

<sup>1</sup> Nest shape does not affect ant colony performance against a nest invader  
<sup>2</sup> despite altered worker movement and communication

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#### <sup>4</sup> Abstract

The spatial configuration or ‘architecture’ of an animal’s home can significantly affect the behavior of the occupant. However, the geometry of the occupied space may only partly be under the animals’ control, especially if pre-existing structures are used. In social animals in particular, such geometry may affect not only movement of individuals but also facilitate (or negate) cooperative interactions. How then does nest architecture translate to performance and fitness? We address this question by manipulating internal nest shape in a social insect, the ant *Temnothorax rugatulus*. We test the defensive performance of ant colonies against a conspecific nest invader in two distinct nest shapes, where nest invasion risks brood or queen loss. We also compared both worker movement and interaction networks in each nest shape to infer the spread of information about a nest invader. We specifically test the following hypotheses regarding how nest shape may affect performance (1) by changing how well information flows through each nest shape; (2) by changing the physical accessibility of different sections of the nest, including causing ‘traffic jams’, i.e., total movement blocks; or whether, alternatively, (3) nest shape does not impact performance against nest invaders. We found that, while nest shape did affect information flow and traffic jams through the nest, performance itself (time to remove the invader) was not affected by nest geometry. Our findings imply that social animals may be able to flexibly adapt to, and possibly compensate for, different spatial constraints.

<sup>5</sup> **Keywords:** Ants, Nest Architecture, *Temnothorax*, Colony Organization, Nest Entrance, Nest defense

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## 6 Introduction

7 Social animal architectures exist in a diversity of forms, which often have profound effects on the social  
8 interactions of the builders and thus their fitness (Hansell 2019). For example, kinship in social weaver bird  
9 colonies is influenced by how clustered chambers are in collective nests (Collias and Collias 1977; van Dijk  
10 et al., 2014), and social organization is promoted by the physical segregation of reproductive queens and  
11 other tasks in the architectures of naked mole rats (Tofts and Franks 1992; Faulkes and Bennett 2001) and  
12 social insects (Anderson 1984, Wilson and Kinne 1990, Wilson 1992). In these social species, interactions  
13 between individuals are key to essential colony functions, such as food collection or colony defense (where  
14 recruitment plays a critical role: Dornhaus and Powell 2009; Charbonneau et al., 2017; Pinter-Wollman  
15 2015; Fisher and Pinter-Wollman 2021). For example, in ant colonies, nest chambers that are more highly  
16 connected facilitate foraging recruitment by increasing interaction rates between returning and potential  
17 foragers near the entrance (Pinter-Wollman 2015; Vaes et al., 2020).

18

19 Social insect nests are among the most impressive examples of social animal architecture, such as the  
20 nests of *Atta* leaf-cutter ants, which can have up to nine meters depth above or below ground and span  
21 ten meters across (Hölldobler and Wilson 2010). So far, studies of social insect architecture largely focus  
22 on describing their structure and spatial configurations (Jeanne 1975; Seeley et al., 1982; Tschinkel 1987;  
23 London and Jeanne 2001; Tschinkel 2005; Tschinkel 2011). The next interesting question, namely the fitness  
24 consequences of different types of both built and occupied structures, has largely remained unanswered,  
25 with the exception of thermoregulation and humidity control (Noirot and Darlington 2000; Korb 2003; Korb  
26 2010; Bollazi and Roques 2010; Halboth and Roques 2017).

27

28 A defended, stable, nest is one of the primary characteristics of insect sociality. Social insects generally  
29 display cooperative care of young and reproductive division of labor, thus necessitating a nest that can  
30 house the reproductive and the immature individuals, both of which are often immobile - therefore colonies  
31 have been likened to a ‘factory within a fortress’ (Wilson 1968). These nests, with their high concentration  
32 of individuals, are not only attractive to predators (Nonacs 1993; Kaspari and O’Donnell 2003), but also  
33 to conspecifics: ants are particularly fierce competitors and predators of other ants, even their own species  
34 (Wilson 1976, Detrain, and Pasteels 1992, Foitzik et al., 2001, Huang 2010; Bengston In Prep). Social  
35 insects are thus under strong selective pressure to develop defensive strategies, either through their nests  
36 or behaviors (Wilson 1976, Shorter and Rueppell 2012, Powell 2008; Powell et al., 2017). Ants exhibit  
37 many defensive behaviors towards both preventing and removing nest invaders (Droual 1984; Detrain and  
38 Pasteels 1992; Tanner and Adler 2009; Dornhaus and Powell 2010; Huang 2010; Tian and Zhou 2014). It  
39 is thus clear that nests and their geometry can be a key element of social insect colony defense. Here we  
40 are interested in how nest geometry may change the behavior of the individuals within, and whether such  
41 changed behavior then has consequences for colony performance, for example in the task of defending the  
42 nest. We particularly focus on movement and interaction networks of workers in the colony.

43

44 Information flow among workers in social insect is hypothesized to influence the task performance of colonies  
45 (Ratnieks and Anderson 1999; O’Donnell and Bulova 2007; Sendova-Franks et al., 2010; Lanan et al., 2012;  
46 Donaldson-Matasci et al., 2013; Radeva et al., 2017). Information flow may even be a limiting factor  
47 in effective task allocation, where individual workers need to be informed about which tasks need doing  
48 (Radeva et al., 2017), and information enables fast recruitment to tasks (Donaldson-Matasci et al., 2013;  
49 Wild et al., 2021). Some tasks may require large groups of workers to be coordinated, such as when the goal  
50 is to overwhelm competitors at a resource (Lanan et al., 2012). Some studies have indeed demonstrated  
51 that higher interaction rates, and thus presumably higher information transmission, have increased task  
52 performance (Gordon and Mehdiabadi 1999; Greene and Gordon 2007; Pinter-Wollman et al., 2011; Pinter-  
53 Wollman 2015b; Vaes et al., 2020). On the other hand, delays in communication may themselves be

54 informative and help drive adaptive organization (Ratnieks and Anderson 1999). Ant colonies manage  
55 interaction rates for various purposes, such as reducing disease spread through social distancing: disease  
56 spread is reduced by clustering worker interactions and making the network diameter larger (Stroeymeyt et  
57 al., 2018; Pusceddu et al., 2021).

58

59 Worker movement in the nest is also likely to be connected to colony performance. The interaction networks  
60 of workers can be explained by mobility in the nest (Blonder and Dornhaus 2011). Worker movement  
61 therefore can explain task switching where workers not performing tasks move through the nest and encounter  
62 other workers performing tasks, where they may be recruited to a new task (Gordon and Mehdiabadi 1999,  
63 Garrison et al., 2018). Additionally, the placement of brood affects the spatial relationships of workers in the  
64 nest (Franks and Sendova-Franks 1992, Sempo et al., 2006; Pereira et al., 2018). For example, *Temnothorax*  
65 *unifasciatus* workers are hypothesized to place different staged brood members in a “domain of care” directly  
66 related to the amount of care each brood stage needs (Franks and Sendova-Franks 1992). Nest architecture  
67 additionally influences tasks related to movement in the nest, such as in panicked evacuation: exiting at a  
68 corner in a square nest is faster than from the middle of the wall (Shiwakoti et al., 2011), and exiting in a  
69 circular nest is faster when there is an occlusion at the entrance (Burd et al., 2010; Shiwakoti et al., 2011).

70

71 Here we used the rock ant *Temnothorax rugatulus* as a model to answer whether nest geometry affects  
72 colony performance in two artificial nest shapes. We examined overall performance in the collective task of  
73 removing an experimentally introduced conspecific nest invader, by specifically measuring latency to remove  
74 the nest invader related to nest shape, how far the invader penetrated the nest, and the number of defenders.  
75 We also quantified worker interaction networks and worker movement in undisturbed (baseline) and invader  
76 behavioral assays. We tested the hypotheses that any differences in nest defense performance across nest  
77 shapes might be explained either by differences in efficiency of information flow in worker interaction networks  
78 or by differences in the dynamics of worker movement, in particular possible worker ‘traffic jams’.

79

## 80 Methods

### 81 Colony collections

82 From July to October 2017 and February to May 2018 we collected 20 colonies of *Temnothorax rugatulus*  
83 on rocky, semi-steep slopes with pine-oak-juniper forest on the Santa Catalina Mountains (GPS: 32.395,  
84 -110.688), USA, Pima County, Arizona. We found all of our colonies in granite rock-crevices where entire  
85 colonies were collected by aspirating after prying open the nest cavity. The colonies used here, along with  
86 their maintenance and experimental schedule below, are the same as in Chism et al., 2022, which shows  
87 that nest architecture influences the spatial organization of colonies in the nest, but does not address colony  
88 performance.

89

### 90 Initial housing and care

91 Directly after collection, we placed colonies in generic artificial nests resembling their natural nest sites in  
92 rock crevices. These nests consisted of a 2-mm-thick piece of cardboard (75 mm x 50 mm x 2 mm) between  
93 two glass panes (76.2 mm x 50.8 mm x 0.5mm), with a 2mm x 2mm entrance at the center of one long  
94 side leading to an open nest space (35mm x 25mm) (fig. S1). We gave colonies food and water *ad libitum*,  
95 refreshed weekly, for the duration of their housing: water was given through cotton ball stopped, water-  
96 filled 5 ml plastic tubes, and food was given through both 2 ml microcentrifuge tubes of honey water with a  
97 concentration of 1/4 teaspoon of honey per 50ml water, and ~1/8 (approximately 0.075g) of a fresh-frozen  
98 cockroach (*Nauphoeta cinerea*). We further kept colonies on a 12:12 h light cycle (8 a.m. to 8 p.m.), and

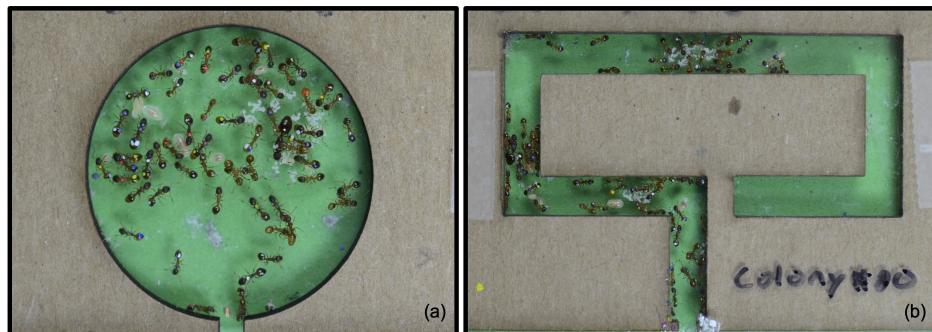
99 constant temperature (approximately 21-24 °C). We kept all artificial nests in open-top plastic containers  
100 (11.1 cm x 11.1 cm x 3.3 cm) with walls lined with ‘insect-a-slip’ (BioQuip 2871A, ‘fluon’) to prevent escape.

101

102 **Experimental setup**

103 We used a circular and a tube-shaped nest cavity (fig. 1) for the experimental phase. We scaled the internal  
104 area of the nests to colony size, giving colonies a consistent density and thus similar ability to utilize their  
105 nest space. In the high-density treatment (treatment 1), we used nest areas of 0.033 mm<sup>2</sup> per worker. We  
106 determined this density by examining the size of a *Temnothorax rugatulus* colony that utilized nearly all the  
107 available nest space in a pre-experimental nest (248 workers, see fig. S1) and doubling the area per worker  
108 to permit flexible nest space use. We further doubled the internal nest area for the low-density treatment  
109 (treatment 2), producing a density half as dense as the high-density treatment (0.066 mm<sup>2</sup> per worker).  
110 These density treatments could result in nature from nest competition: colonies prefer lower worker densities  
111 (Visscher 2007), so high nest competition could result in populations occupying nests with overall higher  
112 worker density; whereas low nest competition could allow colonies to occupy their preferred nests. We  
113 additionally individually marked workers with four identifying marks, one each on the head and prosoma,  
114 and two on the gaster with multiple colors of paints (anesthetizing workers individually with CO<sub>2</sub> and using  
115 Testor’s Pactra® paint) (see fig. 1). We marked ants three to five days before the colony was placed into  
116 experimental nests.

