

¹ Nest shape influences colony organization in ants: colony spatial
² distribution differs from random movement

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⁴ **Abstract**

Many social animals produce architectures that greatly increase the builder's ability to survive across environments. For example, the many forms and functions of ant nests facilitate their ability to occupy nearly every biome. However, nest shape is not always determined by the ants, as some species inhabit cavities with externally determined shapes, such as rock crevices or hollow sticks. In both cases, we can ask not only how ant behaviour can affect the form and function of nests, but also how nest shapes impact the inhabiting ant colony's behaviour. We examined how the spatial organization of *Temnothorax rugatulus* ants differed across given artificial nests with radically different internal shapes. We used geometric measures to describe the emerging spatial relationships of workers, brood, queens, and alates in these two shapes, and show that overall distributions of all types of colony members are influenced by nest shape. However, we also found that individual spatial fidelity zone size, i.e. the areas repeatedly occupied by individuals, which may be linked to their division of labor, are overall not affected by nest shape. This is even though spatial fidelity zone size does vary as a function of distance from the nest entrance and brood center; and that this relationship is affected by both nest shape and density. Finally, we show that workers are found closer to the nest entrance than would be expected in a random walk model in both nest shapes. These findings indicate that ants actively regulate their nest occupation, and that they may compensate for effects of nest architecture constraints. We conclude in this study that physical properties of nests can influence the in-nest spatial organization of ant colonies, which highlights the need to explore nest shape as a direct influence on the organization, movement, and communication of the inhabiting ant colony.

⁵ **Keywords:** Ants, Nest Architecture, *Temnothorax*, Colony Organization, Nest Entrance, Brood

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6 Introduction

7 Social animal architectures benefit individuals by opening a wider range of suitable habitats that they,
8 and their kin, can successfully inhabit. These architectures, i.e. built environmental modifications, range
9 from simple depressions in the ground (e.g. social crab burrows, Laidre et al., 2018) to massive above and
10 below ground structures that span meters across (e.g. Macrotermes termites, Harris 1956). This diversity is
11 unified by the impact each architecture can have on the social behaviour of their occupants: social weaver
12 bird nests promote altruism through spatial and social clustering (van Dijk et al., 2014), kin cohabitation
13 increases with burrow size in social crabs (Laidre et al., 2018, Laidre 2019), and reproductive division of
14 labor is facilitated by both naked mole rat burrows (Tofts and Franks 1992; Faulkes and Bennett 2001) and
15 social insects nests (Wilson and Kinne 1990, Wilson 1992). Ant nests are particularly interesting because of
16 both their representation in nearly every environment, and their diversity in form and function within these
17 environments (Wilson and Kinne 1990, Wilson 1992).

18

19 Here we are interested in how the geometric shape of such built architectures influences the organization of
20 the colony within. Ants have been demonstrated to be distributed non-uniformly across their internal nest
21 space (Sendova-Franks and Franks 1995; Sendova-Franks and Franks 1999; Tschinkel 1999; Tschinkel 2005).
22 For example, colony members may be concentrated in specific sections of the nest (Tschinkel 1999; Tschinkel
23 2005); and workers may use chemical ‘road-signs’ to navigate nest space and differentiate chambers with
24 different functions (Heyman et al., 2017). However, some complex spatial relationships in the nest may
25 be predicted by a random walk model (Sendova-Franks and Van Lent 2002, Davidson and Gordon 2017),
26 implying that the placement of colony members may not be an explicit adaptive strategy. In addition, many
27 ants exploit pre-existing natural structures as nests (e.g. Temnothorax - ‘acorn’ and ‘rock’ ants: Prebus 2017,
28 Cephalotes turtle ants that occupy unmodifiable wood nests: Powell 2008, and rock-dwelling Rhytidoponera
29 metallica ponerine ants: Thomas 2002), which means that the geometry of the available space may not be
30 under the ants’ control. We therefore ask how the internal nest space may influence the spatial organization
31 of workers, queens, and brood in the nest.

32

33 Some studies have previously shown that nest architectural features can greatly affect how workers move
34 and interact in the nest. For example, worker interactions and recruitment increases from movement within
35 more physically connected nest chambers (Pinter-Wollman 2015; Vaes et al., 2020), information processing
36 and exploitation of the environment is decreased in foragers utilizing a nest with multiple entrances (Lehue
37 and Detrain 2019; Lehue and Detain 2020; Lehue et al., 2020a,b; Lehue and Detrain 2020), and panicked
38 workers are slowed by bottleneck effects while evacuating through a single nest entrance (Burd et al., 2010;
39 Wang and Song 2016). These behavioural changes are directly related to a colony’s distribution in the
40 nest, implying consequences of choosing one nest site over another. Here, we examine more basically how
41 nest shape directly influences the spatial positions of colony members, which may translate to changes in
42 performing essential tasks such as foraging.

43

44 Different areas of social insect nests serve different functions. Most prominently in ants, the queen(s) and
45 her brood are typically concentrated in a particular area or chamber(s), referred to as the ‘brood pile’. The
46 location of the brood pile may be influenced by which locations are both more accessible from other nest
47 spaces and possibly more defensible during nest invasions (Varoudis et al., 2018). Worker task allocation,
48 i.e. the types of work performed by different social insect workers, is thus associated with where workers
49 are typically located - individual consistency in their location in the nest is described as ‘spatial fidelity
50 zones’ (Sendova-Franks and Franks 1994; Sendova-Franks and Franks 1995; Sendova-Franks and Franks
51 1999). The locations of these zones for different workers, and the spread of workers across the nest area is a
52 hypothesized mechanism for achieving division of labor in social insects (Robinson et al., 2009). The size of
53 these fidelity zones varies a lot, possibly relating to the type of task available at different locations within

54 the nest (e.g. smaller spatial fidelity zones in workers performing brood care, Sendova-Franks and Franks
55 1995; Jandt and Dornhaus 2009). Inactive workers also occupy smaller fidelity zones, often near the nest
56 center (near/on the brood pile, Charbonneau et al., 2017), and their placement is an indication that they
57 may represent a reserve workforce specializing in brood care (Charbonneau, Poff, et al., 2017; Charbonneau
58 et al., 2017; Leitner and Dornhaus 2019). Thus, the role of different in-nest locations in colony organization
59 varies, and workers occupying space near or on the brood area often have different roles in the colony than
60 workers occupying space in the periphery or near the nest entrance. We therefore focus much of our analyses
61 on the location of colony members relative to the brood pile or the nest entrance.

62

63 Nest-related collective behaviour has been well studied in the *Temnothorax* genus. Rock-dwelling *Temnothorax*
64 have been the subject of studies involving house-hunting (Franks et al., 2002; Dornhaus et al., 2004;
65 Sasaki et al., 2013; Sasaki et al., 2015), collective nest building (Franks et al., 1992; Franks and Deneubourg
66 1997; Aleksiev et al., 2007a; Aleksiev et al., 2007b; Aleksiev et al., 2007c; DiRienzo and Dornhaus 2017),
67 life-history strategy and risk-taking behaviours relating to competition for nest sites (Bengston and Dorn-
68 haus 2014; Bengston and Dornhaus 2015; Bengston et al., 2017), among other topics. We used the rock
69 ant *Temnothorax rugatulus* to examine the influence of nest shape on its occupants because they dwell in
70 pre-existing rock-crevices, producing single-chambered nests that are easily replicated and manipulated in a
71 laboratory setting (Charbonneau and Dornhaus 2015). Worker spatial position in *Temnothorax unifasciatus*
72 nests closely predicts the task they perform, such as brood care near the brood pile (Sendova-Franks and
73 Franks 1995), and *T. unifasciatus* workers will return to their spatial positions in relation to one another in
74 new nests (Sendova-Franks and Franks 1994).

75

76 Here, we determine whether the nest shape affects the in-nest colony organization of *T. rugatulus* colonies.
77 We first compared colony member distributions in two artificial nest designs that differed markedly in
78 physical accessibility. We then asked how in-nest locations of the nest entrance and the center of gravity of
79 the brood distribution or of the physical space as a whole relate to where workers and other colony members
80 are found in the nest. Next, we determined whether the spatial fidelity zones of individual workers are
81 different across nest shapes, relate to the location of nest features or centers, or are conserved individually
82 across nest shapes. Finally, we asked whether worker distributions are predicted by a random walk model.

83

84 Methods

85 Colony collections

86 We collected 20 colonies of *Temnothorax rugatulus* from rocky semi-steep slopes on the Santa Catalina
87 Mountains (GPS: 32.395, -110.688), USA, Pima County, Arizona from July to October 2017 and February
88 to May 2018. The second half of the colonies (IDs 11-20) were collected during the colonies' reproductive
89 period, which allowed us to examine alate spatial organization. The first ten colonies had only workers and
90 (non-alate) queens (and brood), whereas seven of the latter ten colonies had alates (i.e. winged queens and
91 males). We found all our colonies in a pine-juniper zone (altitude approximately 2500m) inhabiting granite
92 rock-crevices where entire colonies were collected by aspiration after prying open the nest cavity.

