

Social, spatial and temporal segregation in an ant society

Lauren E. Quevillon^{1,2*}, Ephraim M. Hanks^{1,3}, Shweta Bansal⁴ and David P. Hughes^{1,2,5}

1 Center for Infectious Disease Dynamics, Penn State University, University Park, Pennsylvania, USA, 2 Department of Biology, Penn State University, University Park, Pennsylvania, USA, 3 Department of Statistics, Penn State University, University Park, Pennsylvania, USA, 4 Department of Biology, Georgetown University, Washington, D.C., USA 5 Department of Entomology, Penn State University, University Park, Pennsylvania, USA

* Email: leq103@psu.edu

1 **Introduction:**

2 Sociality can be risky. A chief cost of social living is increased transmission of
3 infectious diseases, due to higher population densities combined with greater
4 contact between susceptible and infected individuals (1,2,3,4). This greater
5 encounter rate has led to a growing interest in the role of social contact structure
6 in infectious disease transmission (5,6,7,8,9,10,11) To capture the dynamics of
7 disease spread within dense groups, epidemiological models are shifting from
8 the principle of mass action, in which infected and susceptible individuals are
9 assumed to mix randomly, to explicitly incorporating patterns of interaction
10 through which infectious agents are transmitted (12). Understanding how
11 interactions impact epidemiology has real-world applications. The growing global
12 connectivity of human communities, coupled with closer proximity to
13 domesticated and wild populations of animals and plants, will impact the
14 incidence of infectious diseases worldwide. Thus, predicting and mitigating the
15 spread of infectious diseases by understanding transmission flow through social
16 contact networks remains a chief One Health imperative (13,14).

17 Despite the increased propensity for disease transmission inherent to
18 group living, some social organisms have largely overcome this issue. Social
19 insects of the Order Hymenoptera (ants, bees, wasps) are ecologically dominant
20 in almost all terrestrial environments, despite their incredibly dense societies and
21 high degree of genetic relatedness (15). This is not for want of infectious agents-
22 social insects are host to a wide array of pathogens and parasites (16,17,18).
23 Social insects are thought to overcome intense infection pressures through a

24 series of prophylactic and inducible defenses collectively termed “social” or
25 “collective” immunity (19,20). These defenses range from the immunological to
26 the behavioral, including the way colonies are organized and tasks are allocated
27 to workers (21,22,23).

28 The social and spatial segregation of workers most susceptible to infection
29 is often cited as a major mechanism of disease prophylaxis in social insect
30 colonies (24,25). However, it is unclear if such segregation does indeed occur.
31 We remain unsure because observing individual behavior within a realistic colony
32 has been a formidable task. Here we pursue this avenue of inquiry by testing for
33 the presence of social and spatial segregation in colonies of the carpenter ant,
34 *Camponotus pennsylvanicus*, using analysis of ant social networks combined
35 with individual movement data. *C. pennsylvanicus* is an ant species that has
36 evolved to nest inside dead trees; we mimicked this by maintaining colonies
37 inside wood under complete darkness. We focused on the oral exchange of food,
38 trophallaxis, as the key social interaction of interest because colonies must
39 balance efficient resource flow with mitigating disease spread (26). If social
40 segregation does occur, we would expect to see its signature represented in the
41 trophallactic interactions between castes.

42 Through the integration of biologically realistic behavioral observations
43 with network and spatial models centered on individual behavior, we ask if ant
44 castes are indeed segregated within the colony. Studies of social insects have
45 greatly benefitted from network analysis because it links local interactions
46 between individuals to the emergent, colony-wide properties that they produce

47 (27,28,29). Several network metrics are of particular relevance to disease
48 transmission, including degree and betweenness centrality. Degree centrality, the
49 number of unique individuals that a given focal ant interacts with, summarizes
50 that individual's exposure to and potential transmission of infectious agents (11).
51 Although understanding the position of an individual within their social network is
52 important, knowing their spatial context is also crucial for disease flow. Recent
53 advances in automated tracking ability have enhanced our understanding of
54 colony-wide properties such as spatial segregation of different castes (30).
55 However, no empirical studies to date have explicitly linked individual behavior,
56 social network position, and spatial location in a single study. For this reason we
57 combine our network analyses with a statistical analysis of ant movement within
58 the nest.

59 Finally, we also study the duration and temporal order of trophallaxis
60 interactions because although network and spatial position of individuals are
61 considered important for disease dynamics, the timing of ant-ant interactions is
62 also likely important. We find a number of patterns counter to the strongly
63 prevailing view of social immunity. Within the colony conditions for disease
64 spread would appear ideal. However, by integrating network, spatial and
65 temporal views we find that barriers to disease spread likely exist. It is through
66 this integration of spatial and network analyses with time that might best inform
67 our understanding of disease flow in other complex societies.