117 ## here() starts at /Users/gregchism/Library/Mobile Documents/com~apple~CloudDocs/Desktop/AntColonyPer



118 **Figure 1.** Nest shapes used for this study, a circle (a) and tube (b) nest.

119

120 **Experimental timeline**

121 We had three distinct experimental phases which we employed in both experimental density treatments.  
122 Note that half of the colonies experienced the high worker density treatment and the other half experienced  
123 the low worker density treatment; all colonies experienced both nest shapes (the circular and tube-shaped  
124 nest). Food and water were continually provided as described above.

125

126 *Pre-experimental nest acclimation* - We gave colonies three days to acclimate after a forced emigration (see  
127 nest defense performance, below) into their first experimental nest. We randomly assigned nest shapes such  
128 that ten of our colonies experienced the circle nest first and assigned the other ten colonies the tube nest  
129 first.

130

131 *Nest assignment I* - Following the three day acclimation period, we photographed the nest interior of colonies  
132 in their first nest assignment, daily at 10am for 16 days, following which we removed the top pane of glass

134 to expose their nest cavity, which makes emigration into a new nest more desirable than remaining in their  
135 current exposed nest space. We repeated the Pre-experiment acclimation procedure before starting data  
136 collection for the second nest assignment.

137  
138 *Second pre-experimental nest acclimation* - Following the ‘Nest assignment I’ phase, we removed the top pane  
139 of glass to expose their nest cavity, which makes emigration into a new nest more desirable than remaining  
140 in their current exposed nest space. We offered a new nest of the other type (circle or tube) and repeated  
141 the Pre-experiment acclimation procedure.

142  
143 *Nest assignment II* - We again photographed colonies once per day for 16 days.

144  
145 **Video capture**

146 Following the 16 day photographing period above, we recorded control (baseline) and nest invader assay  
147 videos to capture each colony’s response to an invader and to determine how activity and communication  
148 networks differed from the control settings. We repeated this nest invader assay five times to assess each  
149 colony’s response against an invader (recorded at least 8 hours apart). All videos were recorded at 10am  
150 with an HD camera (Nikon D7000 with 60mm lens).

151  
152 *Nest invader assays*: We began each assay with a baseline five-minute video to capture normal worker  
153 movements and interactions, and then followed with a five-minute nest invader assay, where an ‘invader’ (*T.*  
154 *rugatulus*) worker found foraging in the arena of another non-experimental colony from the same population)  
155 was forcibly inserted through the experimental nest entrance, using forceps. Note that we used all invader  
156 assay videos where the invader was both discernible and in the nest, for a total of N = 109 out of 180  
157 possible videos. Our final invader assay video sample size for each nest shape and density combination were  
158 Circle/High = 29, Circle/Low = 28, Tube/High = 22, Tube/Low = 30.

159  
160 *Movement tracking (ABCTracker)*: We used videos from a colony’s first invader assay in each nest for ant  
161 movement tracking. We tracked ants in one set of five-minute baseline and invader assay videos of ten colonies  
162 in each nest shape (five colonies from each density treatment) for a final sample size of 20 baseline and 20  
163 invader assay videos. We analyzed each video using a semi-automated tracking software (ABCTracker:  
164 abctracker.org; Rice et al., 2020), which includes an initial marking phase to scale the distances and speeds  
165 of the ants (producing ‘tracks’), followed by automated tracking and a functionality for user corrections. We  
166 tracked the spatial positions of each worker in each nest shape across all videos (24 fps), which the tracker  
167 outputs as cartesian coordinates (x, y), trajectory (along a 360° axis), and movement speed (px/s) for every  
168 frame.

169 **Nest sections**

170 We used the image analysis software *Fiji* (used for all image analysis throughout, Schindelin et al., 2012),  
171 on screenshots from each video to divide each nest into eight equal-area sections from the nest entrance  
172 to the back of the nest (see fig. S2). We chose not to use more than eight bins because these nest section  
173 assignments were potentially large enough to capture and segregate different worker tasks.

174  
175 **Scaled distances in the nest**

176 *Distance to the nest entrance*: We calculated the shortest linear distance within the available nest cavity  
177 from each colony member to the nest entrance (fig. S3). Specifically: in the circular nest, we calculated

178 each colony member's distance to the nest entrance (per a reference x and y coordinate). In the tube nest,  
179 where a direct (i.e., straight-line) path was often not possible within the available nest space, we found each  
180 colony member's distance to the nest section closest to the entrance using reference coordinates for that  
181 nest section (see Nest Sections), where we then added the shortest distance from that nest section to the  
182 entrance through reference coordinates (fig. S3).

183

184 *Distance scaling:* Actual possible distances varied across nests since the nest dimensions were scaled to  
185 colony size to keep a consistent worker density across colonies. To be able to compare movement speeds of  
186 workers in relation to the nest entrance, we scaled all calculated distances by setting the shortest distance  
187 from the back of each nest shape to the entrance as 1, notably this scaling differs from Chism et al., 2022  
188 which utilized the shortest distance from the back of the tube nest. Notably, speed was scaled only by  
189 ant-lengths (see Traffic Jams)

190

191 **Nest defense performance** We assessed how long colonies took to remove a conspecific nest invader to  
192 determine whether this was influenced by (1) nest shape, (2) the distance the invader penetrated the nest,  
193 or (3) worker defense recruitment.

194

195 *Latency to remove the invader (performance):* We quantified the duration the invader was in the nest by  
196 subtracting the timestamps for the insertion and removal (fully outside of the nest) of the invader.

197

198 *Invader nest penetration:* We determined the farthest point in which the invader penetrated the nest in  
199 each invader assay video and then calculated the distance from the entrance by converting the screenshot  
200 from pixels to metric using reference distances from each image (bottom-left and top-right nest corners: top  
201 y-coordinate - bottom y coordinate = 5cm). We then measured and scaled the invader's shortest distance  
202 to the nest entrance using the same method as above.

203

204 *Worker recruitment (max attacking workers):* We used the maximum number of workers that attacked  
205 the invader in the nest as a proxy for worker defense recruitment. Attacking workers were identified as  
206 individuals that were seen biting the invader for any duration.

207

### 208 **Worker social networks**

209 We used the movement tracking data, five-minutes (constant over colonies and treatments) from ABCTracker  
210 to quantify interactions between workers in each video as unweighted adjacency matrices that were then  
211 converted into static interaction networks. In our networks, the total number of workers in each colony  
212 were nodes and the unweighted worker-worker interactions were edges. We utilized unweighted networks  
213 to determine how information might pass between workers in each of our nest shapes, but the number of  
214 interactions (i.e. represented by a weighted network) were not considered because some ants were immobile  
215 and interacted the entire video.

216

217 *Worker interaction matrices:* We filled adjacency matrices with either direct (workers facing one another)  
218 or indirect (any other proximity) interactions, operationally defined as below.

219

220 Worker proximity: Our tracking software ABCTracker places a rectangular box around each detected ant  
 221 worker that approximates the length and width of the worker. A worker's head was defined as the front of  
 222 the ABCTracker assigned rectangular box and the body was defined as the side of the ABCTracker assigned  
 223 rectangular box. We used this to determine distance between and relative position of ants, in which ants  
 224 could be either direct (head-to-head) or indirect (head-to-body or body-to-body), but note that the final  
 225 networks were derived from all interactions together. Specifically, (1) workers must be within a distance  
 226 measuring 1.5 multiplied by the average box length over the entire video, or (2) the distance from the focal  
 227 worker's body to the target worker's body must be within a distance measuring 0.5 multiplied by the average  
 228 box width of the target worker.

229  
 230 Final matrices and networks: We added all interactions and changed the final matrix cells to a 1 for the  
 231 presence of any interaction and 0 for no interactions between worker pairs, producing unweighted adjacency  
 232 matrices for each video. We formed our networks from these final matrices.

233  
 234 *Comparative network measures:* We used several network measures to describe the topology of our base-  
 235 line and invader assay networks, allowing us to assess whether nest shape affected the pattern of worker  
 236 interactions in baseline and invader assays.

237  
 238 Harmonic centrality ( $C$ ): The distance, in terms of number of edges, nodes are from all others in a network:  
 239 equation 1 (Rochat 2009). Once calculated, we assessed whether each worker's distance from the nest  
 240 entrance (see above) at the beginning of each video influenced these values. Higher harmonic centrality in  
 241 workers near the entrance at the time of invasion could indicate that these workers were more central in  
 242 spreading information about the invader to all others.

(1)

$$C = \sum_{i \neq j} \frac{1}{d_{ij}}$$

243 Where  $i$  and  $j$  represent links between node pairs, and  $d_{ij}$  represents the shortest path between individuals  
 244  $i$  and  $j$  (Dijkstra's algorithm: Dijkstra 1959). Note that disconnected nodes are undefined and produce an  
 245 infinite  $d_{ij}$  value when calculating closeness centrality, but by taking the reciprocal of  $d_{ij}$  here these instead  
 246 are zeros.

247  
 248 Global network efficiency ( $E_{Glob}$ ): We first calculated average efficiency  $E(G)$  or the average of the inverse  
 249 shortest path lengths between  $i$  and  $j$  pairs in a network, which multiplies  $C$  from equation 1 by  $\frac{1}{n(n-1)}$   
 250 because  $E(G)$  considers each node twice as  $i$  and  $j$  (equation 2: Latora and Marchiori 2003; Buhl et al.,  
 251 2004). For a graph ( $G$ ), efficiency is the ratio between  $E(G)$  and  $E(K_n)$ , where  $K_n$  is a complete graph  
 252 of order  $n$  (a graph with the same number of vertices as  $G$ , but one with  $\frac{n(n-1)}{2}$  possible edges). Global  
 253 efficiency ( $E_{Glob}$ ) is therefore the average efficiency  $E(G)$  for all paths ( $i \neq j$ ) in a fully connected graph  $G$   
 254 (equation 3: Latora and Marchiori 2003; Ek et al., 2015). Higher global efficiency indicates that information  
 255 about the conspecific nest invader could spread faster within a network.

(2)

$$E(G) = \frac{1}{n(n-1)} \sum_{i \neq j} \frac{1}{d_{ij}}$$

(3)

$$E_{Glob} = \frac{E(G)}{E(K_n)}$$

256 Where  $n$  is the total number of nodes in a network.

257

258 Gamma connectivity ( $\gamma$ ): We calculated network connectivity as the number of possible edges present  
259 compared to all possible in each of our networks (the gamma index):

(4)

$$\gamma = \frac{m}{3(n - 2)}$$

260 Where  $m$  is the total number of edges for every network measure and  $n$  is the total number of nodes.

261

262 Reciprocity ( $r$ ): We calculated the ratio of reciprocal interactions ( $L \leftrightarrow$ ) over the total interactions between  
263 workers ( $L$ ). Reciprocal interactions may be required to communicate more complex forms of information.

(5)

$$r = \frac{L \leftrightarrow}{L}$$

264

265 Transitivity ( $T_g$ ): We determined the probability that the network has interconnected adjacent nodes,  
266 represented as triplets, which reveals the presence of tightly connected node communities. Transitivity (also  
267 known as clustering coefficient) is the ratio between observed triplets ( $T_0$ ) over all possible triplets ( $T_1$ ). We  
268 used this measure to examine how clustered interactions in our networks are.

(6)

$$T_g = \frac{T_0}{T_1}$$

269

### 270 **Worker activity**

271 We assessed each worker's movement speed (a proxy for activity) in every video. We standardized movement  
272 speeds as the length (pixels) of the boxes that ABCTracker assigned to ants in each video (ant-lengths), and  
273 then converting px/s into ant-lengths/s.

