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# Analysing plant-pollinator interactions with spatial movement networks

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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

26 3) First we explain how spatial movement networks can be derived theoretically and experimentally to  
27 describe bee foraging decisions.

28

29 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

33 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 pollination  
35 ecology research.

36

37 **Keywords:** bumblebees; foraging; honeybees; movement ecology; pollination; route optimization;  
38 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  
47 ecosystems (Goulson *et al.*, 2015; Klein *et al.*, 2017). Central to understanding the impact of pollinator  
48 loss on plant reproduction is the foraging behaviour of bees (Thomson, 1986; Waser, 1986). Most  
49 bees are central-place foragers, meaning that they collect food (nectar and pollen) to provision their  
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  
56 flowers (Ohashi *et al.*, 2007), making short trips after encountering highly rewarding flowers (Chittka *et*  
57 *al.*, 1997) or keeping constant heading directions between visiting flowers (Pyke & Cartar, 1992).  
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,  
61 foragers of many bee species tend to develop stable foraging routes (sometimes called traplines in  
62 analogy to the fixed circuits that trappers follow when examining a number of traps distributed widely  
63 in space) that they follow for several hours or days (e.g. Euglossine bees: Janzen, 1971; bumblebees:  
64 Heinrich 1976; Thomson *et al.*, 1997; honeybees: Buatois & Lihoreau, 2016). This routing behaviour is  
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*:  
69 Ohashi *et al.*, 2007; *Bombus terrestris*: Lihoreau *et al.*, 2012a; *Apis mellifera*: Buatois & Lihoreau,  
70 2016), an optimisation behaviour analogous to solving the Travelling Salesman Problem in graph  
71 theory (Cook, 2012). This mathematical problem is notoriously difficult (if not impossible) to solve for  
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  
77 1971), to what extent these observations in simplified experimental conditions can be extrapolated to  
78 the field, where individuals may interact to exploit numerous highly variable resources scattered  
79 across large landscapes, is an open question.

80 Field data on such multi-destination routes among flower patches are even more complex and  
81 challenging to analyse, and conventional behavioural metrics do not suffice to capture detailed  
82 information about routing behaviour (Thomson *et al.*, 1997; Makino & Sakai 2004; Makino & Sakai  
83 2005; Makino 2013; Lihoreau *et al.*, 2016). We argue that network statistics derived from graph theory

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  
86 between flowers, foragers form movement networks embedded in space (Barthélemy, 2011), where  
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,  
100 2016). Several analytical packages (e.g. *igraph*, *sna*, *tnet* packages in R, graph-tool in Python,  
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  
115 automated movement tracking, now allow for collecting high resolution spatial and temporal data on  
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  
118 radars: Lihoreau *et al.*, 2012b; QR tags: Crall *et al.* 2015; 3D video tracking: Ings & Chittka, 2008).  
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

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).

138 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  
148 sucrose solution collected from a training flower over another three foraging bouts. The forager was  
149 then tested with all test flowers placed in a specific spatial arrangement (see experimental arrays in  
150 Figure 2). During the test, each flower provided the same amount of sucrose solution, chosen so that  
151 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  
154 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  
158 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;  
160 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  
162 experiments 3 and 6 (Lihoreau *et al.*, 2012b; Buatois & Lihoreau, 2016). Details about the spatial

163 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**

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

172

### 173 *Local network measures*

174 At a local level, we calculated the “weighted clustering coefficient”, which assesses the degree to  
175 which nodes tend to cluster together (Barrat *et al.*, 2004). Here a high clustering value indicates that  
176 neighbouring flowers of a given flower are themselves highly connected, i.e. frequently re-visited  
177 (Figure 1B).

178 We used the “Kleinberg’s authority score” (Kleinberg, 1999) to measure the relative  
179 importance of a node in a network (Figure 1C). Given  $A$ , an individual movement matrix across  
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  
183 Kleinberg’s authority score indicates that a specific flower is more often visited than all its neighbour  
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).