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69

70 **Results:**

71 Two colonies of *Camponotus pennsylvanicus*, each containing 75 workers and
72 their queen, were individually housed in an experimental set-up consisting of a
73 wooden nest (area = 63cm²) separated from a foraging arena (area = 63cm²) by
74 a 4m long maze. Inside the foraging arena, ants had *ad-libitum* access to 20%
75 sucrose solution, water, and a protein source. This 4m separation between the
76 nest and the foraging arena ensured a clear behavioral separation between ants
77 allocated to foraging versus internal nest tasks. Video filming for behavioral
78 analysis was accomplished using a video camera (GoPro Hero2 with modified IR
79 lens, www.ragecameras.com) mounted over the wooden nest illuminated under
80 infrared (IR) light. Ants are unable to perceive light in the IR end of the spectrum
81 and thus its presence was not observed to affect their behavior. Two colonies
82 were filmed over 8 consecutive nights within +/- 30 minutes of 21:00 (when *C.*
83 *pennsylvanicus* actively forages, L. Quevillon personal observation). An observer
84 watched this playback on a large computer monitor (size) to facilitate behavioral
85 scoring. Each individual ant was observed for trophallaxis events (two ants orally
86 exchanging liquid) for the initial 20 minutes of each recording for each night,
87 leading to a total of over 400 hours of observation (76 ants x 2 colonies x 0.33
88 hours x 8 nights). The identities of the individuals interacting, the start and stop
89 time of their interaction, and the location of their interaction within the nest was
90 recorded. Additionally, the behavioral identity of the ant during the course of the
91 recording (ie. whether it was an active forager, inactive forager, nest worker, or
92 queen, see methods) was also recorded.

93 *Static network analysis*

94 There was a significant difference in trophollaxis count between worker
95 types. Foragers engaged in more trophollaxis events than did either nest workers
96 or the queen, although there was no significant difference between active and
97 inactive foragers (post-hoc Tukey HSD on one-way ANOVA, Fig. 1A) However,
98 when the duration of these events is compared across worker types, nest
99 workers had on average the longest trophollaxis exchange, and this was only
100 statistically different from inactive foragers (post-hoc Tukey HSD on one-way
101 ANOVA Fig 1B).

102 Static, unweighted network analyses were conducted on the trophollaxis
103 interaction for a single colony using the package ‘iGraph’ (31) implemented in R
104 (32). Active foragers (ants who were observed to forage during the video
105 recording) had a higher degree (number of unique individuals with which they
106 engaged with through trophollaxis) than inactive foragers, nest workers, or the
107 queen. This represents an average of 2 additional unique individuals that
108 foragers exchanged food with compared to the queen. While the queen had an
109 average degree of 1, the identity of the individual she interacted with was not
110 consistent across nights.

111

112 *Ant movement and spatial analysis*

113 For each colony, individual ant movement patterns were investigated by
114 randomly choosing five known foraging ants, five known nest workers, and the
115 queen to have their spatial movement data recorded. The wooden nest in which

116 ants were housed was gridded to a resolution of 1cm^2 , and the cell locations
117 where the majority of the ant's body was located as well as the time stamp when
118 it was in that location were recorded for the entire 20-minute duration of the video
119 for each of the 8 nights. The residence time spent in each cell was recorded and
120 summed over all ants to determine nest spatial use. In both colonies, the queen
121 Residence times in each cell and transitions to neighboring cells were used to fit
122 a continuous-time discrete-space random walk model for ant movement
123 behavior, where were used to calculate a movement or transition rate between
124 cells.

125 The average spatial usage of foragers, nest workers, and the queen is
126 given in Fig. 3. Foragers occupied a greater proportion of the nest than did either
127 non-foraging nest workers or the queen. The queen was largely immobile in both
128 colonies, though in one colony (Col10), the queen spent some time in 3 of the 4
129 chambers of the nest.

130 To test for differences in movement behavior, we used a continuous-time
131 discrete-space Markov chain model for ant movement (33) that allows for testing
132 differences in movement behavior between worker types in response to spatial
133 covariates. We tested for differences in overall mean movement rates between
134 foraging and non-foraging nest workers, and for changes in movement rates
135 when in the same chamber as the queen. We also tested for directional bias in
136 movement behavior toward or away from the queen (i.e., queen avoidance).
137 Results of this analysis show that in both colonies non-foraging ants are more
138 mobile (have higher movement rates) than are foraging ants while in the nest

139 ($p < 10^{-10}$, T-test). There was no evidence of directional queen avoidance by
 140 foraging or non-foraging ants in either colony, but there was strong evidence in
 141 one colony (RG2) that foraging ants move faster when near the queen than when
 142 in another chamber ($p < 10^{-14}$, T-test) and that non-foraging ants tend to move
 143 slower when in the same chamber as the queen ($p < 0.01$, T-test). In the second
 144 colony (Col10), non-foraging ants also tend to move slower when near the queen
 145 ($p < .01$, T-test), but there is small evidence that foraging ants move faster near
 146 the queen ($p < .3$, T-test). This discrepancy is likely due to the increased
 147 movement of the queen in the second colony, which obscures the spatial
 148 movement signal.