274

275 *Movement speeds over time*: We averaged the movement speed of every worker at two-second intervals in  
276 each video to determine how worker movement changed in response to nest invasion. Temnothorax rugatulus  
277 are slow moving ants (many workers are always inactive: Charbonneau et al., Dornhaus 2017) so we averaged  
278 movement in a two-second window to reduce redundancy and noise in the data.

279

280 *Movement speeds over distance from the nest entrance*: We averaged all worker movement speeds within  
281 0.05 scaled distance from the entrance bins (from 0-1, see Invader nest penetration above) to determine how  
282 worker movement speed relates to distance from the nest entrance - see above for distance calculations.

283

### 284 **Traffic jams**

285 We estimated the likelihood of traffic jams in the nest by examining the variation in worker movement speeds  
286 over both assay time and nest space. Variation in speed across time could represent time periods of slow  
287 and fast movement, and variation in speed across space could represent parts of the nest that movement  
288 bottlenecked in, either indicating that traffic jams occurred.

289 *Traffic jams across space:* We calculated and averaged interquartile ranges (3rd quartile - 1st quartile) for  
290 all baseline and invader assay video two-second intervals from above to examine movement speed variability  
291 over time. Our final data sets were one average two-second interval interquartile range value for each video  
292 ( $N = 40$  videos). Higher variation in movement across present at specific areas of the nest could indicate  
293 that slow moving workers are restricting traffic flow, whereas low variation could indicate that traffic flows  
294 freely throughout the nest.

295

296 *Traffic jams over time:* We calculated and averaged the interquartile ranges from each 0.05 scaled distance  
297 bin from above to examine the variability in movement speeds across nest space in each video. Our final  
298 data sets were one average 0.05 scaled distance bin interquartile range value for each video ( $N = 40$  videos).  
299 Higher movement speed variation (larger average interquartile ranges) would indicate, for example, that  
300 some workers moved very slowly (or not at all) and others more quickly throughout the assay. This high  
301 variation in worker movement speeds could indicate that traffic jams are occurring, whereas less variation  
302 (or more similar) movement speeds could indicate that traffic flows freely.

303

304 Worker density in nest sections and traffic: We determined how worker density (number of workers in nest  
305 sections) related to worker movement speeds as another indicator for traffic jams, such that higher worker  
306 densities could restrict worker movement in individual nest sections.

307

### 308 **Data processing**

309 We conducted all data processing using the statistical software R (v4.1.1; R Core Team 2017) and RStudio  
310 (v1.2.5042; Allaire 2012), specifically the tidyverse language ('tidyverse' v1.3.1: Wickham et al., 2019). All  
311 original data and analysis scripts are publicly available on github at <https://github.com/Gchism94/AntColonyPerformance>

312

313 *Network generation:* We both produced and analyzed our networks using the R package 'igraph' (v1.2.6;  
314 Csardi and Nepusz 2006), except harmonic centrality, which was calculated using the R package 'CINNA'  
315 (v1.1.53; Ashtiani et al., 2019).

316

317 Factors influencing colony performance: We used linear mixed effects (henceforth LME) models to test  
318 whether nest shape, invader nest penetration (max scaled distance from the nest entrance), and defense  
319 recruitment (max attacking workers) affected colony performance in removing a conspecific nest invader.  
320 We conducted these models with the R package 'lme4' (v1.1-27.1; Bates et al., 2014), where p values were  
321 calculated through the R package 'lmerTest' (v3.1-3; Kuznetsova et al., 2017). Our LMEs here and below had  
322 colony identification (henceforth colony ID) as a random effect, and by comparing the variation explained by  
323 the fixed effects alone (marginal  $R^{2+}$ ) and with the random effect included (conditional  $R^{2+}$ ), we determined  
324 the amount of variation that colony ID explained (marginal and conditional  $R^{2+}$  values calculated through  
325 the R package 'MuMIn' (v1.43.17; Bartoń 2020)).

326

327 *Comparative network measures* Harmonic centrality and distance to the entrance: We used a linear regression  
328 to test whether worker scaled distance to the nest entrance at the beginning of the invader assay affected  
329 the number of interactions workers had in networks (harmonic centrality) in every video. We specifically  
330 examined the individual terms and two-way interaction combinations between worker scaled distance to the  
331 nest entrance, nest shape, and experimental trial, and the three-way interaction between these terms.

332

333 Network efficiency: We used a linear regression to test the global efficiency of worker interaction networks in  
334 each nest shape, experimental trial, and the interaction between the two terms. We log transformed network  
335 efficiency to account for all values being extremely small, producing a strongly right-tailed data distribution.

336

337 We used linear regressions to determine how our comparative network measures gamma connectivity, reciprocity,  
338 and transitivity (clustering) differed across nest shape, experimental trial, and the interaction between the two terms.  
339

340

341 *Worker activity:* We used LME models to examine both average worker speed (two-second intervals) across  
342 our nest shapes and experimental trials.

343

344 *Worker traffic jams over time and nest space:* We used LME models to examine both the variation (average  
345 interquartile ranges) in worker movement speed through time (two-second intervals) and in average worker  
346 speed (two-second intervals) in relation to worker scaled distance from the nest entrance (0.05 scaled distance  
347 from the entrance intervals) in our nest shapes and experimental trials.

348

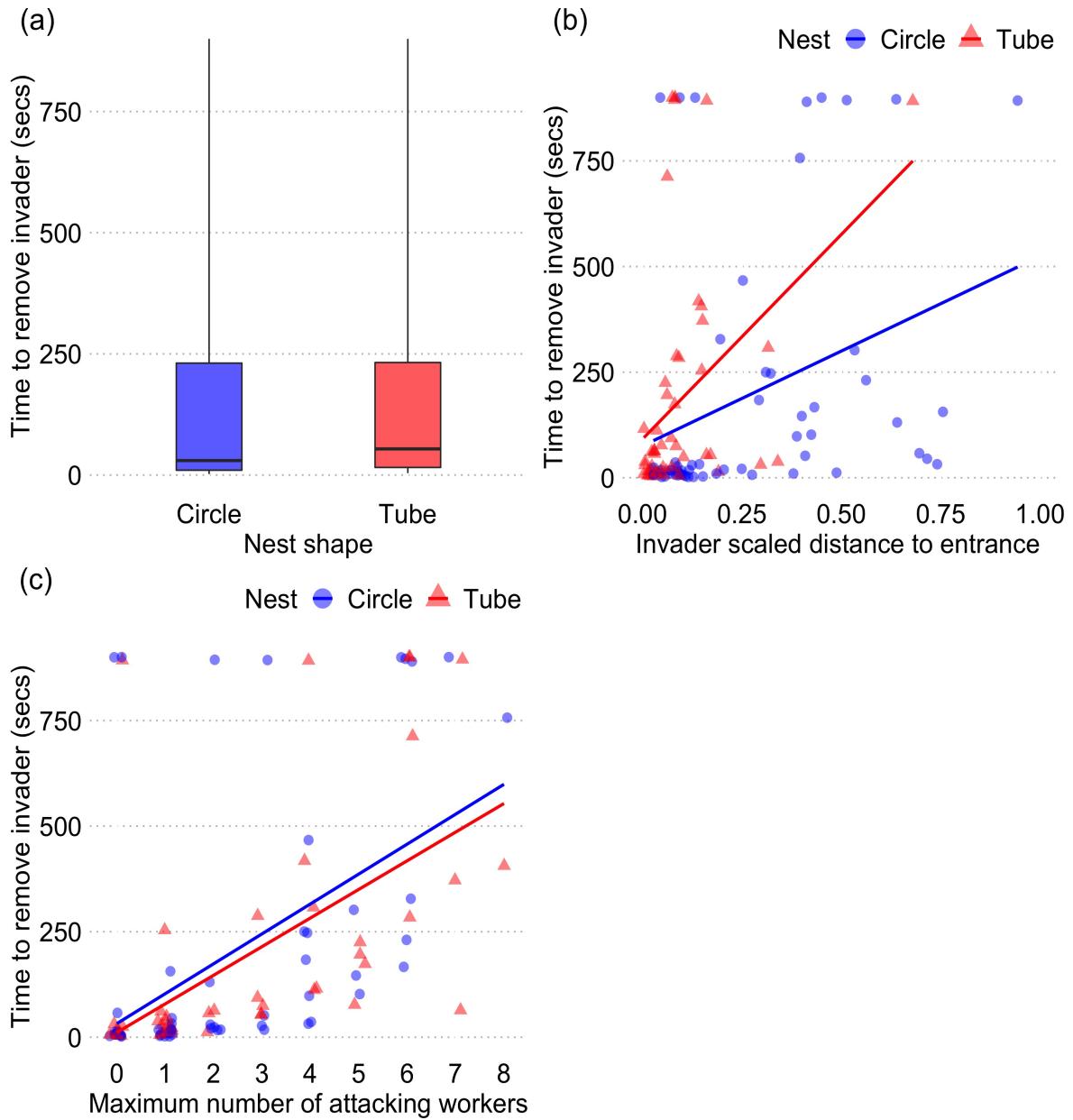
349 *Worker density in nest sections and traffic:* We used a LME model to examine worker density (proportions  
350 in each nest section) in the nest affects worker movement speeds. We considered the relationships between  
351 average worker movement speeds against worker density, and the interactions between worker density and  
352 nest shape, worker density and experiment trial, and the three-way interaction between worker density, nest  
353 shape, and experimental trial.

354

## 355 Results

### 356 *Colony performance against a nest invader*

357 Invader removal was influenced by invader penetration and colony defense recruitment. We found that nest  
358 shape does not affect invader removal time (seconds) ( $p = 0.746$ , fig. 2a, table S1), but invaders were  
359 removed slower in relation to both farther invader nest penetration ( $p < 0.001$ ; fig. 2b, table S2) and higher  
360 worker recruitment ( $p < 0.001$ ; fig. 2c, table S3). Therefore, invader removal time was slower both when the  
361 invader penetrated farther into the nest and more workers were recruited to remove the invader. The mean  
362  $\pm$  standard deviation for invader removal time was  $199 \pm 314$  seconds in the circular nest and  $181 \pm 274$   
363 seconds in the tube nest.