93

94 Initial housing and care

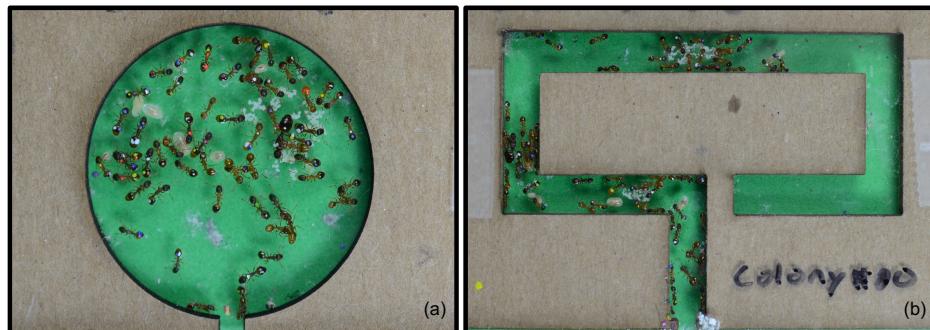
95 We initially placed all colonies in generic artificial nests resembling their natural nest sites in rock crevices.
96 These nests consisted of a 2-mm-thick piece of cardboard (75 mm x 50 mm x 2 mm) sandwiched between
97 two glass panes (76.2 mm x 50.8 mm x 0.5 mm), with a 2 mm x 2 mm entrance at the center of one long
98 side leading to an open nest space (35 mm x 25 mm) (Charbonneau et al., 2015; Appendix, Fig. A1). We

99 gave colonies food and water *ad libitum*, refreshed weekly, for the duration of their housing: water was given
100 through water-filled cotton ball stopped 5 ml plastic tubes, and food was given through 2 ml microcentrifuge
101 tubes of honey water with a concentration of 1/4 teaspoon honey per 50ml water, and 1/8 (approximately
102 0.075 g) of a fresh-frozen cockroach (*Nauphoeta cinerea*). We kept colonies on a 12:12 h light cycle (8
103 a.m. to 8 p.m.), constant temperature (approximately 21-24 °C). We placed all artificial nests in open-top
104 plastic containers (11.1 cm x 11.1 cm x 3.3 cm) with walls lined with 'insect-a-slip' (BioQuip 2871A, 'fluon')
105 to prevent escape. We individually marked CO²⁺ anesthetized workers with four identifying marks with
106 multicolored paints (Testor's Pactra® paint): one each on the head and prosoma, and two on the gaster
107 (Charbonneau and Dornhaus 2015). We marked ants three to five days before the colony was placed into
108 experimental nests.

109

110 **Experimental setup**

111 We used two nest types, a circle, and a tube-shaped nest, for the experimental phase (Fig. 1). We gave
112 colonies comparable nest densities by scaling the internal area of the nest cavities to colony size (number of
113 workers). We examined the size of a *Temnothorax rugatulus* colony that utilized nearly all of the available
114 nest space in a pre-experimental nest (248 workers, Appendix, Fig. A1) and doubled the area per worker to
115 permit flexible nest space use (0.033 mm² per worker) - we termed this the high worker density treatment.
116 We further produced a low worker density treatment by doubling the nest area allocated to each worker
117 compared to that first treatment, producing a density half as dense as the high-density treatment (0.066
118 mm² per worker). These density treatments could result in nature from nest competition: colonies prefer
119 nests that allow lower worker densities (Visscher 2007), but high nest competition could result in populations
120 occupying nests with overall higher density; whereas low nest competition could allow colonies to occupy
121 their preferred nests.



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

124

125 **Experimental timeline**

126 We had three distinct experimental phases which were used for both density treatments. Note that half
127 of the colonies experienced the high nest density treatment, and the other half experienced the low nest
128 density treatment.

129

130 *Pre-experimental nest acclimation* - We gave colonies three days to acclimate after a forced emigration into
131 either their circle or tube experimental nest. We randomly assigned nest shapes such that ten of our colonies
132 experienced the circle nest first and assigned the other ten colonies the tube nest first.

133

¹³⁴ *Nest assignment I* - Following the three-day acclimation period, we photographed colonies in their first nest
¹³⁵ assignment for 16 days.

¹³⁶

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

¹⁴¹

¹⁴² *Nest assignment II* - We repeated the methods from Nest assignment I for the second nest type for each
¹⁴³ colony.

¹⁴⁴

¹⁴⁵ ***Photo collection***

¹⁴⁶ We took daily photos to capture colony organization in both nest shapes over both Nest assignment I and II
¹⁴⁷ (see experimental timeline) with an HD camera (Nikon D7000 with 60 mm lens). We used 10 photos from
¹⁴⁸ each nest assignment for analyses.

¹⁴⁹

¹⁵⁰ ***Photo analysis***

¹⁵¹ We assigned cartesian coordinates to each colony member on the digital images and standardized (i.e. trans-
¹⁵² lated them back into real distances in mm) them with the image analysis software Fiji (Schindelin et al.,
¹⁵³ 2012). To standardize coordinates, we converted each coordinate from pixels to metric using a known
¹⁵⁴ distance between reference coordinates from each image (5cm between the bottom-left and top-right nest
¹⁵⁵ corners).

¹⁵⁶

¹⁵⁷ ***Nest sections and colony member densities***

¹⁵⁸ We divided each nest into eight equal-area sections from the nest entrance to the back of the nest (1-8, see
¹⁵⁹ Appendix, Fig. A2). We chose not to use more than eight bins because these nest section assignments were
¹⁶⁰ potentially large enough to capture and segregate different worker tasks. We determined all colony member
¹⁶¹ densities by calculating what proportion of total observed individuals of that type on the same image were
¹⁶² found in that section.

¹⁶³

¹⁶⁴ ***Scaled distances in the nest***

¹⁶⁵ *Distance to the nest entrance*: We calculated the shortest linear distance within the available nest cavity
¹⁶⁶ from each colony member to the nest entrance (Appendix, Fig. A3). Specifically: In the circle nest, we
¹⁶⁷ calculated each colony member’s distance to the nest entrance (per a scaled reference x and y coordinate)
¹⁶⁸ using the formula: $\sqrt{((\text{colony member } x - \text{entrance } x)^2 + (\text{colony member } y - \text{entrance } y)^2)}$ (Appendix,
¹⁶⁹ Fig. A3a).

¹⁷⁰

¹⁷¹ In the tube nest, where a direct (i.e. straight-line) path was often not possible within the available nest
¹⁷² space, we found each colony member’s distance to the nest section closest to the entrance using reference
¹⁷³ coordinates for that nest section, where we then added the shortest distance from that nest section to the
¹⁷⁴ entrance through reference coordinates (Appendix, Fig. A3).

¹⁷⁵

176 Correcting corner cuts at the entrance: Near the entrance of both nests (in nest section 1), a colony
177 member's distance to the nest entrance can cut through the corner where the entrance tunnel opens into
178 the nest (Appendix, Fig. A4). We solved this by determining whether a colony member's distance would
179 cut through the corner and then assigning an alternative distance to the entrance where necessary.

180

181 *Distance to the brood center:* We found the distances of each mobile colony member (workers, queens, and
182 alates) in every observation to the center of the brood pile (Appendix, Fig. A5), which has been characterized
183 as a nest center in *Temnothorax* ants (Sendova-Franks and Franks 1995). We calculated the brood center
184 as the average brood x and y coordinates in the nest section with the most brood items in each observation.
185 Calculating the overall center of gravity of brood across the entire nest would often take this 'center' outside
186 of the nest space boundaries in the tube nest. We then found the distance from each mobile colony member
187 to this brood center. Specifically:

188 In the circle nest, we found each colony member's shortest distance to the brood center for that observation:
189 $\text{sqrt}((\text{colony member x} - \text{brood center x})^2 + (\text{colony member y} - \text{brood center y})^2)$ (Appendix, Fig. A5a).

190

191 In the tube nest, we calculated the shortest distances of mobile colony members and the brood center to
192 one another if a direct line path was available within the available nest area. Otherwise, we used a method
193 similar to worker mean distance to nest sections (e.g. a colony member in nest section 3 and the brood center
194 in nest section 5, Appendix, Fig. A5b): $\text{sqrt}((\text{colony member x} - \text{xref4})^2 + (\text{colony member y} - \text{yref4})^2)$
195 $+ (\text{sqrt}((\text{brood center x} - \text{xref4})^2 + (\text{brood center y} - \text{yref4})^2))$.

196

197 *Distance scaling:* Actual possible distances varied across nests since the nest dimensions were scaled to
198 colony size to keep a consistent worker density across colonies. To be able to compare overall distribution
199 of colony elements in nests, we scaled all calculated distances by setting the shortest distance from the back
200 of the tube nest shape to the entrance as 1, resulting in 0.34 as the maximum distance possible in the circle
201 nest shape.

202

203 ***Site fidelity***

204 We calculated site fidelity for each paint-marked worker that had at least seven observations: 136 out of
205 941 possible workers in the high-density treatment and 247 out of 838 possible workers from the low-density
206 treatment. This loss occurred in part because although all workers were initially individually painted, many
207 lost parts of their marks.

208

209 *Fidelity and occurrence zone assignment:* We divided each of the eight nest sections described above by
210 three into twenty-four total zones (Appendix, Fig. A6). We assigned all workers with identifiable unique
211 color markings to these zones, but only zones with 15% of the total observations were included in each
212 worker's spatial fidelity zone size (possible zone occupancy therefore was between 15% and 100% of total
213 observations for that worker), whereas all zones were included to calculate a worker's overall space usage
214 (henceforth termed 'occurrence zone'). We only included workers with at least seven observations because
215 if fidelity zones of workers with six or fewer observations are included, this will create a strong positive
216 association between fidelity zone size and sample size based on our calculation method (Appendix, Fig.
217 A7).

218

219 *Fidelity and occurrence zone size:* We determined fidelity and occurrence zone sizes adding the occupied
220 zones for a total possible twenty-four zones. We used this method to calculate zone sizes (instead of, for

example, a minimum convex polygon: Jandt and Dornhaus 2009; Charbonneau et al., 2017) as an easy method for calculating the area occupied by each worker across our nest spaces, and to ensure that we did not overestimate tube nest zone sizes. Note that this relative-size measure will scale with colony size as total nest area is adjusted to colony size. We additionally considered the true area (cm^2) of worker site fidelity in each nest shape (Fig. 6), and how these scales with colony size (Fig. 7).

We used the agent-based simulation software Netlogo (v6.2.2; Wilenski 1999) to simulate a random walk in our tube and circle nests to test whether our observed worker distributions could be explained by random movement.

Model parameters: We simulated 100 workers in each of our simulations. We defined nest sizes through the same method as our experimental setup, representing our high- and low-density experimental treatments. We started our simulations with all agents at position (37.5, 2), which represents two pixels above the nest entrance (37.5, 0). Our agents picked a heading at random (out of 360°) at each time step. If there was either an agent moving towards the same pixel space in the nest, or a black pixel ahead (which defines space outside of the nest) the agent selects a new random step direction towards an unoccupied nest space.