149

150 **Table 1:** Inference on ant movement parameters in a continuous-time random
 151 walk model of ant movement in two ant colonies (See Fig. 3)

	<u>Effect</u>	<u>Estimate</u>	<u>Std. Error</u>	<u>p-val (T-test)</u>
RG2	F	-2.2599	0.02222	$< 10^{-15}$
	NF	-0.92576	0.02035	$< 10^{-15}$
	F*DFQ	-0.19091	0.02453	$< 10^{-14}$
	NF*DFQ	0.04115	0.01435	0.00416
	F*GDFQ	-0.01581	0.03571	0.65801
	NF*GDFQ	-0.00106	0.01023	0.91763
	Col10	F	-3.18845	0.04111
NF		-1.63299	0.03379	$< 10^{-15}$
F*DFQ		-0.04696	0.04114	0.25371
NF*DFQ		0.12605	0.03304	0.00014
F*GDFQ		-0.02391	0.04120	0.56160
NF*GDFQ		-0.00770	0.03221	0.81119

152

153

154 *Time-ordered (time-dependent) social network analysis*

155 Social network data has traditionally been analyzed as a time-aggregated
156 or static graph, in which the timing of interactions and their order is ignored.
157 However, this timing and order is crucially important for dynamic flow processes,
158 such as disease transfer (34). We re-analyzed the ant interaction data using the
159 package ‘timeordered’ (35) implemented in R. This specifically incorporates the
160 time stamp of interactions when computing network metrics, and allows for a
161 much more biologically meaningful picture of intra-colony interactions of import to
162 disease. Fig. 4 shows a representative time-ordered network graph. Based on
163 the timing of interactions, returning foragers were never actually observed to
164 interact in a way necessary for disease transmission.

165 **Discussion:**

166
167 The results of this study provide a comprehensive view of social, spatial
168 and temporal segregation of different ant types within the colony. Static network
169 analyses revealed that actively foraging ants engage in social food exchanges
170 with more individuals than either nest workers or the queen. This is a surprising
171 finding given that actively foraging ants have the highest disease exposure of all
172 ants, and thus social immunity theory would predict that their contact with
173 susceptible nest mates should be minimized (17,19). When the duration of
174 trophallaxis events is taken into account, however, there are not statistically
175 significant differences between foragers and nest workers. This could be a
176 function of the biological limits to oral food transfer in *C. pennsylvanicus* and is
177 worth further investigation. This component of the trophallaxis data is in

178 accordance with what social immunity theory would predict (ie. foragers don't
179 engage long, and therefore dangerous, trophollaxis interactions with nest mates).

180 In addition to social position of ants within the colony, we were also
181 interested in the spatial activity of such ants. Analysis of nest spatial usage
182 showed that foragers are spatially promiscuous, nest workers are less so, and
183 the queen hardly moves. While the queen's lack of movement syncs well with our
184 predictions from social immunity studies, the expansive movement of the
185 foragers is counterintuitive; theory predicts that foragers should be avoiding
186 internal areas of the nest. However, in one colony (RG2) , it does appear that
187 foragers may be modulating their speed in response to their social environment.
188 When foraging ants were in the same chamber as the queen, they moved faster
189 than their nest worker counterparts. By moving faster near the most important
190 individuals in the colony, foragers may be reducing the potential transmission of
191 any infectious agents that they may have been exposed to.

192 The static network analyses of colony social organization and the spatial
193 promiscuity of foragers to queens reveal an ant society not particularly well suited
194 to the prevention of disease transmission. However, the inclusion of temporal
195 data makes this situation far less dire than what it appears. When the timing and
196 order of trophollaxis interactions are taken into account, foragers and the queen
197 never interact in a way that could lead to the biologically meaningful transfer of
198 disease (ie. after a forager has come back into the nest after a foraging trip,
199 carrying some pathogen that might transfer to the queen via close proximity or
200 oral food exchange). Thus, the timing of social interactions coupled with

201 movement rates provides evidence for behavioral prophylaxis within *C.*
202 *pennsylvanicus* colonies.