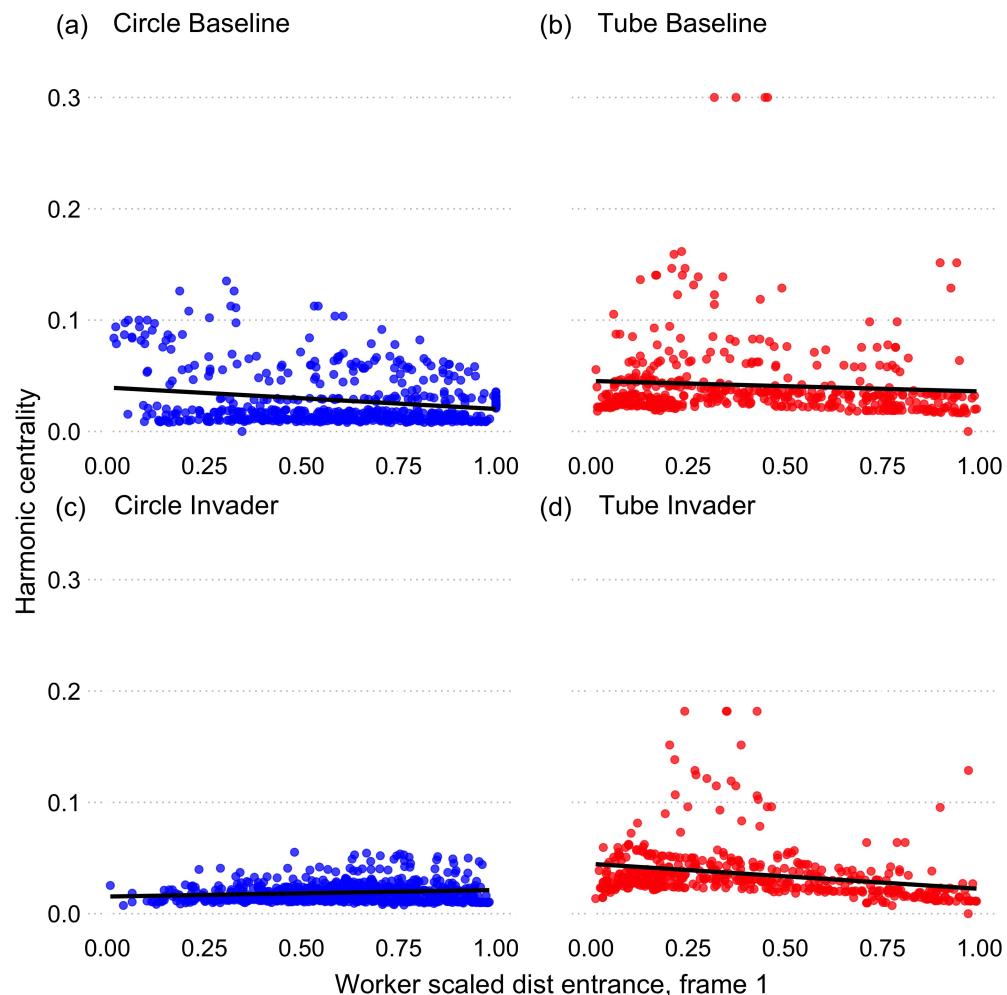
364  
365 **Figure 2.** Effect of nest shape (a), worker nest penetration (b), defense recruitment (c) on invader removal  
366 time. The data is from all replications of the invader assay for each colony, in each nest shape where the  
367 invader was discernible (up to five replicates). In all plots, data points represent one video and are jittered.  
368

369 Colony identification explained substantial invader removal performance model variation. We found that  
370 colony ID explained 3.2% (virtually all) of model variation predicting the effect of nest shape on invader  
371 removal (Marginal  $R^{2+} = 0.001$ , Conditional  $R^{2+} = 0.033$ , table S1). We also found that colony ID explained  
372 6.6% of model variation predicting the effect of nest shape and worker nest penetration on invader removal  
373 time (Marginal  $R^{2+} = 0.109$ , Conditional  $R^{2+} = 0.175$ , table S2), and 2.4% of model variation towards the  
374 effect of worker recruitment and nest shape on invader removal time (Marginal  $R^{2+} = 0.292$ , Conditional  
375  $R^{2+} = 0.316$ , table S3).

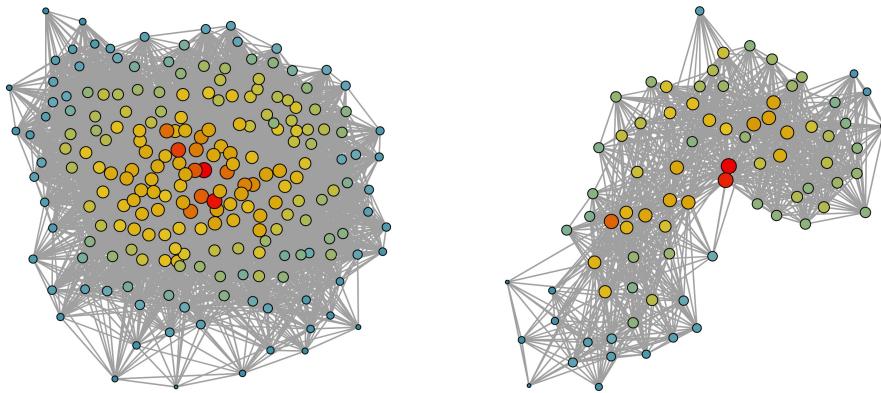
376

377 **Worker interaction networks**378 *Workers near the entrance when the invader was introduced were more central in networks*

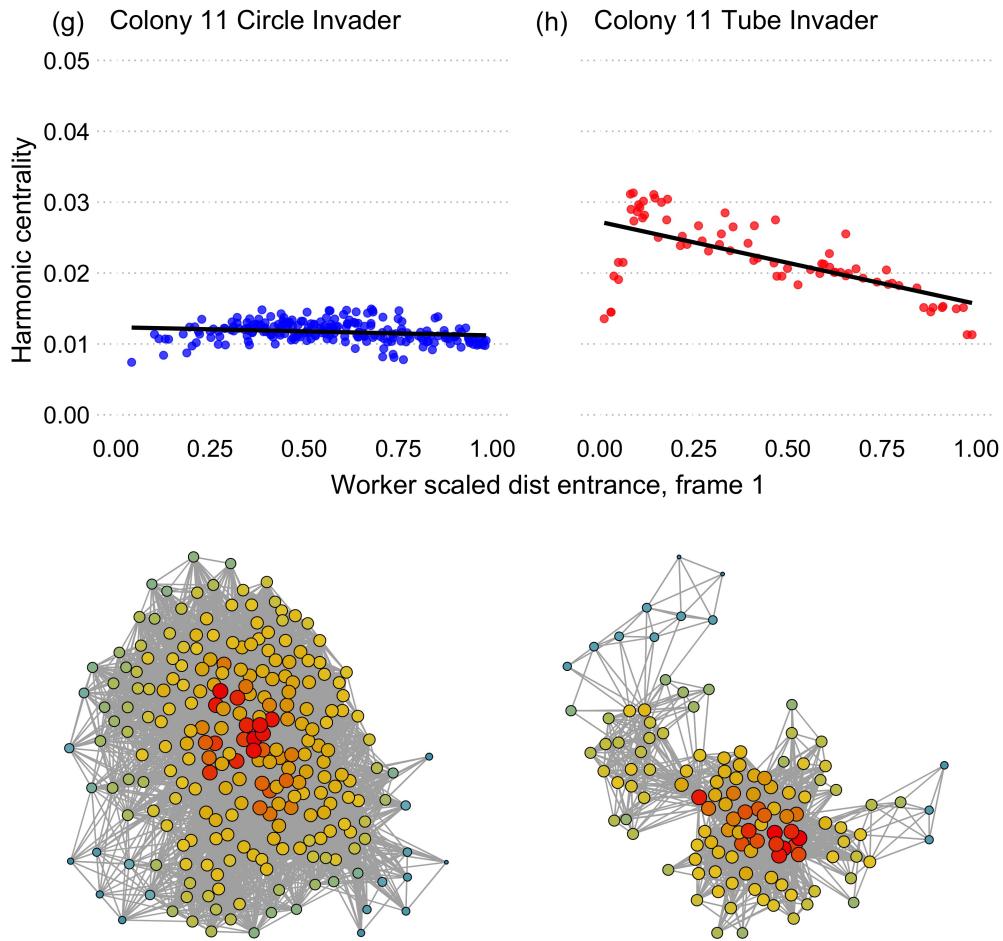
379 We found that workers had higher harmonic centrality values (interacted with more individuals) when they  
 380 were closer to the nest entrance at the time the invader was introduced ( $p < 0.001$ ; fig. 3a-d, table S4). We  
 381 also found that workers had higher harmonic centrality in both the circular nest ( $p < 0.001$ ; fig. 3, table S4),  
 382 and baseline assay ( $p < 0.001$ ; fig. 3, table S4). We however found that the negative relationship between  
 383 worker distance to the nest entrance and harmonic centrality was stronger in the invader assay ( $p = 0.011$ ;  
 384 fig. 3, table S4). Therefore, workers near the entrance interacted with more workers particularly during nest  
 385 invasion.



386



387



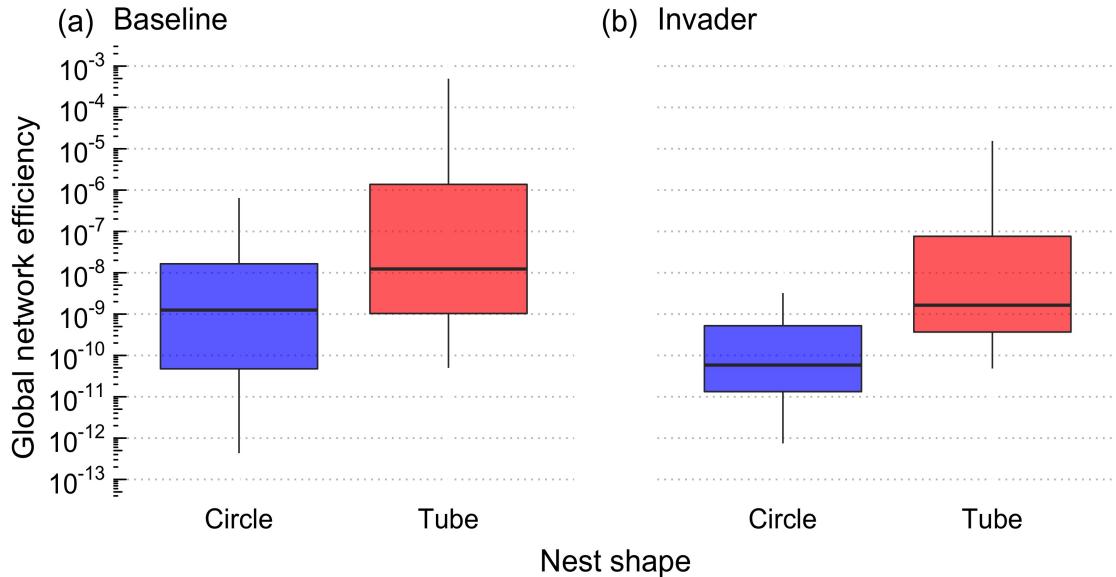
388

389 **Figure 3.** Relationship between worker distance to the entrance, considered at frame 1 when the nest  
 390 invader was introduced (x-axis) and worker network centrality (harmonic centrality, y-axis) for all colonies  
 391 (a-d) and colony no. 11 (e-h). Worker interaction networks for colony no. 11 (e-h) shows workers as nodes  
 392 (cooler smaller nodes representing smaller values of harmonic centrality and warmer larger nodes represent  
 393 larger values) and interactions between worker pairs as edges (gray lines).

394

395 *Nest shape affected how efficient information spread through worker interaction networks*

396 We found that worker interaction networks were more efficient at spreading information in the tube nest  
 397 ( $p = 0.021$ ; fig. 4, table S5). However, network efficiency did not differ across experimental trials ( $p = 0.138$ ;  
 398 fig. 4, table S5). Therefore, information possibly spreads faster in the tube nest, but nest invasion did not  
 399 change this relationship.



**Figure 4.** Network efficiency and nest shape in the baseline (a) and invader (b) assays. Global efficiency determines how effectively information would flow through the network - log scaled, as the values are very small, and the data are severely right-tailed.

*All worker interaction networks were similarly connected*

We found that gamma connectivity did not differ with nest shape ( $p = 0.060$ ; fig. S4, table S6), or experimental trial ( $p = 0.275$ ; fig. S4, table S6).

*Worker interactions were more reciprocal in the invader assay*

We found that network reciprocity did not differ with nest shape ( $p = 0.821$ ; fig. S5, table S7), but was overall higher in the invader assay ( $p = 0.002$ ; fig. S5, table S7).