Simulations: We produced 1000 simulations for the four nest types (small circle, small tube, large circle, large tube), resulting in 4000 total simulations. Our simulations ran for 50,000-time steps, which we determined, by visualizing results (e.g. Figs. 3,4), was sufficient time for agents to evenly distribute throughout the nest in each simulation type

We conducted all data processing using the statistical software R (v4.1.1; R Core Team 2017) and RStudio (v1.2.5042; Allaire 2012), specifically the Tidyverse language ('tidyverse' v1.3.1: Wickham et al., 2019). All original data and analysis scripts are publicly available on Github:

https://github.com/Gchism94/Nest_Arch_ColonyOrganization.

Colony distributions: We used linear mixed effects models to examine colony member distributions (proportion in each nest section) in our experimental nests using the R package 'lme4' (v1.1-27.1; Bates et al., 2014), where P values were calculated through the R package 'lmerTest' (v3.1-3; Kuznetsova et al., 2017). We report the relationships between colony member distributions across nest sections (quadratic term) and nest shape though the interactions term (nest * nest section), and report how nest density affects this relationship through a three-way interaction term (Nest * Nest section * Nest density). Our linear mixed effects models here and below (unless noted otherwise) had colony identification (hereby termed 'ColonyID') as a random effect, and by comparing the variation explained by the fixed effects alone (marginal R^2) and with the random effect included (conditional R^2), we determined the amount of variation that ColonyID explained (marginal and conditional R^2 values calculated through the R package 'MuMIn' v1.43.17; Bartoń 2020).

Scaled distances: We used linear mixed effects models to examine the relationships between nest shape and colony member scaled distances from the nest entrance and mobile colony member distances to the brood center. We examined how nest density affects the relationships between colony member distances and nest shape through an interaction term (Nest * Nest density).

265

266 Worker site fidelity: We used linear mixed effects models to test the effect of nest shape, nest density, and
267 colony identification on worker spatial fidelity and occurrence zone sizes. Our linear mixed effects models
268 predicting fidelity and occurrence zone sizes had worker color identification (hereby termed ‘AntID’) as a
269 random effect. We also consider the true zone sizes (cm^{2+}) and how they relate to colony size through linear
270 mixed effects models.

271

272 Worker site fidelity and distances in the nest: We used linear mixed effects models to test the effect of
273 either worker mean scaled distance to the nest entrance and nest shape, or the effect of worker mean scaled
274 distance to the brood center and nest shape on site fidelity. We assessed how nest density influenced these
275 relationships through the interaction terms between nest distances and nest density, and the three-way
276 interaction between nest distances, nest shape, and nest density. We lastly used an ANOVA to test whether
277 workers had spatial fidelity in relation to the brood center across nest shapes.

278

279 Random walk simulations: We used a quadratic regression (nest section is a quadratic term) to compare
280 worker and simulated distributions across nest sections in both nest shapes. We then used a generalized
281 linear regression with a binomial fit to compare the peak distributions of workers and simulated distributions
282 in nest sections: we assigned a 1 to the nest section with the peak proportion of workers and agents and a
283 0 to all other nest sections. We further compared observed and simulated distributions using two-sample
284 Cramér-von Mises’s tests (calculated through the R package ‘twosamples’ v1.1.1, Dowd 2020), with False
285 Discovery Rate corrected p-values (Benjamini and Hochberg 1995). We chose this distribution test because
286 it compares sums of square differences across entire distribution pairs, whereas the more commonly used
287 Kolmogorov–Smirnov test largely compares the center of the distribution and is therefore less sensitive to
288 differences in the tails (Anderson 1962). Finally, we used a linear regression to compare observed worker
289 and simulated agent scaled distances from the nest entrance.

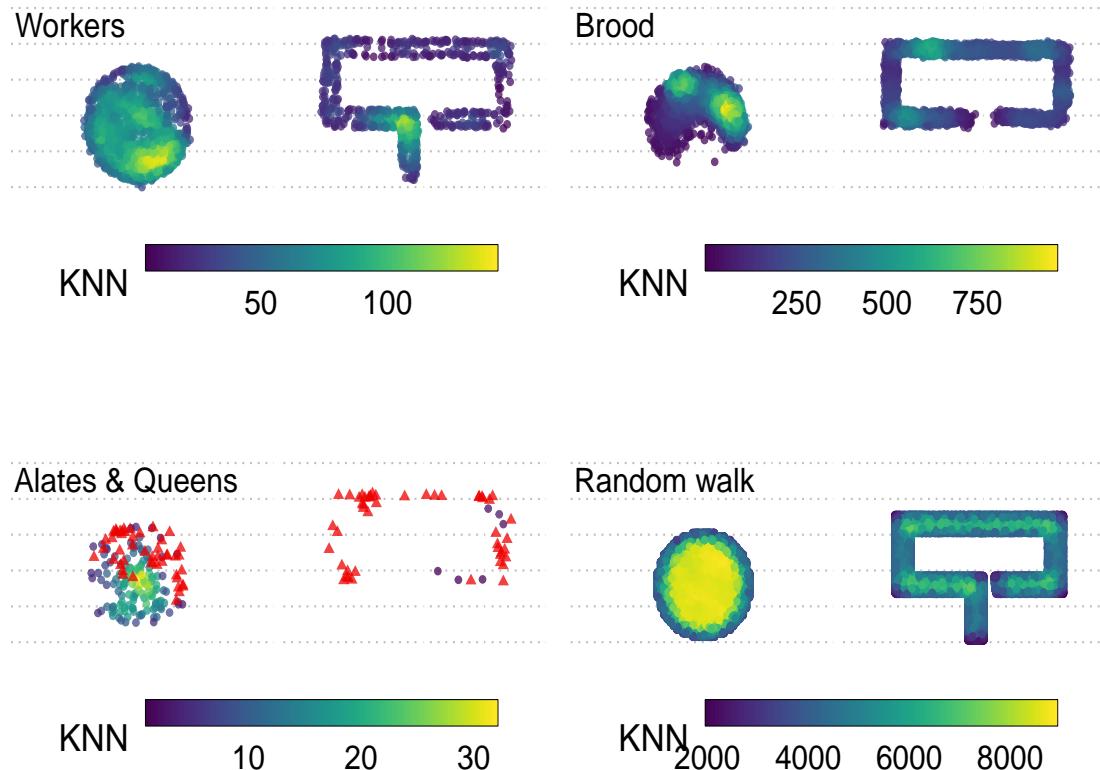
290

291 Results

292 **Colony distributions**

293 *Nest shape influenced how colony members were distributed across the nest*

294 Broadly, all types of colony members (i.e. workers, brood, queens, and alates) were distributed differently
295 in the two nest shapes (Fig. 2).

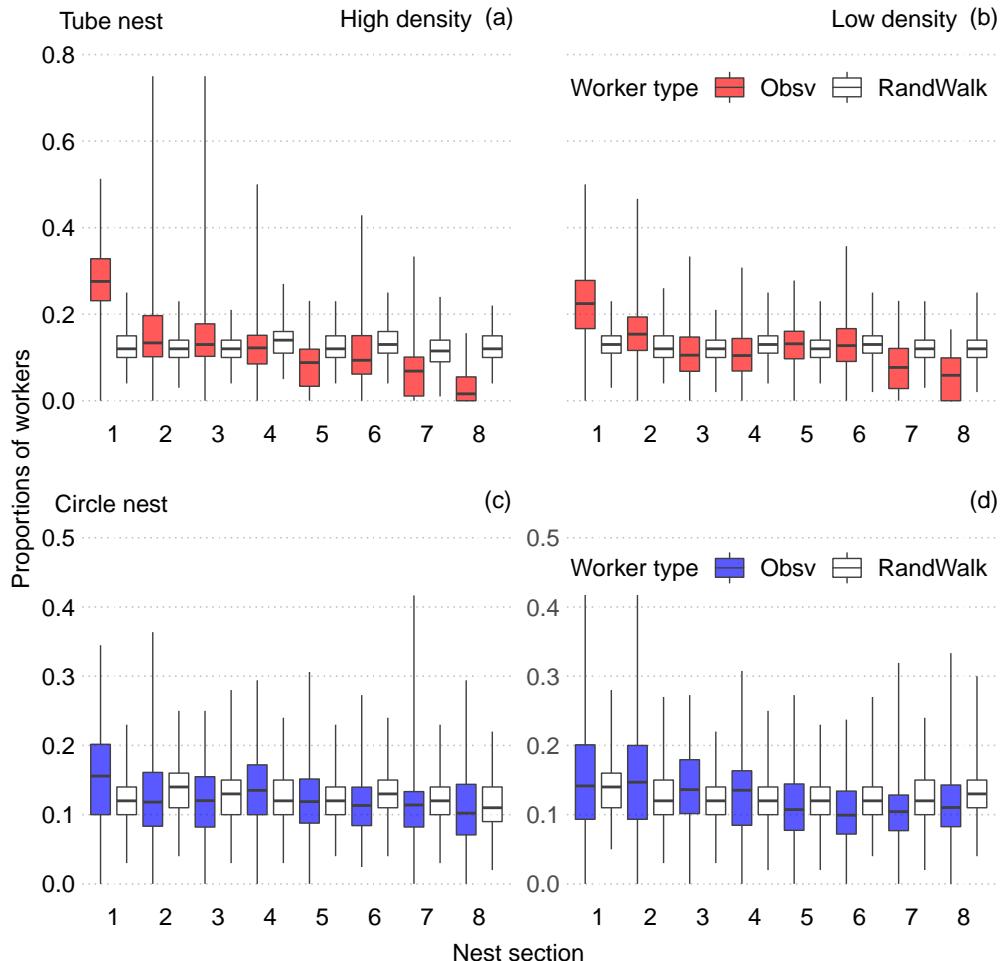


296

297 **Figure 2.** Illustration of colony distribution in nests for one example colony (no. 11). Shown are the
 298 distributions of workers (top-left), brood and queens (top-right, queens are red triangles), alates (bottom-
 299 left), and the low density Netlogo random walk simulated workers (bottom-right). Distributions in circle
 300 nests are on the left and the tube nest on the right. Each point is colored by the number of k-nearest
 301 neighbors (KNN) within a radius that's standardized by the data range (indicated by respective legends
 302 below each sub-figure). Colony 11 was selected to illustrate all colony members since not all colonies possess
 303 alates. The scatter plot grid for every colony, and respective Netlogo simulation, can be found as Appendix,
 304 Figs. 12-31.