187 We calculated the “weighted betweenness centrality”. This metric reflects the importance of a  
188 node as intermediary of the network, based on the number of shortest paths connecting all pairs of  
189 nodes that pass through the focal node (Opsahl 2009). In a bee movement network a high weighted  
190 betweenness centrality characterises a flower that is acting as a bridge among multiple other flowers  
191 (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  
197 coefficients vary between 0 and 1 and need no normalization. In these conditions, an optimal network  
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),  
205 which represent triadic patterns of connection between nodes in a directed network. Network motifs  
206 can be representative of various biological processes such as information flow (Nandi *et al.*, 2014),  
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  
211 be used to better understand the mechanisms of network functionality (Kovanen *et al.*, 2011), here we  
212 used a discrete approach by counting all the 16 possible triadic motifs to connect three flowers  
213 (including the nest) observed at each foraging bout and thus not strictly related to the exact temporal  
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),  
218 these global measures inform us about the overall efficiency of the routes developed by bees.

219

220 **Data analyses**

221

222 *Local network measures*

223 All analyses were conducted in the statistical environment R (i.e. version 3.2.3). For each foraging  
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  
226 “betweenness\_w” in the *tnet* package (Opsahl, 2009) and the function “authority.scores” in the *igraph*  
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)  
230 and all the interactions among these predictors as fixed effects. We used individual identity nested in  
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,  
234 weighted betweenness centrality and authority scores) because their values were constrained  
235 between 0 and 1. We also applied a zero inflation method using the Beta Inflated (BEINF) family  
236 function from the *gam/ss* package (Rigby & Stasinopoulos 2005). Model selection of the Beta  
237 regression mixed models are shown in the supplementary materials (Tables S1, S2 and S3). Because  
238 network metrics are correlated, we applied a Bonferroni correction by setting the alpha level of  
239 significance at 0.017 (Tylianakis *et al.*, 2007).

240

241

242



### 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  
246 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  
248 graph A, B, C) can also occur and being represented for a maximum of 7 times in a network with 7  
249 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  
252 flowers: 21 and 7; for 6 flowers: 12, 6; for 5 flowers: 5, 5; as indicated by red horizontal lines in Figure  
253 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  
255 error) using the observed frequency for each motif and for each dataset as response variable, the  
256 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  
260 also calculated non-network measures used in previous studies for assessing the ability of bees to  
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  
264 visited. Both measures of route efficiency are expected to decrease with increasing network efficiency,  
265 and reach a minimum in an optimal movement network. We applied a GLMM for count data to study  
266 the impact of experience (foraging bout) on the number of revisits to flowers and a linear mixed effect  
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 ( $\text{estimate}_{\text{bout}} = 0.066$ , 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  
275 tendency was stronger in large spatial scale arrays ( $\text{estimate}_{\text{small\_array}} = -0.067$ , se = 0.005,  $t = -13.55$ ,  
276  $P < 0.001$ ). Interestingly, in small spatial scale arrays bumblebees showed higher average weighted  
277 betweenness centrality ( $\text{estimate}_{\text{honeybees}} = -1.172$ , se = 0.164,  $t = -7.15$ ,  $P < 0.001$ ) and a tendency to  
278 develop optimal networks faster ( $\text{estimate}_{\text{honeybees}} = -0.020$ , se = 0.008,  $t = -2.533$ ,  $P = 0.011$ ) than  
279 honeybees (Figure 4).

280 The average Kleinberg’s authority scores also increased as bees accumulated experience in  
281 the six experiments ( $\text{estimate}_{\text{bout}} = 0.083$ , se = 0.03,  $t = 2.781$ ,  $P = 0.004$ ), meaning that all flowers  
282 became equally important in the network. For both bee species, the average authority scores were

283 lower in small spatial scale arrays than in large spatial scale arrays (estimate<sub>small\_arrays</sub> = -0.446, se =  
284 0.103,  $t = -4.334$ ,  $P < 0.001$ ). However, honeybees had larger average authority scores than  
285 bumblebees in the small spatial scale arrays (estimate<sub>small\_arrays\_honeybees</sub> = 0.582, se = 0.111,  $t = 5.229$ ,  
286  $P < 0.001$ ) 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  
293 experience (estimate<sub>honeybees\_small\_arrays</sub> = 0.407, se = 0.148,  $t = 2.752$ ,  $P = 0.006$ ). This again illustrates  
294 the much reduced route optimisation efficiency of honeybees in comparison to bumblebees at small  
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*