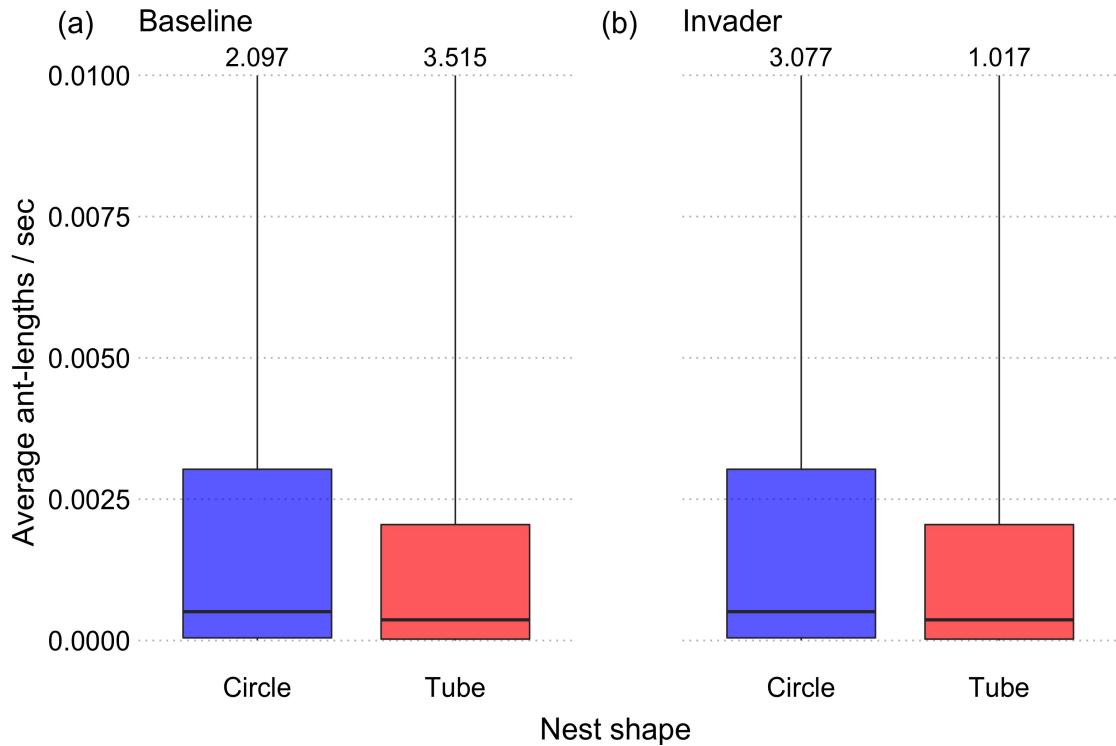
*All worker interaction networks were similarly clustered*

We found that network transitivity did not differ across nest shapes ( $p = 0.344$ ; fig. S6, table S8), or experimental trials ( $p = 0.946$ ; fig. S6, table S8).

#### **Worker activity**

*Worker movement speeds were different across both nest shape and experimental trial*

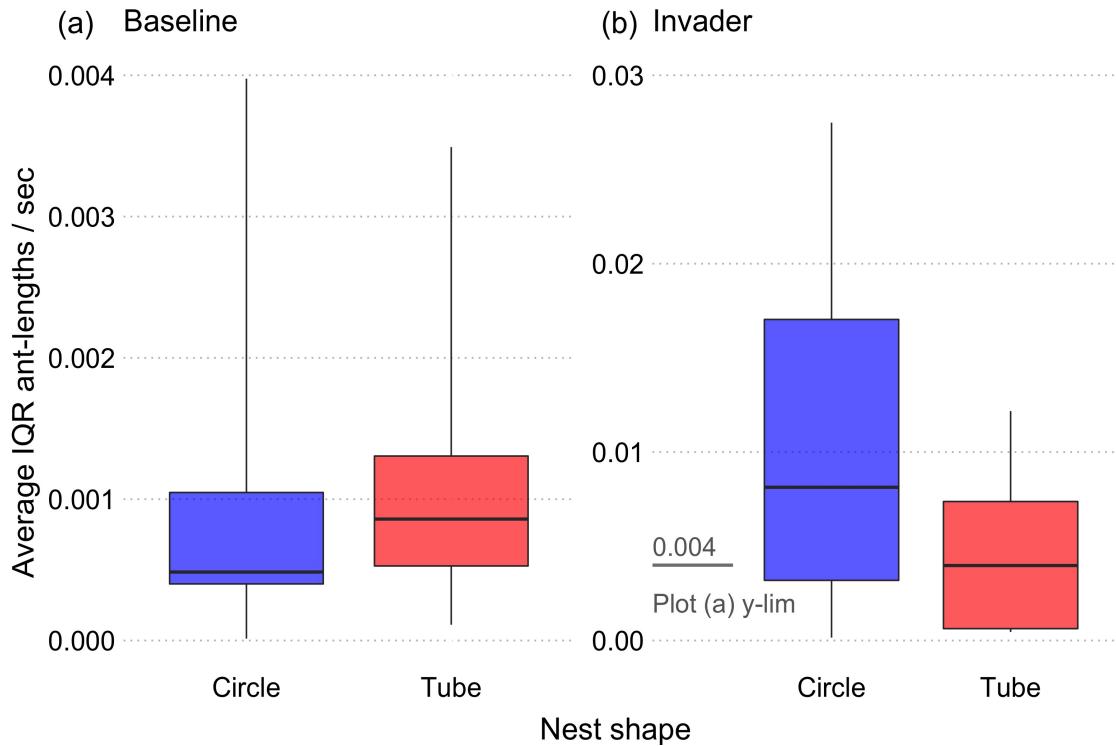
We found that average worker movement speeds over our five-minute videos were higher in the tube nest shape ( $p < 0.001$ ; fig. 5, table S9) and invader assay ( $p < 0.001$ ; fig. 5, table S9). Further, average worker speeds were higher in the beginning of invader assay videos in the circular nest compared to the tube nest ( $p < 0.001$ ; fig. 5, table S9). Therefore, workers moved overall faster in the tube nest and invader assay. The mean  $\pm$  standard deviation for the observations of two-second average worker speeds in our videos was  $31567 \pm 6525$  for the baseline assay and  $29935 \pm 5780$  for the invader assay.



**Figure 5.** Average worker movement speeds (ant-lengths/second) across both nest shape and experimental trial. Each data point is the average speed of each worker over two-seconds. Here and in all following box plots, boxes represent first and third quartiles, bars represent the median, whiskers are the data range, and numbers represent the max value for that box plot. Note that the y-axis is limited at 0.01 average ant-lengths/second.

*Traffic jams through nest space were more likely in the invader assay*

We found that the variation (average two-second IQR) in worker movement speed across our five-minute videos was much higher in the invader assay ( $p < 0.001$ ; fig. 6, table S10). We also found that the tube nest had higher movement speed variation in the baseline assay, but variation in invader assays was higher in the circular nest ( $p < 0.001$ ; fig. 6, table S10). Therefore, traffic jams through nest space likely occurred more during nest invasion, but especially so in the circular nest.

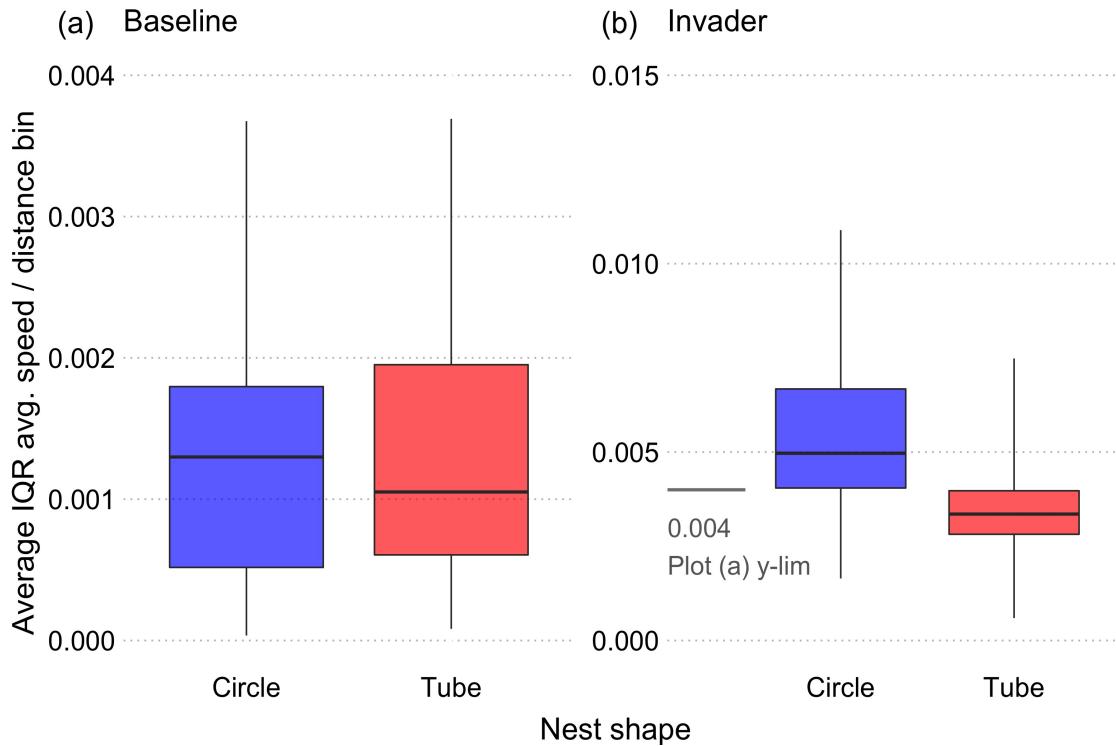


437  
**Figure 6.** Traffic jams: variation in ant speed through nest space. The average interquartile range of  
438 average movement speeds within two-second bins for all colonies across nest shape and experimental trial.  
439 Higher movement speed variation at specific time points could indicate that very slow moving (or stationary)  
440 workers cause traffic jams throughout the nest periodically. There is one point per colony, and overarching  
441 boxplot shows variation among colonies.  
442

443  
**444 Colony identification explained half of the variation in raw worker movement speed**

445 We found that colony ID explained 6.7% of the average worker speed over time model variation (Marginal  
446  $R^2+ = 0.052$ , Conditional  $R^2+ = 0.119$ , table S9), and 0% of the average interquartile ranges of worker  
447 speeds over time model variation (table S10).

448  
449 Traffic jams through time were more likely in the invader assay. We found that the variation (average 0.05  
450 scaled distance from the entrance interval IQR) was higher in the circular nest shape ( $p = 0.036$ ; fig. 7, table  
451 S11), and was significantly higher in the invader assay ( $p < 0.001$ ; fig. 7, table S11). We also found that  
452 the relationship between nest shape and worker movement speeds throughout the nest was not different in  
453 each experimental trial ( $p = 0.107$ ; fig. 7, table S11). Therefore, there were more traffic jams in the circular  
454 nest over time, but especially so in the invader assay. The mean  $\pm$  standard deviation for the number of  
455 workers in each 0.05 scaled distance from the nest entrance interval was  $299 \pm 119$  for the baseline assay and  
456  $138 \pm 64.4$  for the invader assay.



