experience (foraging bout) on the number of revisits to flowers and a linear mixed effect model (LMM) for the travelled distance divided by the number of flowers visited. Both models were ran for each experiment using individual identity as random effect. Both models were run for each experiment using individual identity as random effect. Both models were run for each experiment using individual identity as random effect. Both models were run for each experiment using individual identity as random effect. Both models were run for each experiment using individual identity as random effect. Both models were run for each experiment using individual identity as random effect. Both models were run for each experiment using individual identity as random effect. Both models were run for each experiment using individual identity as random effect. Both models were run for each experiment using individual identity as random effect. Both models were run for each experiment using individual identity as random effect. Both models were run for each experiment using individual identity as random effect. Both models were run for each experiment using individual identity as random effect. Both models were run for each experiment using individual identity as random effect. Both models were run for each experiment using individual identity as random effect. Both models were run for each experiment using individual identity as random effect.

	Estimate ± SE	statistics	p-value
Experiment 1			
Number of flowers re-visits	-0.018 ± 0.001	(z) -25.55	< 0.001
Distance / Number of unique flowers visited	-0.052± 0.007	(t) -7.71	< 0.001
Experiment 2			
Number of flowers re-visits	-0.022 ± 0.002	(z) -12.79	< 0.001
Distance / Number of unique flowers visited	-0.093 ± 0.017	(t) -5.39	< 0.001
Experiment 3			
Number of flowers re-visits	-0.026 ± 0.005	(z) -4.78	< 0.001
Distance / Number of unique flowers visited	-1.491 ± 0.342	(t) -4.359	< 0.001
Experiment 4			
Number of flowers re-visits	-0.021 ± 0.006	(z) -5.67	< 0.001
Distance / Number of unique flowers visited	0.002 ± 0.014	(t) 0.123	0.902
Experiment 5			
Number of flowers re-visits	-0.01 ± 0.002	(z) -4.953	< 0.001
Distance / Number of unique flowers visited	0.011 ± 0.036	(t) 0.310	0.756
Experiment 6			
Number of flowers re-visits	-0.026 ± 0.005	(z) -5.61	< 0.001

Distance / Number of unique flowers visited

-0.321 ± 0.135

0.019

(t) -2.38