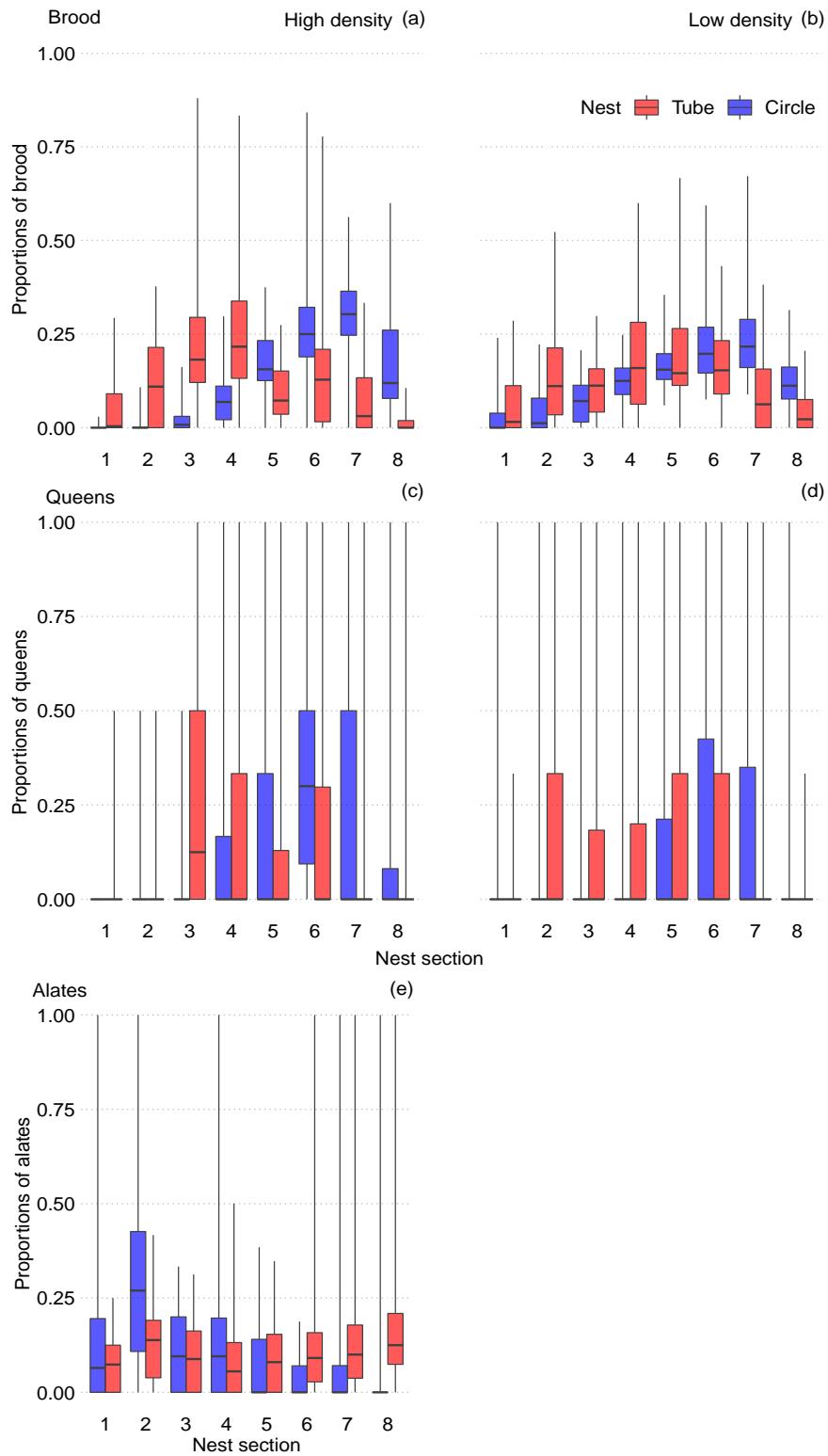
305

306 Workers and brood were found in nest sections closer to the entrance, in relative terms, in the tube nest
 307 than in the circle nest (Workers: $\beta + SE = 0.003 + 0.001$, $t_{3178} = 4.334$, $P < 0.001$; Fig. 3, Appendix,
 308 Table A1), (Brood: $\beta + SE = -0.008 + 0.001$, $t_{3170} = -6.122$, $P < 0.001$; Figs. 4a-b, Appendix, Table A2),
 309 but this was not seen in queens ($\beta + SE = -0.004 + 0.003$, $t_{3178} = -1.403$, $P = 0.161$, Figs. 4c-d, Appendix,
 310 Table A3) or alates ($\beta + SE = -0.001 + 0.004$, $t_{448} = -0.150$, $P = 0.881$; Fig. 4e, Appendix, Table A4).
 311 However, both queens (Appendix, Table A3) and alates (Appendix, Table A4) were not evenly distributed
 312 in either nest shape. All colony member distributions across nest sections were therefore different between
 313 the two nest shapes, suggesting that colonies have an occupation strategy different than evenly distributing
 314 across nest space.



315

316 **Figure 3.** Workers tend to be clustered near the entrance in both nest types, but this is more pronounced
 317 in the tube nest: shown here are the worker ('Osv', solid colors) and Netlogo simulated random walk result
 318 ('RandWalk', white) proportions of workers in each nest section of the tube (a, b; red) and circle (c, d; blue)
 319 nests (each section has the same area across both nest types, but is scaled with colony size). These figures
 320 also show the high (a, c) and low (b, d) nest density treatment. Here, and in all following boxplots, boxes
 321 represent first and third quartiles (75% of the data), the bar within boxes is the median, and whiskers are
 322 the data range. Sample size was 3192 worker proportions in nest sections across 20 colonies.



323

324 **Figure 4.** Brood and queens, but not alates, are generally found further from the entrance than workers in
325 both nest types shown here are the difference in brood (a, b), queen (c, d) and alate (e) proportions across

326 tube (red) and circle (blue) nest sections. The brood and queen densities are represented in both the high
327 (a, c) and low (b, d) nest density treatments. Note that here, and below, alates were only present in the
328 low nest density treatment. Sample sizes for colony members in nest sections: brood = 3184, queen = 3192,
329 alates = 456.

330

331 *Brood in low nest density were more evenly distributed in each nest shape*

332 Represented by a three-way interaction between nest shape, nest density, and nest section, brood were
333 distributed more evenly at the low density treatment in both nest shapes, and they were found closer to the
334 back of the circle nest at high nest density when compared to the tube nest shape (Brood: $\beta + SE = 0.004$
335 $+ 0.002$, $t_{3170} = 2.513$, $P = 0.012$; Figs. 4a-b, Appendix, Table A2). In contrast, there was no significant
336 three-way interaction between nest shape, density, and section in workers ($\beta + SE = -0.002 + 0.001$, $t_{3178} =$
337 -1.695 , $P = 0.090$; Fig. 3, Appendix, Table A1), or queens ($\beta + SE = 0.001 + 0.004$, $t_{3178} = 0.308$, $P =$
338 0.758 , Figs. 4c-d, Appendix, Table A3). Therefore, brood care may be distributed across more nest space
339 in lower densities, but mobile colony members occupy nest space similarly across densities.

340

341 *No differences between colonies in overall colony member distributions in nest sections*

342 In our models, 0.00% of variation in the proportions of workers, brood, queens, and alates in nest sections
343 is explained by ColonyID (Appendix, Tables A1-4).

344

345 ***Distances from specific points in the nest***

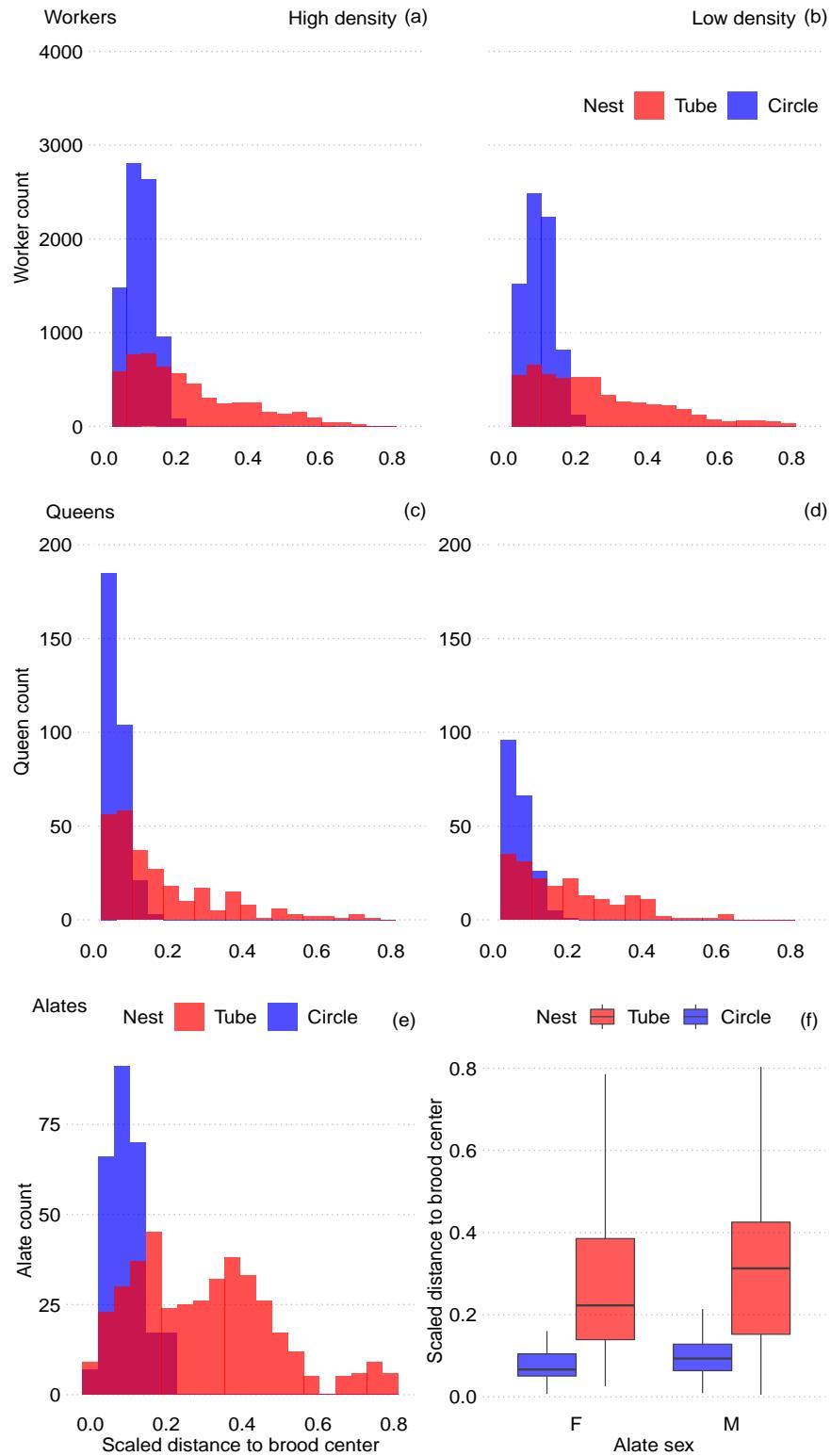
346 *Nest shape affected colony member distance to the nest entrance*