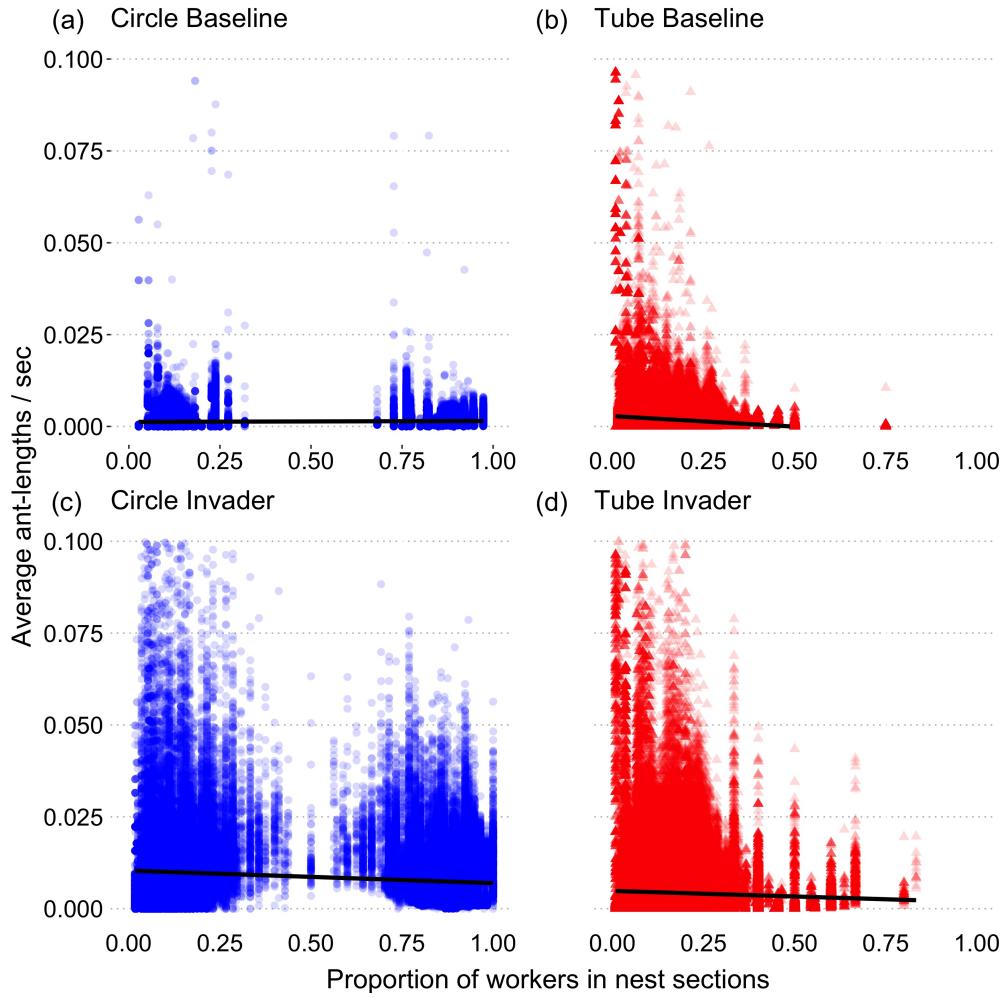
457

458 **Figure 7.** Traffic jams: variation in speed across ants through time. The average interquartile range of  
 459 average movement speeds in each 0.05 distance bin (see fig. S8), ranging from 0 (the entrance) to 1 (the back  
 460 of the nest), for all colonies across nest shape and experimental trial. Higher movement variation in specific  
 461 areas of the nest could indicate where slow moving workers are restricting traffic flow over time. Note that  
 462 the y-axis limit for the baseline assay (a) is represented in the invader assay panel (b) as a gray horizontal line.  
 463

464

465 *Worker density promotes slower worker movement (traffic jams) in nest sections*

466 We found that average worker movement speed (two-second intervals) was lower in nest sections with higher  
 467 proportions of workers ( $p < 0.001$ ; fig. 8, table S12). We further found that this negative relationship was  
 468 stronger in the tube nests ( $p < 0.001$ ; fig. 8 table S12), and in the invader assay ( $p < 0.001$ ; fig. 8, table  
 469 S12). Therefore, traffic jams occurred more over time in nest sections with higher worker density. The mean  
 470  $\pm$  standard deviation of the proportion of workers in nest sections was  $0.231 \pm 0.248$  for the baseline assay  
 471 and  $0.238 \pm 0.252$  in the invader assay.



472

473 **Figure 8.** The relationship between worker density in nest sections and worker movement speed in each  
 474 nest shape and experimental trial. Each nest is binned into eight even area sections where the proportion  
 475 of workers in each nest section is determined to approximate density throughout the nest. Each data point  
 476 is movement speed determined at every time step, black lines represent linear fits.

477

478 *Colony identification slightly influenced models predicting traffic jams over time*

479 We found that colony ID explained 5.9% of the model variation for average interquartile ranges of average  
 480 worker speeds in 0.05 scaled distance to the entrance bins (Marginal  $R^{2+} = 0.464$ , Conditional  $R^{2+} = 0.523$ ,  
 481 table S11). We further found that colony ID explained 3.8% of the model variation examining for worker  
 482 density in the nest and average worker movement speeds (Marginal  $R^{2+} = 0.143$ , Conditional  $R^{2+} = 0.182$ ,  
 483 table S12).

484

## 485 Discussion

486 Nest architecture does not influence *Temnothorax rugatulus* ant colony performance against a conspecific  
 487 nest invader: we found that the removal of nest invaders was slower when invaders penetrated the nest  
 488 farther (fig. 2b) and when more defending workers were recruited (fig. 2c), but invader removal was not

489 influenced by nest shape (fig. 2a). We contrastingly found significant nest shape related differences in how  
490 well assumed information about the nest invader may spread throughout worker interaction networks (figs.  
491 3,4). We also show that traffic jams may occur during nest invasion, represented by higher worker movement  
492 variation both through nest space (fig. 6) and worker density in nest sections (fig. 8) that may bottleneck  
493 worker movement over time. Therefore, nest shape influences the movement and communication of *T.*  
494 *rugatulus* workers in response to a nest invader, but this does not translate to slower invader removal.

495

496 Nest architecture can directly influence worker task allocation in colonies through interaction networks  
497 (Blonder and Dornhaus 2011; Pinter-Wollman et al., 2011; Charbonneau et al., 2013). For example, a  
498 worker's location in an entrance chamber can directly increase interactions with other workers and promote  
499 forager recruitment to food (Pinter-Wollman et al., 2011; Pinter-Wollman 2015b; Lehue and Detrain 2019;  
500 Lehue and Detrain 2020; Lehue et al., 2020a,b). In this study, we examined how nest shape influenced  
501 information spread about a conspecific nest invader through *T. rugatulus* worker interaction networks. We  
502 found that workers interacted with more individuals in the network (higher harmonic centrality) when they  
503 were closer to the nest entrance at the time that the invader was introduced (fig. 3), suggesting that these  
504 workers spread information about the invader to more workers at the beginning of nest invasion. Further,  
505 worker interaction networks in tube nests were more efficient at spreading information (fig. 4), though  
506 network efficiency values were all very low due to some workers (or worker groups) being disconnected  
507 from the main network. Our results suggest that a colony's performance in removing a nest invader is not  
508 restricted by information flow.

509

510 Nest shape can significantly affect worker traffic jams in the nest (Burd et al., 2010; Shiawakoti et al., 2014;  
511 Wang and Song 2016), which can consequently cause higher disease transmission (Pie et al., 2004; Naug  
512 2008; Pusceddu et al., 2021) or slower nest evacuation (Burd et al., 2010; Wang and Song 2016, Wang et al.,  
513 2016, Ji et al., 2018). We found that worker traffic jams (defined in our study as higher variation in worker  
514 movement speeds) occurred more in our circular nests both across nest space (fig. 6) and time (fig. 7), but  
515 especially so in the invader assay (figs. 6,7). Additionally, worker movement speeds were slower in nest  
516 sections with higher worker density (fig. 8), but only the tube nest shape held this relationship in both  
517 assays (figs 8b, d). Despite these relationships, our *Temnothorax rugatulus* colonies removed a nest invader  
518 similarly across nest shapes. Ant defensive strategies may be robust against traffic jams, or may change  
519 with experience, both reducing the impact that colony traffic has on defense in different nest shapes.

520

521 Nest architecture has been hypothesized to significantly affect how colonies perform in their nest, posing  
522 consequences from occupying one nest shape over another (Pinter-Wollman 2015; Lehue and Detrain 2019;  
523 Lehue and Detrain 2020; Lehue et al., 2020a,b). However, in a separate study utilizing the same colonies  
524 as here, worker spatial fidelity zones were resilient to changes in nest shape (Chism et al., 2022, Preprint).  
525 Here, we measured performance as a colony's latency to remove a novel conspecific invader, where we argue  
526 that longer invader removal time increases the risk of queen and brood mortality, or brood stealing seen in  
527 *Temnothorax* ants (Pamminger et al., 2012; Jongepier et al., 2014). Nest defense in *Temnothorax* ants could  
528 be effective across a diversity of nest shapes, and invader removal may be related to colony aggression and  
529 nest site competition (e.g., latitudinal variation: Bengston and Dornhaus 2015). Successful nest defense and  
530 invader elimination could be important to *T. rugatulus* colonies, since colonies fight opponent colonies harder  
531 when the opponent has more brood (Chapin et al., 2022). We hypothesize that *Temnothorax* ants can adjust  
532 their occupation strategy and are resilient to changes in nest occupation. Both the approach and results  
533 of our study could possibly be extended to other eusocial animal architects, such as wasps, bees, termites,  
534 and naked mole rats, providing insights into how the structural components of their built architectures (i.e.,  
535 burrows, nests) can influence occupant behaviors such division of labor.

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541 **References**

- 542 Allaire, J. (2012). RStudio: integrated development environment for R. Boston, MA, 770(394), 165-171.  
543 Available from: <http://www.rstudio.com/>
- 544 Ashtiani, M., Mirzaie, M., and Jafari, M. (2019). CINNA: an R/Cran package to decipher Central Infor-  
545 mative Nodes in Network Analysis. *Bioinformatics*, 35(8), 1436-1437. doi: 10.1093/bioinformatics/bty819
- 546 Anderson, M. (1984). The evolution of eusociality. *Annual Review of Ecology and Systematics*, 15(1),  
547 165-189. doi: 10.1146/annurev.es.15.110184.001121
- 548 Baracchi, D., and Cini, A. (2014). A socio-spatial combined approach confirms a highly compartmentalised  
549 structure in honeybees. *Ethology*, 120(12), 1167-1176. doi: 10.1111/eth.12290
- 550 Bartoń, K. (2013). MuMIn: multi-model inference. R package version 1.43. 17. Vienna, Austria: The  
551 Comprehensive R Archive Network (CRAN). 2013. Available from: <https://CRAN.R-project.org/package=MuMIn>
- 552 Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using  
553 lme4. *Journal of Statistical Software*, 67(1), 1-48. doi: 10.18637/jss.v067.i01
- 554 Bengston, S. E., and Dornhaus, A. (2015). Latitudinal variation in behaviors linked to risk tolerance  
555 is driven by nest-site competition and spatial distribution in the ant *Temnothorax rugatulus*. *Behavioral  
556 Ecology and Sociobiology*, 69(8), 1265-1274. doi: 10.1007/s00265-015-1939-4
- 557 Blonder, B., and Dornhaus, A. (2011). Time-ordered networks reveal limitations to information flow in  
558 ant colonies. *PLoS One*, 6(5), e20298. doi: 10.1371/journal.pone.0020298
- 559 Bollazzi, M., and Roces, F. (2010). Leaf-cutting ant workers (*Acromyrmex heyeri*) trade off nest ther-  
560 moregulation for humidity control. *Journal of Ethology*, 28(2), 399-403. doi: 10.1007/s10164-010-0207-3
- 561 Bruce, A. I., Pérez-Escudero, A., Czaczkes, T. J., and Burd, M. (2019). The digging dynamics of ant  
562 tunnels: movement, encounters, and nest space. *Insectes Sociaux*, 66(1), 119-127. doi: 10.1007/s00040-018-  
563 0657-0
- 564 Buhl, J., Gautrais, J., Solé, R. V., Kuntz, P., Valverde, S., Deneubourg, J. L., and Theraulaz, G. (2004).  
565 Efficiency and robustness in ant networks of galleries. *The European Physical Journal B-Condensed Matter  
566 and Complex Systems*, 42(1), 123-129. doi: 10.1140/epjb/e2004-00364-9
- 567 Burd, M., Shiwakoti, N., Sarvi, M., and Rose, G. (2010). Nest architecture and traffic flow: large  
568 potential effects from small structural features. *Ecological Entomology*, 35(4), 464-468. doi: 10.1111/j.1365-  
569 2311.2010.01202.x
- 570 Cao, T. T. (2013). High social density increases foraging and scouting rates and induces polydomy in  
571 *Temnothorax* ants. *Behavioral Ecology and Sociobiology*, 67(11), 1799-1807. doi: 10.1007/s00265-013-1587-  
572 5
- 573 Chandrasekhar, A., Gordon, D. M., and Navlakha, S. (2018). A distributed algorithm to maintain and  
574 repair the trail networks of arboreal ants. *Scientific Reports*, 8(1), 1-19. doi: 10.1038/s41598-018-27160-3
- 575 Chandrasekhar, A., Marshall, J. A., Austin, C., Navlakha, S., and Gordon, D. M. (2021). Better tired  
576 than lost: Turtle ant trail networks favor coherence over short edges. *PLoS Computational Biology*, 17(10),  
577 e1009523. doi: 10.1371/journal.pcbi.1009523