301 While bees initially used the 16 possible motifs to link flowers, they gradually reduced the number of  
302 motifs to only use two of them by the end of training (motifs 2 and 6), a behaviour that is characteristic  
303 of route optimisation (Figure 3). This tendency was less pronounced for honeybees at small spatial  
304 scales (Figures 3D and 3E). Analyses of the frequency usage of each motif confirmed that honeybees  
305 at small spatial scales often presented opposite tendencies than honeybees at large spatial scales or  
306 bumblebees at all spatial scales (Figure 3D: motifs 3, 7, 8 and 15; Figure 3E: motifs 4, 5, 10 and 15)  
307 (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  
321 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*

323 *al.*, 2016). Here we show how spatial network analyses can be developed to study the movement  
324 patterns of individual bees exploiting multiple feeding locations at various spatial and temporal scales  
325 in simplified experimental conditions. We argue that this approach may prove particularly helpful to  
326 analyse pollinator movements in more complex and ecologically realistic experimental designs and to  
327 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  
332 contrast the average clustering of flowers decreased with time as bees started to develop optimal or  
333 suboptimal stable movement networks. This tendency for optimisation of spatial movement networks  
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  
336 studies (e.g. Saleh & Chittka, 2007; Lihoreau *et al.*, 2012a; Buatois & Lihoreau 2016), we found that  
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  
339 spatial networks at large spatial scales, suggesting that they use more complex optimisation  
340 movement rules in more costly conditions. These results were confirmed with more conventional  
341 statistical approaches (e.g. flower re-visits, travel efficiency), thereby validating our approach.  
342 Importantly, the global network approach, based on motif analyses, brought new insights into the  
343 spatial behaviour of bees. For instance the foraging patterns of honeybees were characterised by  
344 frequent back and forth movements between flowers (Figure 6D - i.e. motifs 7 & 8) and  
345 disproportionate usage of specific flowers or local hubs (Figure 6D – i.e. motif 4).

346 The aim of this exploratory study was to introduce spatial network analyses for characterising  
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  
353 are hardly detectable with current measures of sequence repeatability (Thomson *et al.*, 1997; Ayers *et al.*,  
354 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  
357 constraints of resource locations, in order to extend our approach to field conditions. Experimentally,  
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  
361 resource diversity in network formation, and bring new insights into how bees integrate memories of  
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  
364 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  
366 during the process of route optimisation. Other network measures, not used here, may also help  
367 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  
370 honeybee movement patterns in relatively simple foraging conditions aims at illustrating how network  
371 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  
378 than honeybees. Bumblebees tended to reach a frequency of each triadic structure that would lead to  
379 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  
381 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  
383 their return to the hive (Von Frisch, 1967; Dornhaus *et al.*, 2006). These insects may thus invest less  
384 in individual sampling and efficient route learning than species lacking the means to communicate  
385 foraging locations, such as bumblebees (Buatois & Lihoreau, 2016). Another possibility is the  
386 difference of typical foraging range between the two species. While bumblebees rarely cover more  
387 than three kilometres to exploit floral resources (Osborne *et al.*, 2008), honeybees can travel more  
388 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  
397 development of pollinator movement networks may also include detailed dynamic temporal analyses  
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  
401 prove useful to integrate rate of change of flower visitation sequences. New metrics could be  
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  
413 pollination processes and the genetic structuralism of plant populations. The development of optimal  
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  
416 Clare *et al.*, 2013; and metabarcoding; Pornon *et al.*, 2016) now allow identification of flower species  
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 al.*,  
419 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  
423 information about how the foraging strategies of bees directly influence pollen transfer and plant  
424 mating patterns across landscapes, and therefore a better assessment of consequences of bee  
425 declines on pollination.

426  
427

#### 428 **Contribution of the authors**

429 ML designed and conducted the experiments; CP built and analysed the networks; CP, RJ, CA, LC  
430 and ML wrote the manuscript. All authors gave final approval for publication. Authors declare no  
431 competing interests.