347 Our tube nest design was long and narrow, thus allowing colony members to be located at much further
348 farther actual distances from the entrance in the tube nest than was possible in the circle nest: (Workers:
349 Appendix, Figs. A8a-b, Table A5), (Brood: Appendix, Figs. A9a-b, Table A6), (Queens: Appendix, Figs.
350 A9c-d, Table A7), (Alates: Appendix, Figs. A9e-f, Table A8). We further found that male alates were
351 closer than queen alates to the nest entrance in both nest shapes (Appendix, Fig. A9f, Table A8). As they
352 settled in the nest, workers (Appendix, Table A5), brood (Appendix, Table A6), and queens (Appendix,
353 Table A6) moved closer to the nest entrance (Appendix, Tables A5-8), but alates moved farther over time
354 (Appendix, Table A8). These results suggest that shorter distances to the entrance are an important factor
355 in *Temnothorax rugatulus* nest occupation.

356

357 *Nest shape affected mobile colony member distance to the brood center*

358 All mobile colony members, i.e. workers, queens, and alates, were also farther from the brood center in the
359 tube nest shape: (Workers: $\beta + SE = 0.103 + 0.002$, $t_{26669.530} = 45.530$, $P < 0.001$; Figs. 7a-b, Appendix,
360 Table A9), (Queens: $\beta + SE = 0.090 + 0.011$, $t_{1067.062} = 8.143$, $P < 0.001$; Figs. 7c-d; Appendix, Table
361 A10), (Alates: $\beta + SE = 0.211 + 0.018$, $t_{2.895} = 11.672$, $P = 0.002$; Fig. 7e, Appendix, Table A11). We also
362 found that male alates were found farther from the brood center than queen alates (Appendix, Table A11).
363 We lastly found that mobile colony member distances to the brood center did not change over the course
364 of the experiment (Appendix, Tables A9-11). Therefore, nest shapes that elongate nest space (e.g. like our
365 tube nest) likely force mobile colony members to distances farther from the brood center.



366
367 **Figure 5.** Worker and queen distributions center near the center of the brood distribution; but in the tube
368 nest, the distances of both from the brood center are much larger. The peak of alate distributions is away

369 from the brood center. Shown on the x-axis are the actual distances from the brood center (see Methods),
370 scaled only by setting the farthest point of the tube nest as a distance of 1 (instead of dividing the nest
371 into sections as in Figs. 3-4). This implies that distances given here are also scaled with colony size. Panels
372 show workers (a, b), queens (c, d), and alates (e, f). The difference between male and female alates is shown
373 in (f), denoted by letters A for females and B for males. Differences in distributions are additionally shown
374 across high (a, c) and low (b, d) nest densities. Sample sizes for colony member distances from the brood
375 center: workers = 26733, queens = 1080, alates = 676.

376

377 *Nest density increased worker and brood distance to the entrance in each nest shape*

378 Workers and brood were found farther from the nest entrance in both nest shapes at low nest density, but
379 this was more pronounced in the tube nest shape (Workers: Figs. 3a-b, Appendix, Figs. A8a-b, Table A5),
380 (Brood: Figs. 4a-b, Appendix, Figs. A9a-b, Table A6). In contrast, there was no effect of nest density on
381 queen distances from the entrance in each nest (Queens: Figs. 4c-d, Appendix, Figs. A9c-d, Table A7).
382 These results may imply that at low density, colonies do not prioritize being near the entrance, perhaps
383 because of short travel times.

384

385 *Nest density decreased worker distance to the brood center both nest shapes*

386 We saw that workers were farther from the brood center in low nest density, which was more pronounced
387 in the tube nest (Workers: $\beta + SE = 0.034 + 0.003$, $t_{26709.876} = 11.769$, $P < 0.001$; Figs. 5a-b, Appendix,
388 Table A9), but queen distances to the brood center were not different across density treatments (Queens: β
389 $+ SE = 0.001 + 0.013$, $t_{1060.719} = 0.105$, $P = 0.916$; Figs. 5c-d; Appendix, Table A10). Queen placement
390 in nest shapes is likely more influenced by distance to the brood center than the entrance, while workers are
391 possibly more influenced by distance to the nest entrance.

392

393 *Small differences between colonies in distance relationships, particularly in alates*

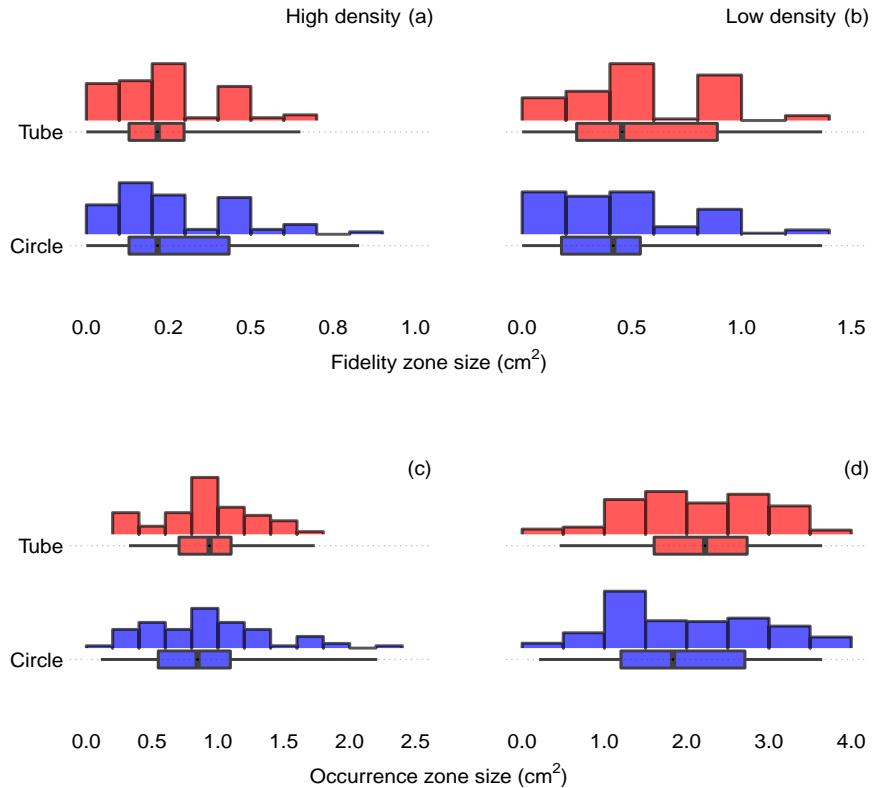
394 For proximity to the nest entrance, the model variation explained by colony ID was 1.5% for workers
395 (Marginal R^2+ = 0.337, Conditional R^2+ = 0.352, Appendix, Table A5), 4.8% for brood (Marginal R^2+ =
396 0.439, Conditional R^2+ = 0.487, Appendix, Table A6), 7.2% for queens (Marginal R^2+ = 0.492, Conditional
397 R^2+ = 0.564, Appendix, Table A7), and 17.2% for alates (Marginal R^2+ = 0.451, Conditional R^2+ =
398 0.623, Appendix, Table A8). Concerning proximity to the brood center, the model variation explained by
399 ColonyID was 2.2% for workers (Marginal R^2+ = 0.279, Conditional R^2+ = 0.301, Appendix, Table A9),
400 8.6% for queens (Marginal R^2+ = 0.249, Conditional R^2+ = 0.335, Appendix, Table A10), and 0.6% for
401 alates (Marginal R^2+ = 0.340, Conditional R^2+ = 0.346, Appendix, Table A11).