203 Through the incorporation of social interactions, individual movement data,
204 and the timing of social interactions, we now have a better understanding of how
205 disease prophylaxis could be accomplished in *C. pennsylvanicus* ant societies.
206 Had the timing of interactions and movement been ignored, a different picture
207 invalidating tenets of social immunity theory would have emerged. This provides
208 further evidence for the growing argument that temporal information and
209 meaningful behavioral interactions should be included into social network
210 analyses if we are to make biologically accurate conclusions (34). Laboratory
211 studies involving animal behavior benefit from the incorporation of environmental
212 complexity and ecological realism. We encourage the continued advancement of
213 experimental set-ups if we are to gain a true understanding of how social insect
214 societies are structured.

215 Having provided a necessary null model of colony organization in the
216 absence of disease, future experiments in which laboratory infections are
217 combined with network analyses will further inform the extent to which colony
218 organization reduces disease transmission in social insect societies. Such
219 studies will also afford us the ability to synchronize theoretical predictions from
220 agent-based modeling approaches (36) with empirical data that will allow for
221 enhanced model parameterization. Social insect societies are a powerful model
222 system for investigating how perturbations in social structure can influence
223 disease transmission dynamics. However, to realize their full potential we

224 advocate for continued inquiry through the use of biologically meaningful
225 behavioral interactions that include temporal information.

226 **Methods:**

227

228 *Ant colony set-up and filming*

229

230 Two queen-right *Camponotus pennsylvanicus* colonies were collected
231 from field sites in Pennsylvania, U.S.A. in December 2012. Seventy-five worker
232 ants were haphazardly selected from each colony and were individually labeled.
233 Labels consisted of numbers printed on photo paper that were affixed to the ants'
234 gasters with optically clear nail polish. The labeling was not observed to alter the
235 ants' behavior or interactions (L. Quevillon, personal observation).

236

237 The labeled ants and the queen were housed in a nest set-up consisting
238 of a four-chambered wooden nest (total area = 63 cm²) that was gridded to a
239 resolution of 1cm² and covered with a plexiglas top. This nest was contained
240 within a filming box so that nest conditions were always dark. The nest was
241 separated from a sand-bottomed foraging arena (total area = 63 cm²) by a 4m
242 long maze. The length of the maze ensured that there was a clear separation
243 between workers allocated to foraging versus internal colony tasks (L. Quevillon,
244 personal observation). Inside the foraging arena, ants had *ad libitum* access to
245 water, 20% sucrose solution and mealworms.

246

247 Each colony was filmed at +/- 30 minutes of 21:00 on 8 consecutive nights
248 in June 2013 using a GoPro Hero2 camera with a modified IR filter
249 (RageCams.com) illuminated under infrared light (Canon CMOS IR light).
250 Infrared light, which ants are unable to perceive (reference), was not observed to
251 affect ant behavior.

252

253 *Video analysis and ant worker classification*

254

255 For each night of filming, the tropholactic interactions of every ant inside
256 the nest were individually observed. Due to degradation of IR light intensity while
257 filming, only the first 20 minutes of each video were analyzed. For each
258 tropholactic interaction that was observed, the ant identities, start time, stop
259 time, and location within the nest were recorded. Additionally, the overall
260 behavioral category of each ant on each day was recorded (i.e. nest worker,
261 forager, non-active forager, queen). Nest workers were ants that were never
262 observed to leave the nest, foragers were ants that actively left the nest during
263 the course of the video segment, and inactive foragers were ants that had been
264 witnessed to leave the nest in video segments on previous days, but which did
265 not leave the nest during the video segment being currently analyzed.

266

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268

269 *Trophollaxis count and duration*

270

271 The number of trophollaxis events and their duration for each individual in
272 Colony 10 was recorded as given above. To test for differences in both
273 trophollaxis count and duration as a function of ant type (ie. forager, inactive
274 forager, nest worker, or queen), a one-way analysis of variance was conducted
275 using .aov in R. Post-hoc tests for differences (Tukey HSD) were then used to
276 determine which ant types had significant differences from each other.

277

278 *Static network analysis*

279

280 Network metrics were analyzed for colony 10 for each night of
281 observation. Unweighted, static network analyses were conducted using the
282 iGraph package (31) implemented in R (32). Metrics analyzed for each individual
283 ant included degree, betweenness centrality, closeness centrality, and Burt's
284 constraint.