432

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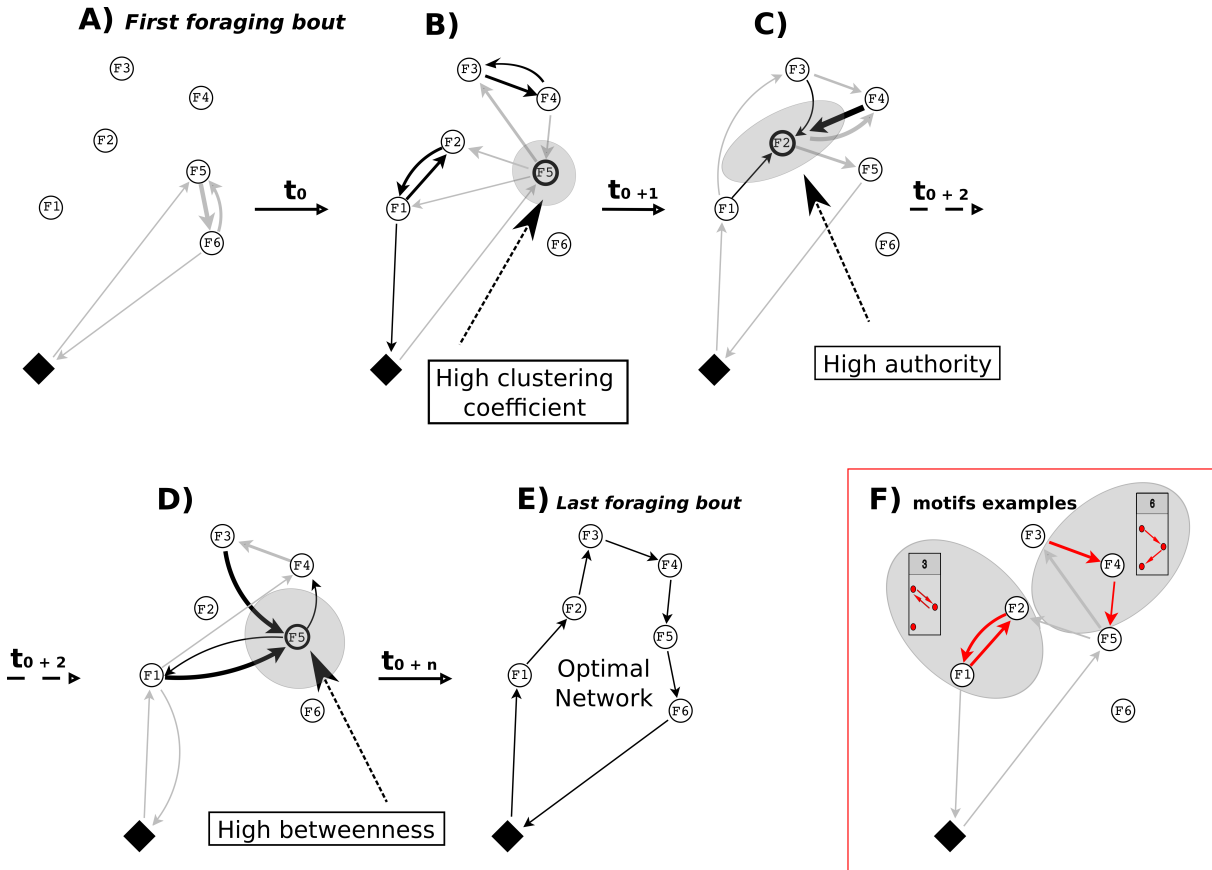
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