402

403 *Worker site fidelity*

404 *Scaled worker site fidelity was influenced by neither nest shape nor nest density*

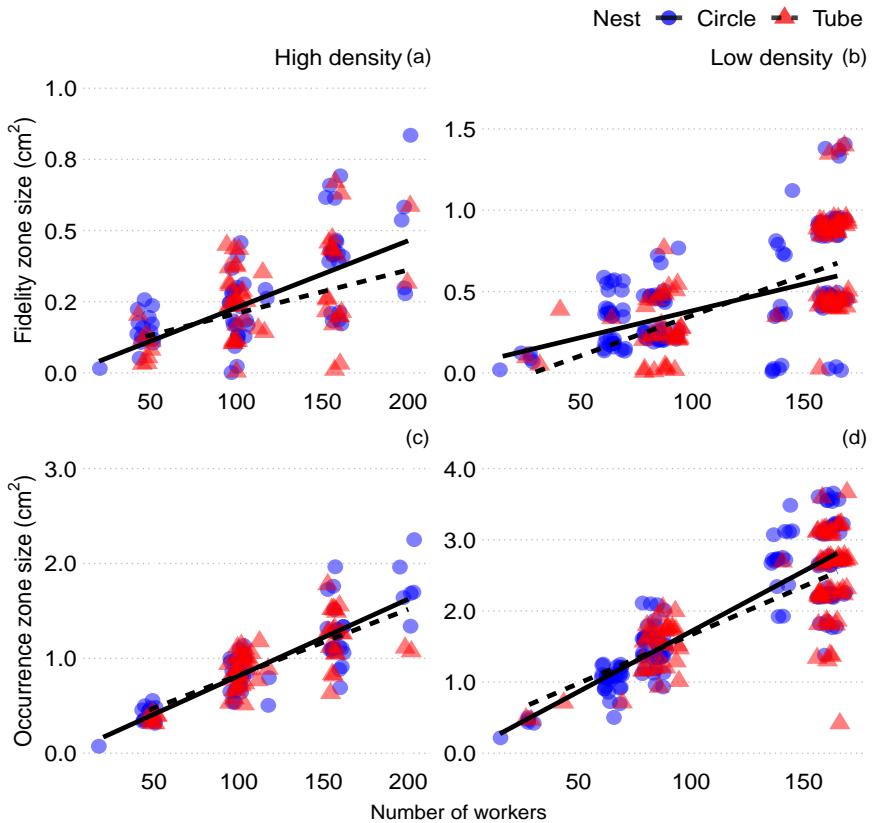
405 Nest shape did not influence worker spatial fidelity zone size as measured in nest sections ($\beta + SE = -0.145$
406 $+ 0.146$, $t_{378.727} = -0.994$, $P = 0.321$; Figs. 8a-b, Appendix, Table A12) or occurrence zone size in this
407 measurement ($\beta + SE = -0.080 + 0.214$, $t_{378.858} = -0.372$, $P = 0.710$; Figs. 8b-c, Appendix, Table A13),
408 nor did nest density (Fidelity zones: $\beta + SE = -0.320 + 0.159$, $t_{14.107} = -2.010$, $P = 0.064$, Appendix,
409 Tables A12) (Occurrence zones: $\beta + SE = 0.282 + 0.248$, $t_{19.358} = 1.137$, $P = 0.270$, Appendix, Tables
410 A13). Therefore, ant workers appear to be able to maintain the same site fidelity regardless of nest shape.



411
 412 **Figure 6.** Comparison of worker fidelity and occurrence zones sizes (cm^2) in the circle (blue) and tube
 413 (red) nests for both the high (a, c) and low (b, d) nest density treatments. Fidelity zone sizes are the same
 414 in each nest shape ($\beta + \text{SE} = -0.022 + 0.044$, $t_{377.968} = -0.494$, $P = 0.622$, Appendix, Table A14) and
 415 across density treatments ($\beta + \text{SE} = 0.148 + 0.082$, $t_{19.161} = 1.799$, $P = 0.088$, Appendix, Table A14).
 416 Occurrence zone sizes are the same in each nest shape ($\beta + \text{SE} = -0.023 + 0.071$, $t_{366.580} = -0.324$, $P =$
 417 0.746, Appendix, Table A15) but are larger in lower nest density ($\beta + \text{SE} = 0.808 + 0.337$, $t_{16.682} = 2.398$,
 418 $P = 0.029$, Appendix, Table A15). Histograms represent the data distribution; boxplots are the first and
 419 third quartiles where the black bar is the median and the whiskers extend to the data range. Please see
 420 the methods for details on how scaled worker fidelity and occurrence zone size calculations, which here were
 421 multiplied by the total area of the nest to achieve unscaled zone sizes. The sample size was 383 marked ants
 422 across 19 colonies.
 423

424 *Worker identity explained some worker site fidelity variation*

425 The model variation explained (notably small) by the random effect ColonyID was 4.6% in fidelity zone
 426 sizes (marginal $R^{2+} = 0.022$, conditional $R^{2+} = 0.068$, Appendix, Table A12) and 6.3% in scaled occurrence
 427 zone sizes (marginal $R^{2+} = 0.015$, conditional $R^{2+} = 0.078$, Appendix, Table A13).

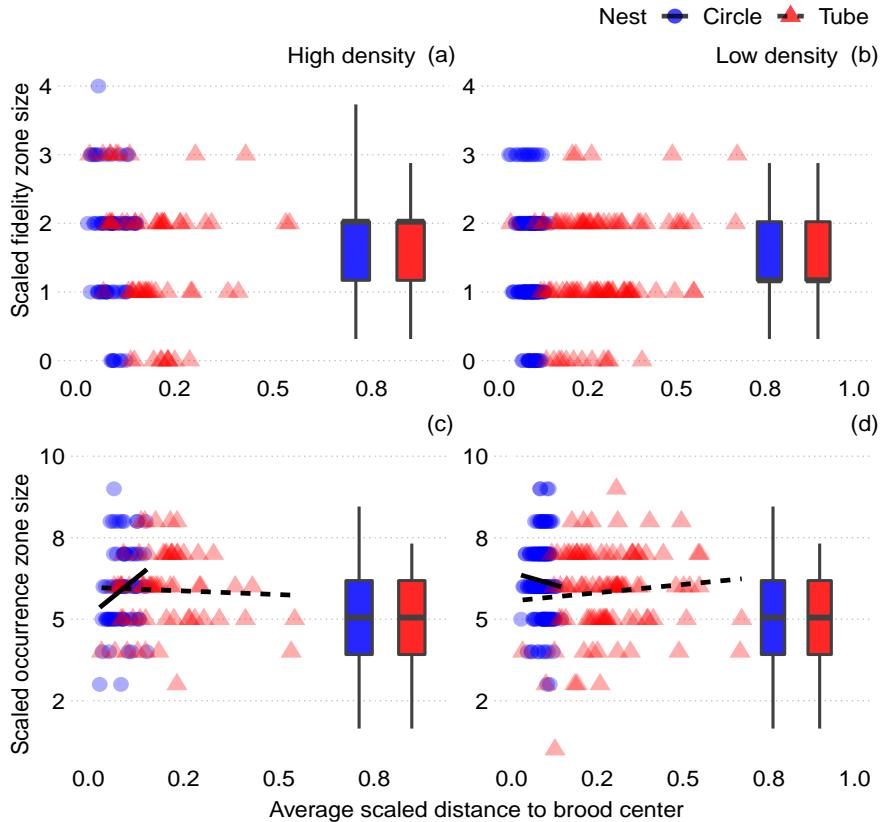


429
 430 **Figure 7.** Relationship between true worker fidelity and occurrence zones sizes (cm²) and colony size in the
 431 circle (blue) and tube (red) nests for both the high (a, c) and low (b, d) nest density treatments. Points are
 432 jittered on the x and y axis. Lines show significant linear relationships, but note that the relationships in
 433 (a, c) and (b, d) are not significantly different (Appendix, Tables A16-17). The sample size was 383 marked
 434 ants across 19 colonies.

435
 436 **Worker site fidelity and distances in the nest**
 437 *No consistent relationship between worker site fidelity and distance to the nest entrance*
 438 We saw that both worker spatial fidelity and occurrence zones had inconsistent relationships with that
 439 worker's distance to the nest entrance both across nest shapes and nest densities ($\beta + SE = 6.710 + 3.339$,
 440 $t_{375} = 2.010$, $P = 0.045$; Appendix, Figs. A10a-b, Table A18), ($\beta + SE = -14.610 + 4.870$, $t_{374.994} =$
 441 -3.000 , $P = 0.003$; Appendix, Figs. A10c-d, Table A19) - e.g. fidelity zones: the relationship in the circle
 442 nest was positive in high density but negative in low density, both of which were the opposite of the tube
 443 nest relationships in their respective nest densities (Figs. Appendix, Figs. A10a-b, Table A18). Therefore,
 444 we did not find a consistent relationship between worker site fidelity and distance to the nest entrance.

445
 446 *Distance to the brood center did not influence spatial fidelity zones*
 447 There was no relationship between worker spatial fidelity zones and distance from the brood center ($\beta + SE$
 448 $= 1.517 + 4.715$, $t_{360.029} = 0.322$, $P = 0.748$; Figs. 8a-b, Appendix, Table A20), but occurrence zones had
 449 an inconsistent relationship both across nest shapes and nest densities ($\beta + SE = 18.951 + 6.889$, $t_{374.920} =$
 450 2.751 , $P = 0.006$; Figs. 8c-d, Appendix, Table A21). We therefore do not find that workers in different

451 areas of the nest have differently sized spatial fidelity zones.



452
453 **Figure 8.** The relationship between scaled individual worker fidelity (a, b) and occurrence (c, d) zone sizes
454 (scaled relative to total nest size, which was related to colony size) and average scaled distance to the brood
455 pile in the circle (blue circles, solid lines) and tube (red triangles, dashed lines) nest shapes. Lines represent
456 significant linear relationships. These differences are additionally compared across the high (a, c)
457 and low (b, d) nest density treatments. There were no significant differences in any scaled zone sizes across nest
458 shapes or density treatments (Appendix, Tables A20-21). The sample size was 383 marked ants across 19
459 colonies.

460

461 *Workers have spatial fidelity in relation to the brood center across nest shapes*

462 Individual workers were found at different distances from the brood center across all observations that they
463 were identifiable (ANOVA: $F_{\sim 380, 2328} = 4.180$, $P < 0.001$, Appendix, Table A22). We therefore can
464 conclude that though workers did not have consistent spatial fidelity zone sizes (Appendix, Table A12),
465 workers held repeatable spatial fidelity in their distances from the brood center.

466

467 *Small differences between colonies in the relationship between worker site fidelity and nest distances*

468 In models of the relationship between worker site fidelity and distance to the entrance, we saw that the
469 variation explained by our random effect (ColonyID) was 3.7% for fidelity zone size (marginal R² = 0.045,
470 conditional R² = 0.082, Appendix, Table A18) and 6.2% for occurrence zone size (marginal R² = 0.041,
471 conditional R² = 0.103, Appendix, Table A19). In models of the relationship between worker site fidelity
472 and distance to the brood center, we saw that the variation explained by our random effects was 3.1%

473 for fidelity zone size (marginal R² = 0.036, conditional R² = 0.067, Appendix, Table A20) and 8.0% for
474 occurrence zone size (marginal R² = 0.038, conditional R² = 0.118, Appendix, Table A21).