- 578 Chang, J., Powell, S., Robinson, E. J., and Donaldson-Matasci, M. C. (2021). Nest choice in arboreal  
579 ants is an emergent consequence of network creation under spatial constraints. *Swarm Intelligence*, 15(1),  
580 7-30. doi: 10.1007/s11721-021-00187-5
- 581 Chapin, K. J., Paat, V. A., and Dornhaus, A. (2022). Brood as booty: the effect of colony size and  
582 resource value in social insect contests. *Behavioral Ecology*, 33(3), 549-555. doi: 10.1093/beheco/arac019
- 583 Charbonneau, D., Blonder, B., and Dornhaus, A. (2013). Social insects: a model system for network  
584 dynamics. In *Temporal networks* (pp. 217-244). Springer, Berlin, Heidelberg. doi: 10.1007/978-3-642-  
585 36461-7\_11
- 586 Charbonneau, D., Poff, C., Nguyen, H., Shin, M. C., Kierstead, K., and Dornhaus, A. (2017). Who are  
587 the “lazy” ants? The function of inactivity in social insects and a possible role of constraint: inactive ants  
588 are corpulent and may be young and/or selfish. *Integrative and Comparative Biology*, 57(3), 649-667. doi:  
589 10.1093/icb/icx029
- 590 Chism, G. T., Nichols, W., and Dornhaus, A. (2022). Nest shape influences colony organization in ants:  
591 spatial distribution and connectedness of colony members differs from that predicted by random movement  
592 and is affected by nest space. *bioRxiv*. doi: 10.1101/2022.06.30.498314
- 593 Collias, E. C., and Collias, N. E. (1964). The development of nest-building behavior in a weaverbird. *The  
594 Auk*, 81(1), 42-52. doi: 10.1111/j.1474-919X.1970.tb00818.x
- 595 Csardi, G., and Nepusz, T. (2006). The igraph software package for complex network research. *Inter-  
596 Journal, Complex Systems*, 1695(5), 1-9. Available from: <https://igraph.org>
- 597 Davidson, J. D., and Gordon, D. M. (2017). Spatial organization and interactions of harvester ants during  
598 foraging activity. *Journal of The Royal Society Interface*, 14(135), 20170413. doi: 10.1098/rsif.2017.0413
- 599 Detrain, C., and Pasteels, J. M. (1992). Caste polyethism and collective defense in the ant, *Pheidole  
600 pallidula*: the outcome of quantitative differences in recruitment. *Behavioral Ecology and Sociobiology*,  
601 29(6), 405-412. doi: 10.1007/BF00170170
- 602 Dijkstra, E. W. (1959). A note on two problems in connexion with graphs. *Numerische Mathematik*, 1(1),  
603 269-271. doi: 10.1007/BF01386390
- 604 Donaldson-Matasci, M. C., DeGrandi-Hoffman, G., and Dornhaus, A. (2013). Bigger is better: hon-  
605 eybee colonies as distributed information-gathering systems. *Animal Behaviour*, 85(3), 585-592. doi:  
606 10.1016/j.anbehav.2012.12.020
- 607 Dornhaus, A., and Powell, S. (2010). Foraging and Defence Strategies. In *Ant Ecology* Oxford University  
608 Press. doi: 10.1093/acprof:oso/9780199544639.003.0012
- 609 Droual, R. (1984). Anti-predator behaviour in the ant *Pheidole desertorum*: The importance of multiple  
610 nests. *Anim. Behavior*. 32, 1054–1058. doi: 10.1016/S0003-3472(84)80221-3
- 611 Ek, B., VerSchneider, C., and Narayan, D. A. (2015). Global efficiency of graphs. *AKCE International  
612 Journal of Graphs and Combinatorics*, 12(1), 1-13. doi: 10.1016/j.akcej.2015.06.001
- 613 Faulkes, C. G., and Bennett, N. C. (2001). Family values: group dynamics and social control of re-  
614 production in African mole-rats. *Trends in Ecology and Evolution*, 16(4), 184-190. doi: 10.1016/S0169-  
615 5347(01)02116-4
- 616 Fisher, D. N., and Pinter-Wollman, N. (2021). Using multilayer network analysis to explore the temporal  
617 dynamics of collective behavior. *Current Zoology*, 67(1), 71-80. doi: 10.1093/cz/zoaa050
- 618 Foitzik, S., DeHeer, C. J., Hunjan, D. N., and Herbers, J. M. (2001). Coevolution in host-parasite systems:  
619 behavioural strategies of slave-making ants and their hosts. *Proceedings of the Royal Society of London.  
620 Series B: Biological Sciences*, 268(1472), 1139-1146. doi: 10.1098/rspb.2001.1627

- 621        Franks, N. R., and Sendova-Franks, A. B. (1992). Brood sorting by ants: distributing the workload over  
 622 the work-surface. *Behavioral Ecology and Sociobiology*, 30(2), 109-123. doi: 10.1007/BF00173947
- 623        Garrison, L. K., Kleineidam, C. J., and Weidenmüller, A. (2018). Behavioral flexibility promotes collective  
 624 consistency in a social insect. *Scientific Reports*, 8(1), 1-11. doi: 10.1038/s41598-018-33917-7
- 625        Gordon, D. M., and Mehdiabadi, N. J. (1999). Encounter rate and task allocation in harvester ants.  
 626 *Behavioral Ecology and Sociobiology*, 45(5), 370-377. doi: 10.1007/s002650050573
- 627        Gordon, D. M. (2010). Ant encounters. In *Ant Encounters*. Princeton University Press. doi: 10.1515/9781400835447
- 628        Greene, M. J., and Gordon, D. M. (2007). Interaction rate informs harvester ant task decisions. *Behavioral  
 629 Ecology*, 18(2), 451-455. doi: 10.1093/beheco/arl105
- 630        Hansell, M. H. (2019). *Animal architecture*. Oxford University Press on Demand. doi: 10.1016/B978-0-  
 631 12-809633-8.90728-3
- 632        Halboth, F., and Roces, F. (2017). The construction of ventilation turrets in *Atta vollenweideri* leaf-cutting  
 633 ants: Carbon dioxide levels in the nest tunnels, but not airflow or air humidity, influence turret structure.  
 634 *PLoS One*, 12(11), e0188162. doi: 10.1371/journal.pone.0188162
- 635        Hölldobler, B., and Wilson, E. O. (2010). *The leafcutter ants: civilization by instinct*. WW Norton and  
 636 Company. ISBN-13: 978-0393338683
- 637        Huang, M. H. (2010). Multi-phase defense by the big-headed ant, *Pheidole obtusospinosa*, against raiding  
 638 army ants. *Journal of Insect Science*, 10(1), 1. doi: 10.1673/031.010.0101
- 639        Jeanne, R. L. (1975). The adaptiveness of social wasp nest architecture. *The Quarterly Review of Biology*,  
 640 50(3), 267-287. doi: 10.1086/408564
- 641        Ji, Q., Xin, C., Tang, S. X., and Huang, J. P. (2018). Symmetry associated with symmetry break:  
 642 Revisiting ants and humans escaping from multiple-exit rooms. *Physica A: Statistical Mechanics and its  
 643 Applications*, 492, 941-947. doi: 10.1016/j.physa.2017.11.024
- 644        Jongepier, E., Kleeberg, I., Job, S., and Foitzik, S. (2014). Collective defence portfolios of ant hosts shift  
 645 with social parasite pressure. *Proceedings of the Royal Society B: Biological Sciences*, 281(1791), 20140225.  
 646 doi: 10.1098/rspb.2014.0225
- 647        Kaspari, M., and O'Donnell, S. (2003). High rates of army ant raids in the Neotropics and implications  
 648 for ant colony and community structure. *Evolutionary Ecology Research*, 5(6), 933-939. Available from:  
 649 <http://www.pages.drexel.edu/~so356/pdfRaidRates.pdf>
- 650        Korb, J. (2003). Thermoregulation and ventilation of termite mounds. *Naturwissenschaften*, 90(5),  
 651 212-219. doi: 10.1007/s00114-002-0401-4
- 652        Korb, J. (2010). Termite mound architecture, from function to construction. In *Biology of termites: a  
 653 modern synthesis* (pp. 349-373). Springer, Dordrecht. doi: 10.1007/978-90-481-3977-4\_13
- 654        Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. (2017). lmerTest package: tests in linear mixed  
 655 effects models. *Journal of Statistical Software*, 82(1), 1-26. doi: 10.18637/jss.v082.i13
- 656        Lanan, M. C., Dornhaus, A., Jones, E. I., Waser, A., and Bronstein, J. L. (2012). The trail less  
 657 traveled: individual decision-making and its effect on group behavior. *PLoS One*, 7(10), e47976. doi:  
 658 10.1371/journal.pone.0047976
- 659        Latora, V., and Marchiori, M. (2003). Economic small-world behavior in weighted networks. *The European  
 660 Physical Journal B-Condensed Matter and Complex Systems*, 32(2), 249-263. doi: 10.1140/epjb/e2003-  
 661 00095-5