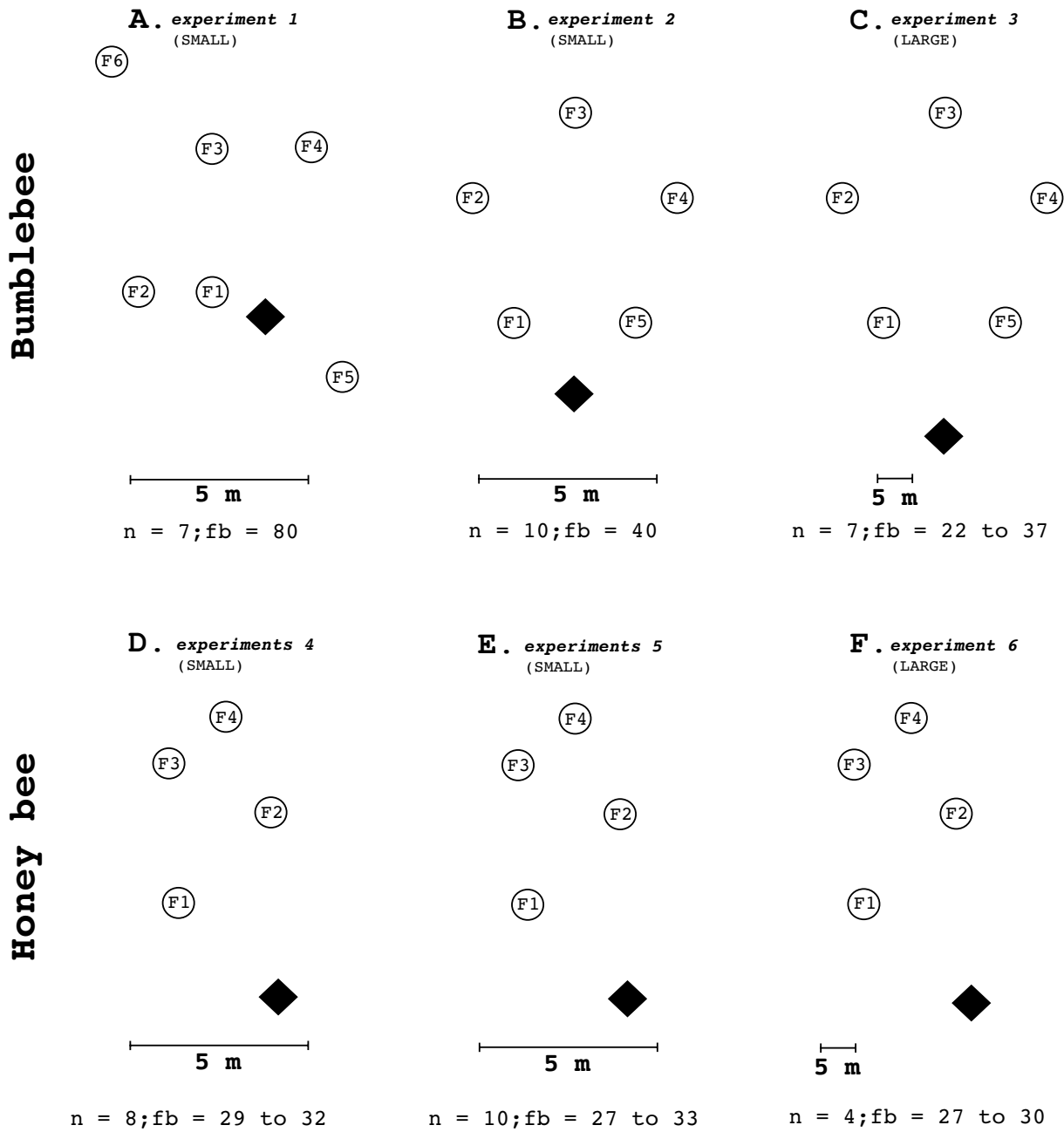
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## Figure legends