475 **Random walk simulations**

476 *Empirical worker distributions differed from those predicted by a random walk*

477 We saw that empirical and random walk simulated distributions were different (Appendix, Tables A23-26).
478 Specifically, simulated agents were more evenly distributed, while real (observed) workers were found closer
479 to the nest entrance (NestSection2 * Nest * WorkerType: $\beta + SE = -0.002 + 0.000$, $t_{35167} = -5.385$, $P < 0.001$; Fig. 3, Appendix, Table A24). The spatial distribution of ant colonies in their nests is thus
481 significantly different from the outcome that would result from a random walk.

482 *Empirical worker peak distributions differed from those predicted by a random walk*

483 We saw that the nest section with the highest proportion of workers (peak worker distribution) was closer
484 to the entrance in the tube nest than the circle nest, whereas simulated peaks were equally likely to be in
485 any nest section (NestSection2 * WorkerType * Nest: $\beta + SE = -0.133 + 0.041$, $z_{35168} = -3.262$, $P = 0.001$;
486 Appendix, Fig. A11, Appendix, Table A25).

487 **Discussion**

488 Available nest shape influences the spatial organization of colonies in the rock-dwelling ant *Temnothorax*
489 *rugatulus*. Overall, ants preferred the areas in their nest that were closer to the entrance and moved closer
490 to the entrance over the 10 days of our measurements. But in the tube-shaped nest, we found that workers,
491 brood, queens, and alates were on average located further from the nest entrance (Figs. 3-4, Appendix,
492 Figs. A8-9) and further from the brood pile compared to the circle nest (Fig. 5), presumably because the
493 circle nest did not allow such longer distances, but maybe also because of a lack of available space closer to
494 these points in the tube nest. Supporting this second interpretation, workers, brood, and queens were found
495 closer to the nest entrance relative to the maximum available distance in that nest type in the tube-shaped
496 nest. None of these patterns are explained by a null model of ants moving around randomly in the same
497 nest shapes (Fig. 3, Appendix, Figs. A8, A11). Nest shape alone could therefore significantly impact the
498 distances between individuals and biologically relevant points in the nest, and thus how ants can interact
499 with each other and their nest space.

500 We found, however, that both worker spatial fidelity zone sizes and overall area used by each worker ('occurrence
501 zone' size) were not affected by nest shape, either when measured in absolute area or relative to total
502 nest size (Figs. 6, 8). We also did not find a consistent relationship of these zone sizes with location in the
503 nest (e.g. distance to the brood pile, Fig. 8). This is different from what has been demonstrated previously
504 in both *Temnothorax unifasciatus* and the bumble bee *Bombus impatiens*; in both species, workers further
505 from the nest center (brood pile) had larger spatial fidelity zones (Sendova-Franks and Franks 1995; Jandt
506 and Dornhaus 2009). However, our result is consistent with earlier studies on our species *T. rugatulus* which
507 showed that while inactive workers tended to both have small spatial fidelity zones and remain near the nest
508 center while inactive, this was not the case when including these workers' active phases (Charbonneau et
509 al., 2017). We however found that individual workers held spatial fidelity over time in relation to distance
510 from the brood center, despite no consistent trends. The site fidelity of social insect workers in the nest
511 may relate to task specialization, as certain tasks are more likely to be performed in specific nest sections
512 (Sendova-Franks and Franks 1995); smaller fidelity zones thus may be connected to and allow for more
513 specialized workers. Given that workers are more crowded in the tube-shaped nest in our experiment, the
514 lack of a consistent effect on worker spatial fidelity zones may suggest that workers actively regulate their
515 zone size even against constraints imposed by nest shape, to maintain a desired association between workers
516 and their tasks. This bears further investigation.

517 Our study confirms that the spatial distribution of colony members in ant nests is not identical to one that
518 would be produced by 'random movement'. We do not propose that it is realistic that ants move randomly;
519 rather, we believe this is an appropriate null hypothesis to clarify whether the outcome of movement is in

fact different from one in which individual actions do not add up to a non-random trend to accumulate in particular areas. In other studies, the outcome of ant movement has sometimes been indistinguishable from similar null hypotheses of randomness (e.g. worker sorting: Sendova-Franks and Van Lent 2002; interactions between potential and returning foragers: Davidson and Gordon 2017). Additionally, movement in the nest from one ant *T. albipennis* can be predicted by movement from another, and faster movement occurs when more ants are in motion (Gallotti and Chialvo 2018). Outside of the nest, random movement that is reinforced by trail pheromones explains ant coordination along specific trails to and from food sources and the nest (Ma et al., 2013; Chang et al., 2021). The non-random nest occupation of our *T. rugatulus* colonies therefore demonstrates not only that individual ants' movement is not random, but that in aggregate, individual movement rules are such that they bias worker movement to produce clustering and heterogeneity in distribution at the colony level, and that the effects of nest shape we find here cannot be produced by passive effects of geometry on random movement.

The total area available in an enclosed ant nest will determine worker density. Both nest area and worker density in the nest have previously been demonstrated to be important in ants, and our results support this. Nest area is an important consideration for nest site selection in *Temnothorax* ants (Pratt and Pierce 2001; Mitrus 2015), where workers measure the size of prospective nests (Pratt 2005). Crowded nests can significantly increase worker energy expenditure in *T. rugatulus* colonies (Cao and Dornhaus 2008), while also both increasing foraging and scouting rates and inducing polydomy (Cao 2013). The consequences of nest density to ant colonies, such as traffic jammed panicked nest evacuation, can be mediated by small structural features at the nest entrance (Burd et al., 2010; Shiawakoti et al., 2014; Wang and Song 2016). Ants may even adaptively regulate contact rate with each other, as a part of strategies for task allocation and information exchange (Pacala et al., 1996; Pinter-Wollman et al., 2012; Pinter-Wollman et al., 2013; Lehue et al., 2020b). Contact rate may also be used to estimate colony or group size (Gordon et al., 1992; Pratt 2005; Dornhaus and Franks 2006). In our study here, we compared colonies at two different worker densities, but also found that local density in nests is heterogeneous and may be driven by overall nest shape and thus access to all parts of the nest. We saw that colony members spread out more in both nest shapes at low worker density, but this effect was more pronounced in the tube nest shape; in addition, we saw that constraints of space led to higher local density near the entrance in the tube nest (e.g. see Figs. 3-4). Our study did not address whether the spatial distribution of colony members in their nests is a passive effect of particular cavity shapes and densities, or a result of adaptive, flexible individual strategies used by *Temnothorax* ants in response to the particular nest spaces encountered. *Temnothorax* ants, in nature, can also modify their nest spaces by adding internal stone walls to the cavity their nest inhabits (Franks et al., 1992; Franks and Deneubourg 1997; Aleksiev et al., 2007a; Aleksiev et al., 2007b; Aleksiev et al., 2007c; DiRienzo and Dornhaus 2017). The purpose of such nest modifications is not well studied, but it is a possibility that these ants actively modify either the available area or the shape of their nest space. Future work may reveal more about how these spatial distributions affect colony performance, and thus spatial properties of both nest cavities and colony distribution matter most to colonies.

Overall, we found that nest shape affected how ants of the species *T. rugatulus* occupied their nests, and therefore traits of nest cavities available in the ants' environment may affect the behaviour of the colonies inhabiting them. Nest geometry may affect the accessibility of the nest entrance and center of the brood pile, and it may affect ant-ant interactions through heterogeneity in worker density in the nest. On the other hand, we found that at least one important characteristic of the distribution of ant workers in their nests, namely the 'spatial fidelity zone size' for ant workers, appears resilient to such changes in nest shape. It is therefore possible that such resilience is the result of evolved strategies by ant workers to deal with such variability in nest geometry. We argue that nest shape and worker density need to be considered when studying topics such as worker site fidelity, task specialization, overall nest space usage, and nest site selection in ant colonies. Both the approach and results of our study could possibly be extended to other social animal architects, such naked mole rats, social crabs, and social weaver birds, providing insights into how the structural components of their built architectures (i.e. burrows, nests) can influence occupant behaviours such as kinship and division of labor.

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- 752 Appendix
- 753 **Table A1.** Proportions of workers in each nest section related to nest shape and physical properties. Here
 754 and below: Sections (NestSect) begin at the nest entrance (1) and complete at the back of the nest (8) and
 755 is a quadratic term. Density is the amount of area allocated for each worker (high = 0.033 cm², low = 0.066
 756 cm² x 2), Day is the observation day along the experimental timeline. Corner is the presence of corners in
 757 the nest section. The random effect ColonyID is colony identification. Asterisks denote interactions, and
 758 bold P values indicate significance. Linear mixed effects model: PropWorker ~ poly(NestSect, degree = 2,
 759 raw = TRUE) * Nest * Density + Day + Corner + (1 | ColonyID)
- 760 **Table A2.** Proportions of brood in each nest section related to nest shape and physical properties. Linear
 761 mixed effects: PropBrood ~ poly(NestSect, degree = 2, raw = TRUE) * Nest * Density + Day + Corner +
 762 (1 | ColonyID)
- 763 **Table A3.** Proportions of queens in each nest section related to nest shape and physical properties. Linear
 764 mixed effects: PropQueens ~ poly(NestSect, degree = 2, raw = TRUE) * Nest * Density + Day + Corner
 765 + (1 | ColonyID)
- 766 **Table A4.** Proportions of alates in each nest section related to nest shape and physical properties. Linear
 767 mixed effects: PropAlates ~ poly(NestSect, degree = 2, raw = TRUE) * Nest + Day + Corner + (1 |
 768 ColonyID)
- 769 **Table A5.** Relationships between workers scaled distance to the nest entrance and nest shape and physical
 770 properties. Distance to the entrance is scaled such that 1 is the back of the tube nest shape. Linear mixed
 771 effects: ScaledDist ~ Nest * Density + Day + Corner + (1 | ColonyID)
- 772 **Table A6.** Relationships between brood scaled distance to the nest entrance and nest shape and physical
 773 properties. Linear mixed effects: ScaledDist ~ Nest * Density + Day + Corner + (1 | ColonyID)
- 774 **Table A7.** Relationships between queens scaled distance to the nest entrance and nest shape and physical
 775 properties. Linear mixed effects: ScaledDist ~ Nest * Density + Day + Corner + (1 | ColonyID)
- 776 **Table A8.** Relationships between workers scaled distance to the nest entrance and nest shape, nest physical
 777 properties, and alate sex. Sex is male or female alates, and ratio is the proportion of male alates over all
 778 alate types in the observation. Linear mixed effects: ScaledDist ~ Nest + Sex + Ratio + Day + Corner +
 779 (1 | ColonyID)
- 780 **Table A9.** Relationships between workers scaled distance to the brood center and nest shape and physical
 781 properties. The brood center is a colony's brood center in each observation. Distance to the brood center
 782 is the absolute value of the difference between the scaled distances of the brood center from each worker to
 783 the entrance and is scaled such that 1 is the back of the tube nest. Linear mixed effects: ToBrood ~ Nest *