285

286 *Spatial movement analysis*

287

288 The time-referenced spatial locations of the queen, 5 forager ants, and 5
289 randomly chosen nest-worker ants were recorded for each night. We used a
290 continuous-time discrete-space agent-based random walk model (33,37) to make
291 inference about ant movement behavior. The CTDS framework is notable in that
292 it allows for inference on both directional (e.g., queen avoidance) and location-
293 based (e.g., variable movement rates in different nest chambers) movement
294 mechanisms. Additionally, Hanks et al., (2013) have shown how inference can be
295 made on CTDS movement models under a standard generalized linear modeling
296 (GLM) framework, which leads to intuitive inference and efficient computation.
297 Drawing on standard continuous-time Markov chain models (e.g., 38), if an ant is
298 in cell i at time t , then define the rate of transition from cell i to a neighboring cell j
299 as $\lambda(ij)$. The total rate $\lambda(i)$ at which ants move (transition) out of cell i is the sum of
300 the rates to all neighboring cells: $\lambda_i = \sum_j \lambda_{ij}$, and when the ant moves, the probability
301 of moving to cell k (instead of to another neighboring cell) is the ratio: λ_{ik} / λ_i .

302

303 To model ant movement behavior near the queen, we will model $\lambda(ij)$ as a
304 function of a spatial covariate which measures the distance from the queen's
305 most used locations (DFQ) at each grid cell (Figure 3). To examine local
306 behavior, the DFQ covariate was set to be constant out of the queen's chamber.
307 The DFQ covariate is location-based and will allow us to model differences in
308 movement rates when near or far from the queen. We also considered a
309 directional covariate, a gradient of the DFQ covariate (GDFQ). The GDFQ
310 gradient is a directional vector that points towards the queen, or along the
311 direction of steepest ascent of the DFQ covariate, and the GDFQ covariate will
312 be different for the transition rates to neighboring cells in different directions, thus
313 allowing for directional preference in ant movement. We also consider potential

314 differences in movement behavior between foraging (F) and non-foraging (NF)
315 ants, with F=1 for foraging ants and F=0 otherwise, and NF=0 for foraging ants
316 and NF=0 otherwise. We model the movement rate $\lambda_k(ij)$ of the k -th ant from cell
317 i to cell j as a function of interactions of these covariates and corresponding
318 regression parameters $\{\beta\}$:

319

$$320 \lambda_k(ij) = \exp\{ F_k\beta_1 + NF_k\beta_2 + (F_k * DFQ_i)\beta_3 + (NF_k*DFQ_i)\beta_4$$
$$321 + (F_k*GDFQ_{ij})\beta_5 + (F_k*GDFQ_{ij})\beta_5 \}$$

322

323 Differences in overall movement rates between foragers and non-foragers will be
324 represented by differences in β_1 and β_2 , with positive values corresponding to
325 higher movement rates. Positive values of β_3 correspond to higher movement
326 rates of foraging ants when far from the queen, and decreased movement rates
327 near the queen. Positive values of β_5 correspond to preferential directional
328 movement by foragers away from the queen (in the direction of the increase in
329 the gradient of DFQ). The parameters β_4 and β_6 correspond to the response of
330 non-foraging ants to DFQ and GDFQ, respectively. Hanks et al. (2013) have
331 shown that inference on the parameters in this movement model can be
332 accomplished using a Poisson GLM, which we fit using the 'glm' command in R.
333 Results are summarized in Table 1.

334

335 *Temporal (time-ordered) network analysis*

336

337 Interactions from the static network analyses were re-analyzed including
338 the time-stamp of when the interactions occurred. Temporal networks were
339 constructed using the package 'timeordered' in R. The time to interaction
340 between foraging ants and the queen was calculated using the function
341 'shortesttimepath'.

References:

1. Alexander, RD. (1974) The evolution of social behavior. *Annu. Rev. Ecol. Syst.* 1974.5:325-383
2. Côté IM and R Poulin. (1995) Parasitism and group size in social animals: a meta-analysis. *Behavioral Ecology* 6:2 (159-165).
3. Arneberg P, A Skorping, B Grenfell, and AF Read. (1998) Host densities as determinants of abundance in parasite communities. *Proc. R. Soc. Lond. B* 265, 1283-1289
4. Altizer S, CL Nunn, PH Thrall, JL Gittleman, Antonovics, AA Cunningham, AP Dobson, V Ezenwa, KE Jones, AP Pedersen, M Poss, and JR Pulliam. (2003) Social organization and parasite risk in mammals: integrating theory and empirical studies. *Annual Review of Ecology, Evolution, and Systematics*, 34: 517-547
5. Newman ME. (2002) The spread of epidemic disease on networks. *Phys. Rev. E* 66, 016128
6. May RM. (2006) Network structure and the biology of populations. *TRENDS in Ecology and Evolution*: 21(7)
7. Bansal S, BT Grenfell, and LA Meyers. (2007) When individual behaviour matters: homogeneous and network models in epidemiology. *J. R. Soc. Interface* 4, doi: 10.1098/rsif.2007.1100
8. Keeling, MJ & P Rohani. (2007) Modeling infectious diseases in humans and animals. Princeton, NJ: Princeton University Press.
9. Read JM, TD Eames, and WJ Edmunds. (2008) Dynamic social networks and the implications for the spread of infectious disease. *J. R. Soc. Interface* 2008 5, 1001-1007
38. Keeling MJ & KTD Eames. (2005) Networks and epidemic models. *J. R. Soc. Interface* 2, 295–307. (doi:10.1098/rsif.2005.0051)
10. Salathé M, M Kazandjieva, JW Lee, P Levis, MW Feldman and JH Jones. (2010) A high-resolution contact network for infectious disease transmission. *Proc. Nat'l. Acad. Sci.* 107:51 22020–22025
11. House T and MJ Keeling. (2012) Epidemic prediction and control in clustered populations. *Journal of Theoretical Biology* 272: 1-7
12. Gonçalves B, Perra N and Vespigiani A. (2013) Social Networks, Contagion Processes, and the Spreading of Infectious Disease. In: *Handbook of Systems Biology Concepts and Insights*. Elsevier Inc.
13. Smith KP and NA Christakis. (2008) Social networks and health. *Annual Review of Sociology* 34.
14. MacKenzie JS, M Jeggo, P Daszak and JA Richt [eds.]. (2013) One Health: The Human-Animal-Environment Interfaces in Emerging Infectious Diseases. Springer-Verlag Berlin Heidelberg
15. Hölldobler B and EO Wilson. (1990) The Ants. Belknap (Harvard University Press), Cambridge, MA.

16. Kistner DH. (1979) Social and evolutionary significance of social insect symbionts, pp. 339-413 in HR Hermann [ed.], *Social Insects*. Academic Press, New York, Vol 1, 437 p.
17. Schmid-Hempel, P. (1998) *Parasites in social insects*. Princeton University Press.
18. Boomsma JJ, P Schmid-Hempel and WOH Hughes. (2005) Life histories and parasite pressure across the major groups of social insects. In: *Insect evolutionary ecology*, ed. F Fellowes, G Holloway and J Rolff. Wallingford, UK: CABI.
19. Cremer S, SAO Armitage and P Schmid-Hempel. (2007) Social Immunity. *Current Biology* 17
20. Cotter SC and RM Kilner. (2010) *Personal immunity versus social immunity*. *Behavioral Ecology*. 21:4
21. Wang D, F Moeller. (1970b) The division of labor and queen attendance behavior of Nosema-infected worker honeybees, *J. Econ. Entomol.* 63, 1539–1541.
22. Hart AG, & FL Ratrieks. (2002) Waste management in the leaf-cutting ant *Atta colombica*. *Behavioral Ecology*, 13(2), 224-231.
23. Naug D and S Camazine. (2002) The role of colony organization on pathogen transmission in social insects. *Journal of Theoretical Biology*. 215
24. Fefferman NH, JFA Traniello, RB Rosengaus and DV Calleri. (2007) Disease prevention and resistance in social insects: modeling the survival consequences of immunity, hygienic behavior, and colony organization. *Behav Ecol Sociobiol.* 61:565–577
25. Cremer S and M Sixt. (2009) Analogies in the evolution of individual and social immunity. *Phil. Trans. R. Soc. B* 364, p.129 -142
26. Blonder B, A Dornhaus (2011) Time-Ordered Networks Reveal Limitations to Information Flow in Ant Colonies. *PLoS ONE* 6(5): e20298. doi:10.1371/journal.pone.0020298
27. Charbonneau D, B Blonder and A Dornhaus. (2013) Social insects: a model system for network dynamics. In: P. Holme and J. Saramaki (eds.), *Temporal Networks, Understanding Complex Systems*, DOI 10.1007/978-3-642-36461-7 11, Springer-Verlag Berlin Heidelberg
27. Fewell JH. (2003) Social insect networks. *Science* 301, 1867
28. Waters JS, JH Fewell. (2012) Information processing in social insect networks. *PLoS ONE* 7(7): e40337. doi:10.1371/journal.pone.0040337
29. Charbonneau D, B Blonder and A Dornhaus. (2013) Social insects: a model system for network dynamics. In: P. Holme and J. Saramaki (eds.), *Temporal Networks, Understanding Complex Systems*, DOI 10.1007/978-3-642-36461-7 11, Springer-Verlag Berlin Heidelberg
30. Mersch DP, A Crespi and L Keller. (2013) Tracking individuals shows spatial fidelity is a key regulator of ant social organization. *Science* 340: 6136 1090-1093.
31. Csardi G, T Nepusz. The igraph software package for complex network research, *InterJournal, Complex Systems* 1695. 2006. <http://igraph.org>