**Figure 1.**

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 ( $t_0$ ,  $t_0 + 1$ ,  $t_0 + 2$ ,  $t_0 + n$ ) 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).

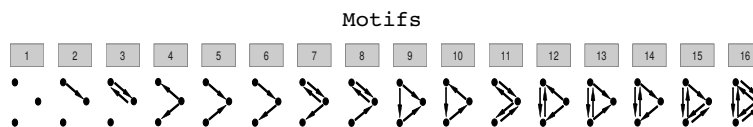
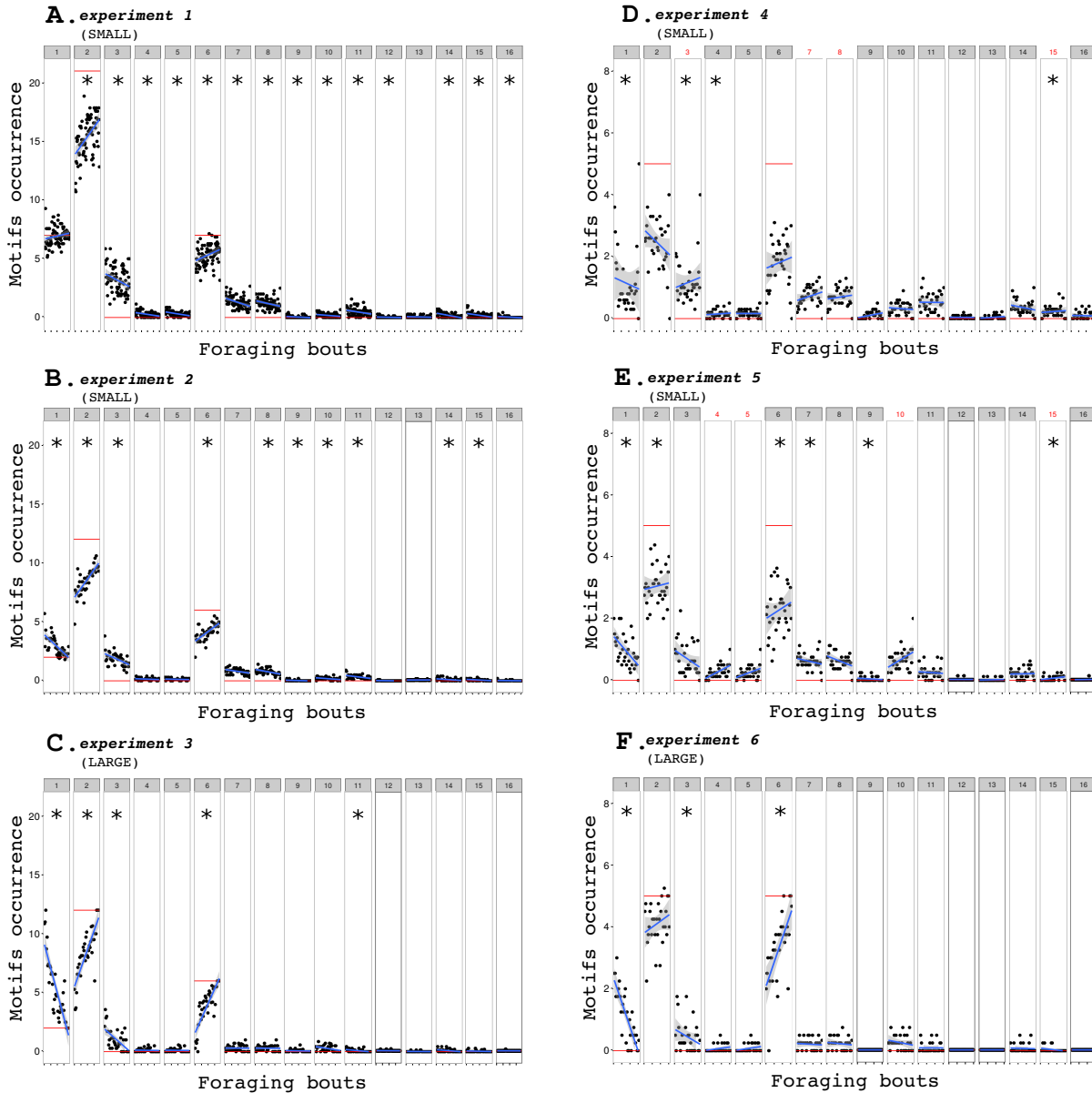


**Figure 2.**

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.*, 2011). C. Experiment 3: bumblebees in the field (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).