784 Density + Day + Corner + (1 | ColonyID) Table A10. Relationships between queen scaled distance to the
785 brood center and nest shape and physical properties. Linear mixed effects: ToBrood ~ Nest * Density +
786 Day + Corner + (1 | ColonyID)

787 **Table A11.** Relationships between alate scaled distance to the brood center and nest shape and physical
788 properties. Linear mixed effects: ToBrood ~ Nest + Sex + Ratio + Day + Corner + (1 | ColonyID)

789 **Table A12.** The relationship between worker spatial fidelity zone size and nest shape. Fidelity zone size is
790 the summation of all zones that a worker was found in (zones must have at least 7 observations and 15% of
791 total observations), with twenty-four total possible zones. Linear mixed effects: SFZ ~ Nest * Density + (1
792 | ColonyID)

793 **Table A13.** The relationship between worker occurrence zone size and nest shape. Occurrence zone size
794 is the ratio between the summation of all zones that a worker was found in (zones must have at least 7
795 observations), and twenty-four (total possible zones). Linear mixed effects: Occur ~ Nest * Density + (1 |
796 ColonyID)

797 **Table A14.** The relationship between true worker spatial fidelity zone size (cm²) and nest shape. Here and
798 below the ratio between the total number of occupied zones with >15% observations (fidelity zone size) and
799 the twenty-four possible zones is multiplied by nest area to produce true fidelity zone size. Linear mixed
800 effects: SFZ_Area ~ Nest * Density + (1 | ColonyID)

801 **Table A15.** The relationship between true worker occurrence zone size (cm²) and nest shape. Here
802 and below the ratio between the total number of occupied zones (occurrence zone size) and the twenty-
803 four possible zones is multiplied by nest area to produce true occurrence zone size. Linear mixed effects:
804 Occur_Area ~ Nest * Density + (1 | ColonyID)

805 **Table A16.** The relationship between true worker spatial fidelity zone size (cm²) and colony size (worker.number).
806 Linear mixed effects: SFZ_Area ~ Number.workers * Nest * Density + (1 | ColonyID)

807 **Table A17.** The relationship between true worker occurrence zone size (cm²) and colony size (worker.number).
808 Linear mixed effects: Occur_Area ~ Number.workers * Nest * Density + (1 | ColonyID)

809 **Table A18.** The relationship between a worker's spatial fidelity zone size and average scaled distance to
810 the nest entrance across nest shapes. Linear mixed effects: SFZ ~ MeanScaledDist * Nest * Density + (1 |
811 ColonyID)

812 **Table A19.** The relationship between a worker's occurrence zone size and average scaled distance to the
813 nest entrance across nest shapes. Linear mixed effects: Occur ~ MeanScaledDist * Nest * Density + (1 |
814 ColonyID)

815 **Table A20.** The relationship between a worker's spatial fidelity zone size and average scaled distance to
816 the brood center across nest shapes. Linear mixed effects: SFZ ~ MeanToBrood * Nest * Density + (1 |
817 ColonyID)

818 **Table A21.** The relationship between a worker's occurrence zone size and average scaled distance to the
819 brood center across nest shapes. Linear mixed effects: Occur ~ MeanToBrood * Nest * Density + (1 |
820 ColonyID)

821 **Table A22.** Individual worker (marked) fidelity in relation to distance from the brood center across all
822 observations. AntIDColNest is each identifiable ant in the study. ANOVA: ToBrood ~ Nest * Density +
823 AntIDColNest

824 **Table A23.** Distribution comparisons between the worker and Netlogo random walk simulation results.
825 Two-sample Cramér-von Mises's test statistics were calculated through 2000 random resample bootstraps of
826 the worker and simulated distributions, from which the P value was derived. False Discovery Rates inherent
827 to multiple comparisons were corrected using the Benjamini-Hochberg method.

828 **Table A24.** The relationship between proportions of worker and Netlogo random walk simulated results
829 in each nest section and nest shape and physical properties. WorkerType is whether the observation was a
830 worker or Netlogo random walk simulated result. Linear regression: PropWorker ~ poly(NestSect, degree =
831 2, raw = TRUE) * Nest * WorkerType * Density + Corner

832 **Table A25.** The maximum worker and Netlogo random walk simulated result proportions in each nest
833 section. Nest sections with the max worker or simulated result proportion in each observation were assigned
834 a 1, where the other sections are 0. Logistic regression: MaxNestSect ~ NestSect * WorkerType * Nest *
835 Density, family = Binomial

836 **Table A26.** Relationships between worker and Netlogo random walk simulated result scaled distance to the
837 nest entrance and nest shape and physical properties. Linear regression: ScaledDist ~ Nest * WorkerType
838 + Density + Corner

839 **Figure A1.** The reference photo used to determine the nest area allocated to each worker in a colony.
840 The colony contains 248 individuals, determined with the image analysis software Fiji. The internal area
841 is approximately 4.11mm², producing 0.017 mm² for each worker, which was doubled to 0.033 mm² to
842 promote more flexible space usage. This value represents the high nest density treatment and was doubled
843 to produce the low nest density treatment: 0.066 mm² for each worker in a colony

844 **Figure A2.** The eight equal-area nest sections for the circle (a) and tube (b) nests (black lines) used to
845 determine the densities of colony members and Netlogo simulations through the nest.

846 **Figure A3.** Visual examples of the shortest distance to the entrance calculation utilized for spatial analysis
847 of colony members and Netlogo simulations in the circle (a) and tube (b) nests.

848 **Figure A4.** The criteria used to determine if a colony member required an alternative linear distance to
849 the nest entrance near the corner formed from the nest entrance tunnel opening into the nest (black solid
850 lines). The red dot represents a colony member, and the black dot represents the entrance. The dashed
851 lines represent the hypotenuses between the colony member and the corner (red) and the corner and the
852 entrance (black). The arcs represent angles, and the black square represents 90°. The solid red polygon
853 indicates nest space that would require this type of alternative calculation of distance to the nest entrance.
854 Please see the methods for a full description.

855 **Figure A5.** Visual example of the shortest distance from an example worker (red dots) to an example
856 brood center (white dots) in the circle (a) and tube (b) nests. Figure A6. The twenty-four equal area zones
857 used to calculate worker site fidelity in the circle (a) and tube (b) nests.

858 **Figure A7.** The relationship between the number of observations that contributed to the sizes of worker
859 fidelity (a, c) and occurrence (b, d) zones. Zones are scaled (a, b) and unscaled (cm²) (c, d). Points are
860 individual workers and are jittered in height and width. The sample size was 383 marked ants across 19
861 colonies.

862 **Figure A8.** When showing the actual distances of workers from the nest entrance (scaled only by colony
863 size, by setting the farthest point of the tube nest as a distance of 1) instead of sections as in Figs. 3-4, it
864 becomes clear how colonies in tube nests are spread out more from the entrance and from each other relative
865 to the circle nest. Shown are worker distributions (a, b) and Netlogo random walk simulation results (c, d)
866 for the circle (blue) and tube (red) nests. These distances are also compared in high (a, c) and low (b, d)
867 nest density treatments. Sample sizes were 30247 workers across 20 colonies and 400000 Netlogo simulated
868 agents across 4000 simulations.

869 **Figure A9.** The differences in brood (a, b), queen (c, d), and alate (e, f) scaled distances from the nest
870 entrance. Differences in these distributions are also shown across the low (a, c) and high (b, d) nest density
871 treatments. The significant difference between each alate sex's scaled distances to the nest entrance is shown
872 in (f), denoted by an A for females and B for males (Appendix, Table A8). Sample sizes for colony member
873 distances from the entrance: workers = 30247, brood = 59459, queens = 1178, alates = 1006.

874 **Figure A10.** The relationship between scaled (see methods) individual worker fidelity (a, b) and occurrence
875 (c, d) zone sizes and average scaled distance to the nest entrance in the circle (blue circles, solid lines) and
876 tube (red triangles, dashed lines) nest shapes, and across the high (a, c) and low (b, d) nest density
877 treatments. These differences are additionally compared across the high (a, c) and low (b, d) nest density
878 treatments. Lines represent significant linear relationships (Appendix, Tables A16-17). There were no
879 significant differences in any scaled zone sizes in each nest shape or across density treatments (Appendix,
880 Tables A12-13). The sample size was 383 marked ants across 19 colonies. The sample size was 383 marked
881 ants across 19 colonies.

882 **Figure A11.** The nest sections with the maximum number of workers are near the entrance in the real ants
883 (a), whereas in the random walk simulation the densest area could be in any nest section (b). Lines represent
884 significant binomial logistic regression fits for high (solid) and low (dashed) nest density treatments, in the
885 tube (red) and circle (blue) nest shapes. Sample size was 3192 worker proportions in nest sections across 20
886 colonies, and 32000 Netlogo simulated agents across 40000 simulations.

887 **Figures A12-31.** The densities of workers, brood, queens (red triangles), and, where present, alates in
888 the circle (left) and tube (right) nest shapes for all experimental colonies. Densities of Netlogo simulated
889 workers are also shown in the high (colonies 1-10) or low (colonies 11-20) nest density treatments. Each
890 point is colored by the number of k-nearest neighbors within a radius that's standardized by the data range,
891 where the sub-figure legends are increasing nearest neighbor values from left to right. Note that alates are
892 not always present in both nest shapes.

893 write.bibtex(file="references.bib")