32. R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.
33. Hanks, EM, MB Hooten, and MW Alldredge. (2013) Continuous-Time, Discrete-Space Models of Animal Movement. arXiv preprint arXiv:1211.1992.
34. Blonder B, TM Wey, A Dornhaus, R James and A Sih. (2012) Temporal dynamics and network analysis. *Methods in Ecology and Evolution* **3**, 958–972
35. Blonder B. (2014) timeordered: Time-ordered and time-aggregated network analyses. version 0.9.4. <http://cran.r-project.org/web/packages/timeordered/timeordered.pdf>
36. Pie M, RB Rosengaus, JFA Traniello. (2004) Nest architecture, activity pattern, worker density and the dynamics of disease transmission in social insects. *Journal of Theoretical Biology*. 226 45-51
37. Hooten, MB, DS Johnson, EM Hanks and JH Lowry. (2010) Agent-based inference for animal movement and selection, *Journal of Agricultural, Biological, and Environmental Statistics*. 15:523-538.
38. Ross SM. (2006) Introduction to Probability Models, 9th Edition. Elsevier.

Figures:

Figure 1: Trophollaxis data for colony 10. a) Trophollaxis count (number of individual trophollaxis events) and b) trophollaxis duration (seconds) as a function of ant worker type. * and ** denote ant worker types that differed significantly in their trophollaxis count or duration (post-hoc Tukey HSD on one-way ANOVA).

Figure 2: Representative Unweighted Static Network Graph . (Colony 10, June 3rd 2013). Circles represent individual ants; queen (black), nest workers (gray), inactive foragers (yellow), and active foragers (blue). Lines between circles represent trophollaxis interactions between those ants.

Figure 3: Segregated Use of Nest Space. Aggregated residence times in ant-days for queens, active foragers, and non-foraging ants from two colonies (RG2 and Colony 10).

Figure 4: Representative Time-Aggregated Network Graph. (colony 10, June 10th, 2013). Each vertical line represents an individual ant, time increases up the vertical axis. Horizontal lines represent trophollaxis events between the individual lines that they connect. The queen is highlighted in red, and a foraging ant that has returned to the nest is highlighted in green. Note that the queen and foraging ant never interact in a temporally meaningful way, despite their overall connectivity within a static network representation.