# Bumblebee

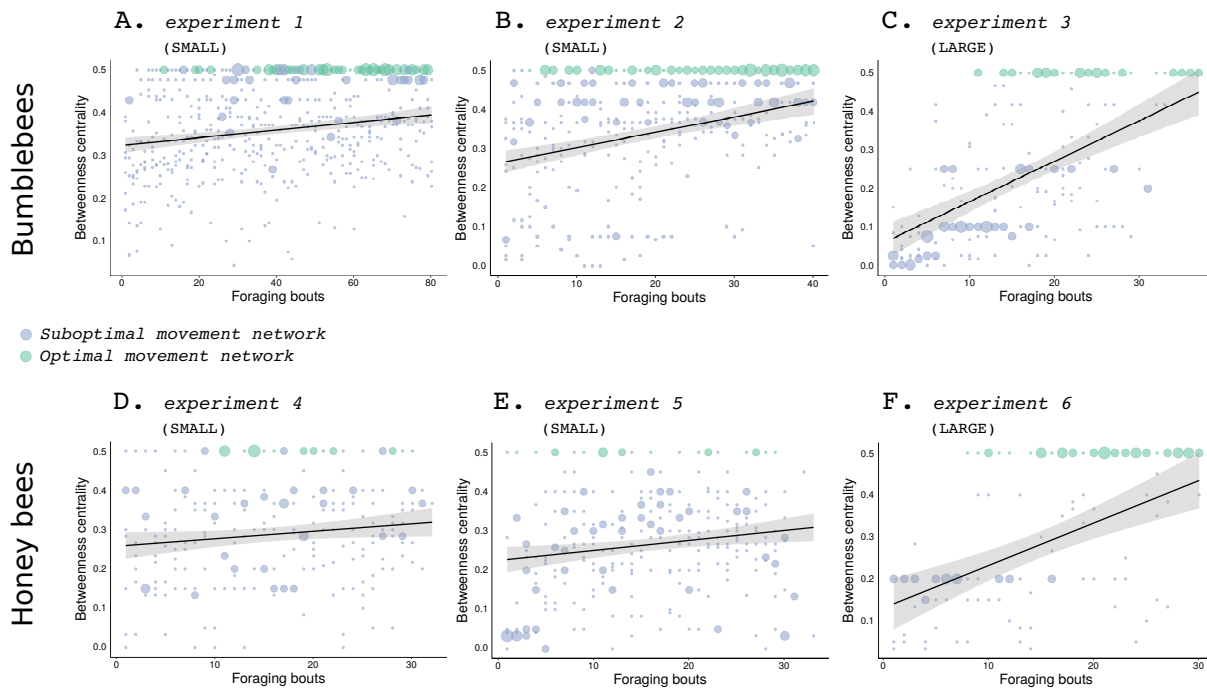
# Honey bee



**Figure 3.**

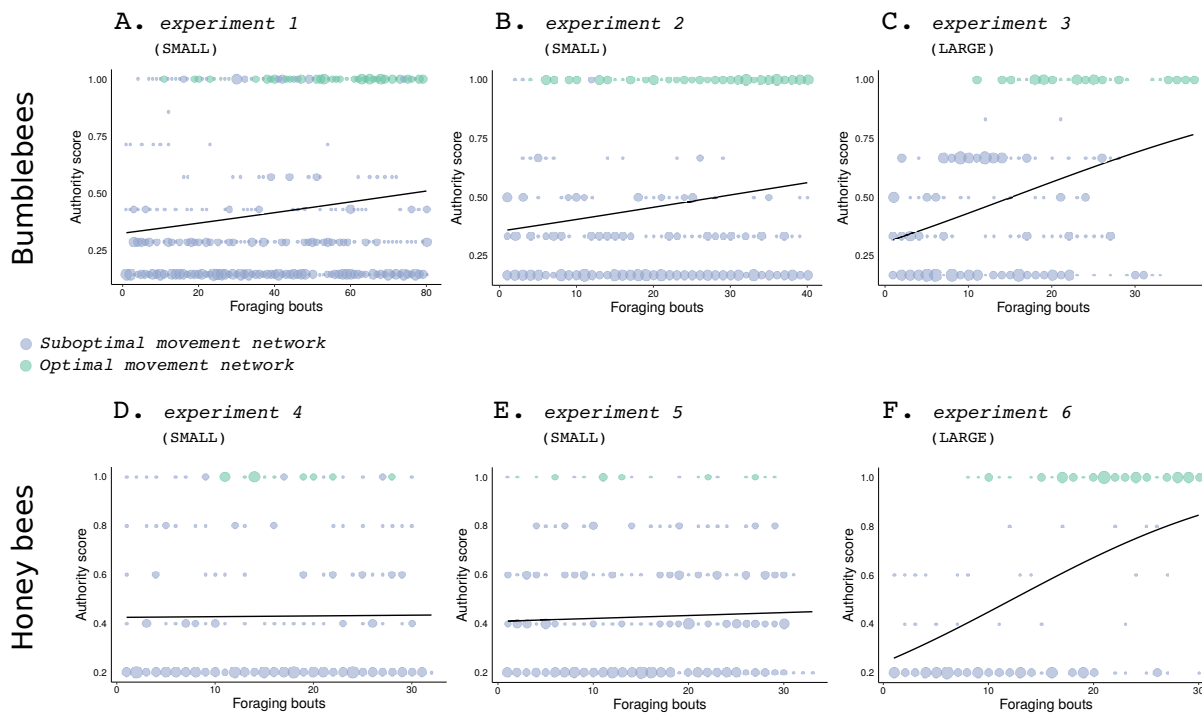
Distribution of all possible network triadic motifs across foraging bouts. For each motif, the x-axis represents the temporally ordered foraging bouts. Red horizontal lines indicate the frequency of each motif expected in the optimal network. Best fitted lines obtained from generalized linear models 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).