- 662 Latty, T., Ramsch, K., Ito, K., Nakagaki, T., Sumpter, D. J., Middendorf, M., and Beekman, M. (2011).  
663 Structure and formation of ant transportation networks. *Journal of The Royal Society Interface*, 8(62),  
664 1298-1306. doi: 10.1098/rsif.2010.0612
- 665 Lehue, M., and Detrain, C. (2019). What's going on at the entrance? A characterisation of the social  
666 interface in ant nests. *Behavioural Processes*, 160, 42-50. doi: 10.1016/j.beproc.2018.12.006
- 667 Lehue, M., and Detrain, C. (2020). Foraging through multiple nest holes: An impediment to collective  
668 decision-making in ants. *PLoS One*, 15(7), e0234526. doi: 10.1371/journal.pone.0234526
- 669 Lehue, M., Collignon, B., and Detrain, C. (2020a). Multiple nest entrances alter foraging and information  
670 transfer in ants. *Royal Society Open Science*, 7(2), 191330. doi: 10.1098/rsos.191330
- 671 Lehue, M., Detrain, C., and Collignon, B. (2020b). Nest entrances, spatial fidelity, and foraging patterns in  
672 the red ant *Myrmica rubra*: a field and theoretical study. *Insects*, 11(5), 317. doi: 10.3390/insects11050317
- 673 London, K. B., and Jeanne, R. L. (2000). The interaction between mode of colony founding, nest  
674 architecture and ant defense in polistine wasps. *Ethology Ecology and Evolution*, 12(1), 13-25. doi:  
675 10.1080/03949370.2000.9728440
- 676 Mersch, Danielle P., Alessandro Crespi, and Laurent Keller. "Tracking individuals shows spatial fidelity is a  
677 key regulator of ant social organization." *Science* 340.6136 (2013): 1090-1093. doi: 10.1126/science.1234316
- 678 Naug, D., and Camazine, S. (2002). The role of colony organization on pathogen transmission in social  
679 insects. *Journal of Theoretical Biology*, 215(4), 427-439. doi: 10.1006/jtbi.2001.2524
- 680 Noirot, C., and Darlington, J. P. (2000). Termite nests: architecture, regulation and defence. In *Termites: evolution, sociality, symbioses, ecology* (pp. 121-139). Springer, Dordrecht. doi: 10.1007/978-94-017-3223-9\_6
- 681 Nonacs, P. (1993). The economics of brood raiding and nest consolidation during ant colony founding.  
682 *Evolutionary Ecology*, 7(6), 625-633. doi: 10.1007/BF01237825
- 683 O'Donnell, S., and Bulova, S. J. (2007). Worker connectivity: a review of the design of worker communication  
684 systems and their effects on task performance in insect societies. *Insectes Sociaux*, 54(3), 203-210.  
685 doi: 10.1007/s00040-007-0945-6
- 686 Pamminger, T., Modlmeier, A. P., Suette, S., Pennings, P. S., and Foitzik, S. (2012). Raiders from the  
687 sky: slavemaker founding queens select for aggressive host colonies. *Biology Letters*, 8(5), 748-750. doi:  
688 10.1098/rsbl.2012.0499
- 689 Pereira, H., Jossart, M., and Detrain, C. (2020). Waste management by ants: the enhancing role of larvae.  
690 *Animal Behaviour*, 168, 187-198. doi: 10.1016/j.anbehav.2020.08.017
- 691 Pie, M. R., Rosengaus, R. B., and Traniello, J. F. (2004). Nest architecture, activity pattern, worker  
692 density and the dynamics of disease transmission in social insects. *Journal of Theoretical Biology*, 226(1),  
693 45-51. doi: 10.1016/j.jtbi.2003.08.002
- 694 Pinter-Wollman, N., Wollman, R., Guetz, A., Holmes, S., and Gordon, D. M. (2011). The effect of  
695 individual variation on the structure and function of interaction networks in harvester ants. *Journal of the  
696 Royal Society Interface*, 8(64), 1562-1573. doi: 10.1098/rsif.2011.0059
- 697 Pinter-Wollman, N. (2015a). Persistent variation in spatial behavior affects the structure and function of  
698 interaction networks. *Current Zoology*, 61(1), 98-106. doi: 10.1093/czoolo/61.1.98
- 699 Pinter-Wollman, N. (2015b). Nest architecture shapes the collective behaviour of harvester ants. *Biology  
700 Letters*, 11(10), 20150695. doi: 10.1098/rsbl.2015.0695
- 701 Powell, S. (2008). Ecological specialization and the evolution of a specialized caste in *Cephalotes* ants.  
702 *Functional Ecology*, 22(5), 902-911. doi: 10.1111/j.1365-2435.2008.01436.x

- 705 Powell, S., and Dornhaus, A. (2013). Soldier-based defences dynamically track resource availability and  
706 quality in ants. *Animal Behaviour*, 85(1), 157-164. doi: 10.1016/j.anbehav.2012.10.020
- 707 Powell, S., Donaldson-Matasci, M., Woodrow-Tomizuka, A., and Dornhaus, A. (2017). Context-dependent  
708 defences in turtle ants: Resource defensibility and threat level induce dynamic shifts in soldier deployment.  
709 *Functional Ecology*, 31(12), 2287-2298. doi: 10.1111/1365-2435.12926 Pusceddu, M., Cini, A., Alberti,  
710 S., Salaris, E., Theodorou, P., Floris, I., and Satta, A. (2021). Honey bees increase social distancing when  
711 facing the ectoparasite Varroa destructor. *Science Advances*, 7(44), eabj1398. doi: 10.1126/sciadv.abj1398
- 712 Radeva, T., Dornhaus, A., Lynch, N., Nagpal, R., and Su, H. H. (2017). Costs of task allocation with local  
713 feedback: Effects of colony size and extra workers in social insects and other multi-agent systems. *PLoS  
714 Computational Biology*, 13(12), e1005904. doi: 10.1371/journal.pcbi.1005904
- 715 Ratnieks, F. L., and Anderson, C. (1999). Task partitioning in insect societies. II. Use of queueing delay  
716 information in recruitment. *The American Naturalist*, 154(5), 536-548. doi: 10.1086/303256
- 717 Rice, L., Tate, S., Farynyk, D., Sun, J., Chism, G., Charbonneau, D., ... and Shin, M. C. (2020). ABC-  
718 Tracker: an easy-to-use, cloud-based application for tracking multiple objects. arXiv preprint arXiv:2001.10072.  
719 doi: 10.48550/arXiv.2001.10072
- 720 Rochat, Y. (2009). Closeness centrality extended to unconnected graphs: The harmonic centrality index  
721 (No. CONF). Available from: [https://infoscience.epfl.ch/record/200525/files/\[EN\]ASNA09.pdf](https://infoscience.epfl.ch/record/200525/files/[EN]ASNA09.pdf)
- 722 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., ... and Tinevez,  
723 J. Y. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676. doi:  
724 10.1038/nmeth.2019
- 725 Seeley, T. D., Seeley, R. H., and Akratanakul, P. (1982). Colony defense strategies of the honeybees in  
726 Thailand. *Ecological Monographs*, 52(1), 43-63. doi: 10.2307/2937344
- 727 Sempo, G., Depickère, S., and Detrain, C. (2006). How brood influences caste aggregation patterns in the  
728 dimorphic ant species *Pheidole pallidula*. *Insectes Sociaux*, 53(2), 241-248. doi: 10.1007/s00040-006-0864-y
- 729 Sendova-Franks, A. B., and Franks, N. R. (1995). Spatial relationships within nests of the ant *Leptothorax*  
730 *unifasciatus* (Latr.) and their implications for the division of labour. *Animal Behaviour*, 50(1), 121-136.  
731 doi: 10.1006/anbe.1995.0226
- 732 Sendova-Franks, A. B., Hayward, R. K., Wulf, B., Klimek, T., James, R., Planqué, R., ... and Franks,  
733 N. R. (2010). Emergency networking: famine relief in ant colonies. *Animal Behaviour*, 79(2), 473-485. doi:  
734 10.1016/j.anbehav.2009.11.035
- 735 Shorter, J. R., and Rueppell, O. (2012). A review on self-destructive defense behaviors in social insects.  
736 *Insectes Sociaux*, 59(1), 1-10. doi: 10.1007/s00040-011-0210-x
- 737 Shiwakoti, N., Sarvi, M., Rose, G., and Burd, M. (2011). Animal dynamics based approach for modeling  
738 pedestrian crowd egress under panic conditions. *Procedia-social and Behavioral Sciences*, 17, 438-461. doi:  
739 10.1016/j.sbspro.2011.04.526
- 740 Shiwakoti, N., Sarvi, M., and Burd, M. (2014). Using non-human biological entities to understand pedes-  
741 trian crowd behaviour under emergency conditions. *Safety Science*, 66, 1-8. doi: 10.1016/j.ssci.2014.01.010
- 742 Stroeymeyt, N., Grasse, A. V., Crespi, A., Mersch, D. P., Cremer, S., and Keller, L. (2018). Social  
743 network plasticity decreases disease transmission in a eusocial insect. *Science*, 362(6417), 941-945. doi:  
744 10.1126/science.aat4793
- 745 Swain, A., Williams, S. D., Di Felice, L. J., and Hobson, E. A. (2022). Interactions and informa-  
746 tion: exploring task allocation in ant colonies using network analysis. *Animal Behaviour*, 189, 69-81. doi:  
747 10.1016/j.anbehav.2022.04.015

- 748 Tanner, C. J., and Adler, F. R. (2009). To fight or not to fight: context-dependent interspecific aggression  
749 in competing ants. *Animal Behaviour*, 77(2), 297-305. doi: 10.1016/j.anbehav.2008.10.016
- 750 Team, R. Core (2017). R: A language and environment for statistical computing. R Foundation for  
751 Statistical Computing, Vienna, Austria. Available from: <https://www.R-project.org/>.
- 752 Tian, L., and Zhou, X. (2014). The soldiers in societies: defense, regulation, and evolution. *International  
753 Journal of Biological Sciences*, 10(3), 296. doi: 10.7150/ijbs.6847
- 754 Tofts, C., and Franks, N. R. (1992). Doing the right thing: ants, honeybees and naked mole-rats. *Trends  
755 in Ecology and Evolution*, 7(10), 346-349. doi: 10.1016/0169-5347(92)90128-X
- 756 Tschinkel, W. R. (1987). Seasonal life history and nest architecture of a winter-active ant, *Prenolepis  
757 imparis*. *Insectes Sociaux*, 34(3), 143-164. doi: 10.1007/BF02224081
- 758 Tschinkel, W. R. (2005). The nest architecture of the ant, *Camponotus socius*. *Journal of Insect Science*,  
759 5(1). doi: 10.1093/jis/5.1.9
- 760 Tschinkel, W. R. (2011). The nest architecture of three species of north Florida *Aphaenogaster* ants.  
761 *Journal of Insect Science*, 11(1). doi: 10.1673/031.011.10501
- 762 Vaes, O., Perna, A., and Detrain, C. (2020). The effect of nest topology on spatial organization and  
763 recruitment in the red ant *Myrmica rubra*. *The Science of Nature*, 107, 1-14. doi: 10.1007/s00114-020-  
764 01675-0
- 765 van Dijk, R. E., Covas, R., Doutrelant, C., Spottiswoode, C. N., and Hatchwell, B. J. (2015). Fine-scale  
766 genetic structure reflects sex-specific dispersal strategies in a population of sociable weavers (*Philetairus  
767 socius*). *Molecular Ecology*, 24(16), 4296-4311. doi: 10.1111/mec.13308
- 768 Visscher, P. K. (2007). Group decision making in nest-site selection among social insects. *Annual Review  
769 of Entomology*, 52, 255-275. doi: 10.1146/annurev.ento.51.110104.151025
- 770 Wang, S., and Song, W. (2016). Experimental study of ant movement in a straight passageway under  
771 stress conditions. *Journal of Insect Behavior*, 29(6), 735-743. doi: 10.1007/s10905-016-9593-x
- 772 Wang, S., Cao, S., Wang, Q., Lian, L., and Song, W. (2016). Effect of exit locations on ants escaping a  
773 two-exit room stressed with repellent. *Physica A: Statistical Mechanics and its Applications*, 457, 239-254.  
774 doi: 10.1016/j.physa.2016.03.083
- 775 Wang, Q., Song, W., Zhang, J., Wang, S., Wu, C., and Lo, S. (2019). Understanding single-file movement  
776 with ant experiments and a multi-grid CA model. *Physica A: Statistical Mechanics and its Applications*,  
777 513, 1-13. doi: 10.1016/j.physa.2018.08.013
- 778 Waters, J. S., and Fewell, J. H. (2012). Information processing in social insect networks. *PLoS One*, 7(7),  
779 e40337. doi: 10.1371/journal.pone.0040337
- 780 Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D. A., François, R., ... and Yutani, H.  
781 (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, 4(43), 1686. doi: 10.21105/joss.01686
- 782 Wild, B., Dormagen, D. M., Zachariae, A., Smith, M. L., Traynor, K. S., Brockmann, D., ... and  
783 Landgraf, T. (2021). Social networks predict the life and death of honey bees. *Nature Communications*,  
784 12(1), 1-12. doi: 10.1038/s41467-021-21212-5
- 785 Wilson, E. O. (1968). The ergonomics of caste in the social insects. *The American Naturalist*, 102(923),  
786 41-66. doi: 10.1086/282522
- 787 Wilson, E. O. (1976). The organization of colony defense in the ant *Pheidole dentata* Mayr (Hymenoptera:  
788 Formicidae). *Behavioral Ecology and Sociobiology*, 1(1), 63-81. doi: 10.1007/BF00299953
- 789 Wilson, E. O., and Kinne, O. (1990). Success and dominance in ecosystems: the case of the social insects  
790 (Vol. 2, pp. I-XXI). Oldendorf/Luhe: Ecology Institute.

791 Wilson, E. O. (1992). The effects of complex social life on evolution and biodiversity. *Oikos*, 13-18. doi:  
792 10.2307/3545511  
793 write.bibtex(file="references.bib")