Figure 1

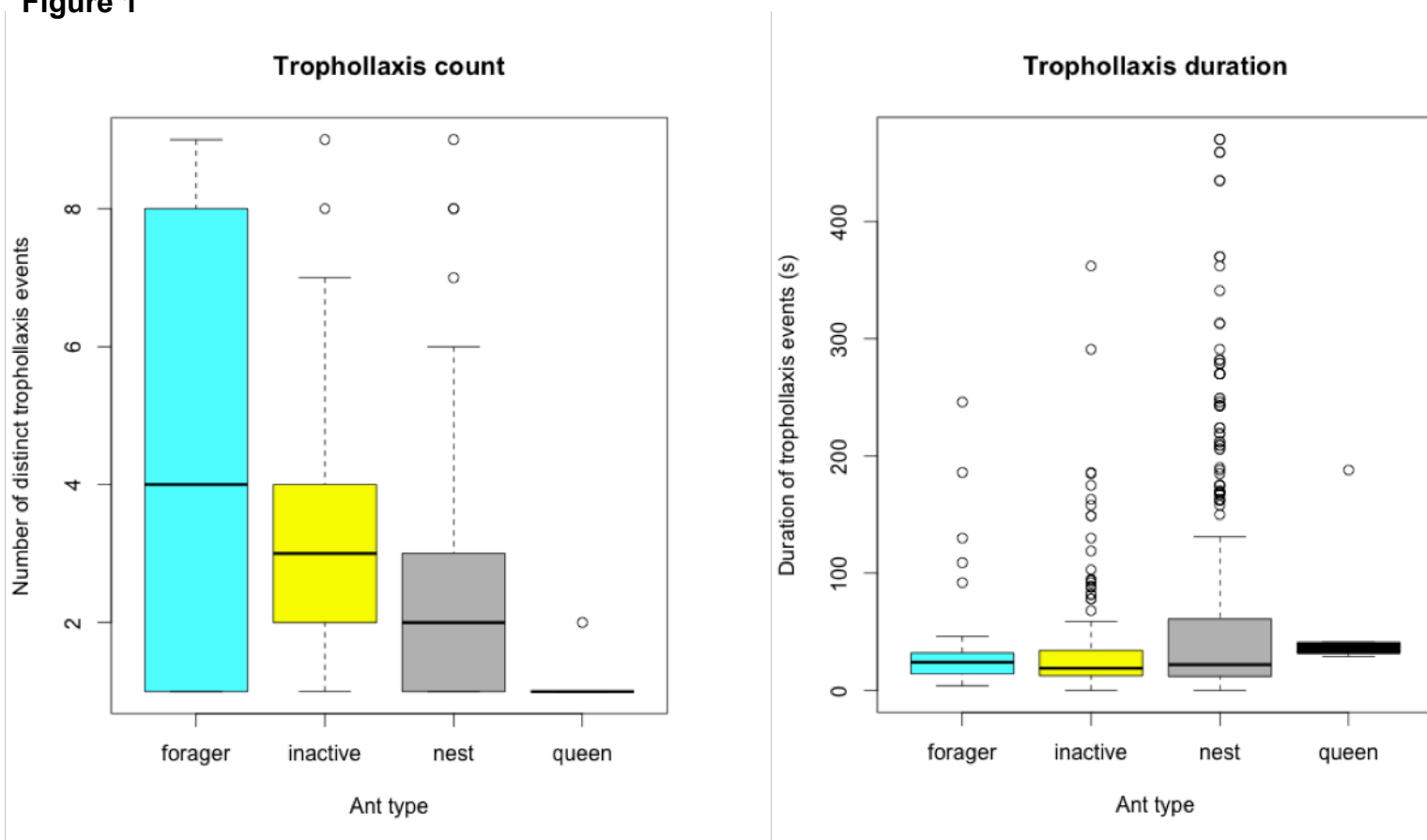


Figure 3

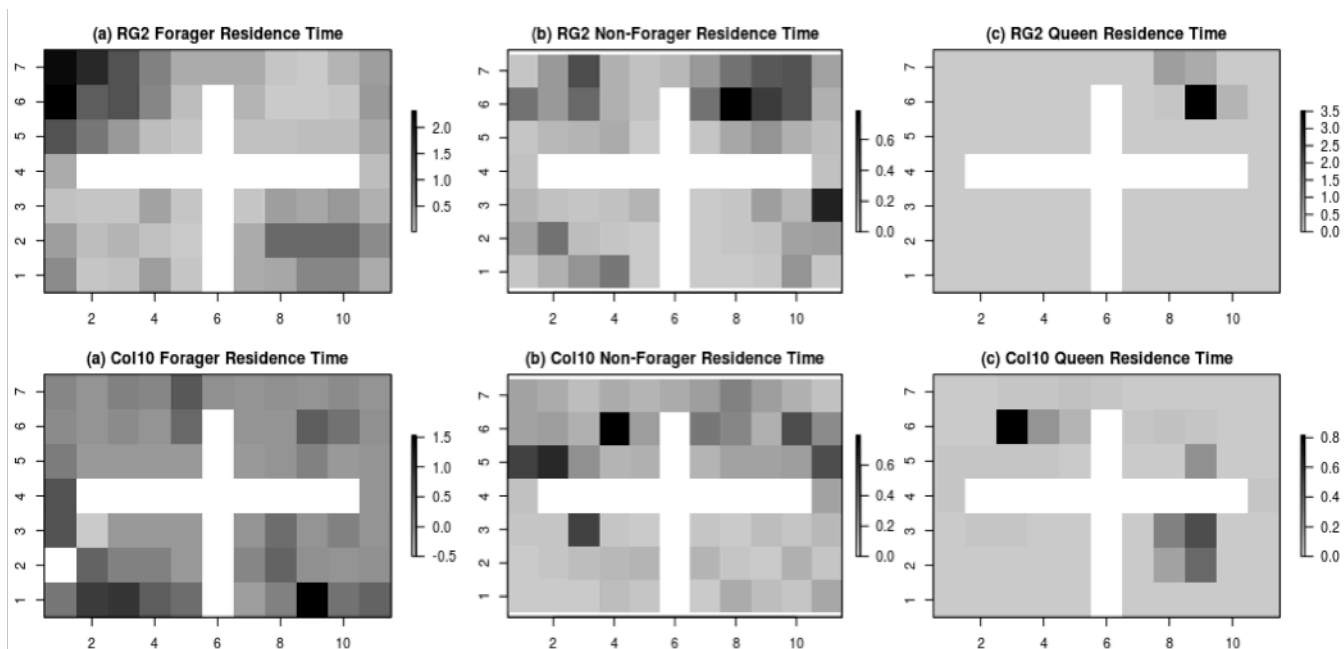


Figure 4