**Figure 4.**

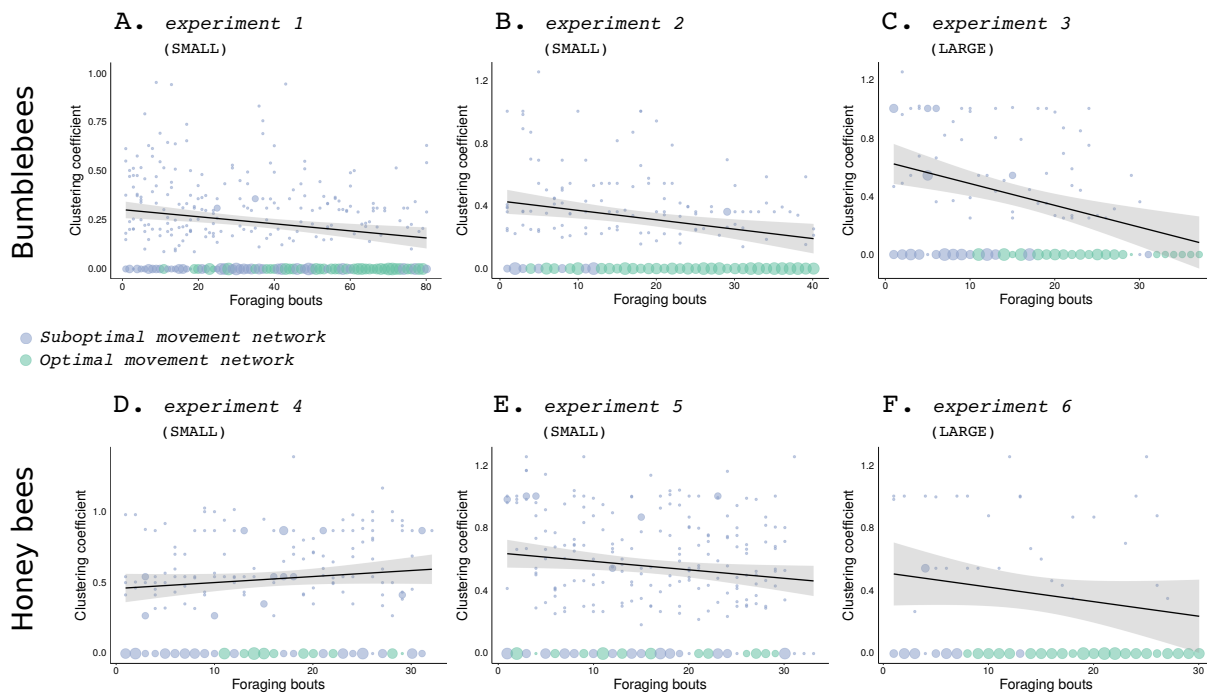
Average weighted betweenness centrality values for each individual bee at each foraging bout. Black lines and grey shaded areas represent respectively the best fitted lines and their standard errors obtained from zeroinflated mixed effect models built using foraging bouts as fixed effect and individual 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).



**Figure 5.**

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).





**Figure 6.**

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.

<b>Beta regression</b>	<b>df</b>	<b>GAIC</b>
~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.

<b>GLMM Proportional model</b>	<b>df</b>	<b>GAIC</b>
~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.

<b>Beta regression</b>	<b>df</b>	<b>GAIC</b>
~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

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

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

	Estimate (SE)	t-values	P-values
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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

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

	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 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. Estimates, their standard errors (SE), the appropriate statistics (z-value for GLMM or t-value for LMM) and P-values are shown.

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

Distance / Number of unique flowers visited

-0.321 ± 0.135

(t) -2.38

0.019

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