Nest shape influences colony organization in ants: spatial distribution and connectedness of colony members differs from that predicted by random movement and is affected by nest space

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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 just 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 despite the fact that spatial fidelity zone size does vary as a function of distance from the nest entrance and the brood center; and that this relationship is affected by both nest shape and density. Finally, we show that workers are found closer to the entrance of the nest than would be expected in a random walk model in both nest shapes. These findings indicate that ants actively regulate which areas of a nest they occupy, 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.

Introduction

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

Here we are interested in how the geometric shape of such built architectures influences the organization of the colony within. Ants have been demonstrated to be distributed non-uniformly across their internal nest space (Sendova-Franks and Franks 1995; Sendova-Franks and Franks 1999; Tschinkel 1999; Tschinkel 2005). For example, colony members may be concentrated in specific sections of the nest (Tschinkel 1999; Tschinkel 2005); and workers may use chemical ‘road-signs’ to navigate nest space and differentiate chambers with different functions (Heyman et al., 2017). However, some complex spatial relationships in the nest may be predicted by a random walk model (Sendova-Franks and Van Lent 2002, Davidson and Gordon 2017), implying that the placement of colony members may not be an explicit adaptive strategy. In

addition, many ants exploit pre-existing natural structures as nests (e.g. *Temnothorax* - ‘acorn’ and ‘rock’ ants: Prebus 2017, *Cephalotes* turtle ants that occupy unmodifiable wood nests: Powell 2008, and rock-dwelling *Rhytidoponera metallica* ponerine ants: Thomas 2002), which means that the geometry of the available space may not be under the ants’ control. We therefore ask how the internal nest space may influence the spatial organization of workers, queens, and brood in the nest.

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

Different areas of social insect nests serve different functions. Most prominently in ants, the queen(s) and her brood are typically concentrated in a particular area or chamber(s), referred to as the ‘brood pile’. The location of the brood pile may be influenced by which locations are both more accessible from other nest spaces and possibly more defensible during nest invasions

(Varoudis et al., 2018). Worker task allocation, i.e. the types of work performed by different social insect workers, is thus associated with where workers are typically located - individual consistency in their location in the nest is described as ‘spatial fidelity zones’ (Sendova-Franks and Franks 1994; Sendova-Franks and Franks 1995; Sendova-Franks and Franks 1999). The locations of these zones for different workers, and the spread of workers across the nest area is a hypothesized mechanism for achieving division of labor in social insects (Robinson et al., 2009). The size of these fidelity zones varies a lot, possibly relating to the type of task available at different locations within the nest (e.g. smaller spatial fidelity zones in workers performing brood care, Sendova-Franks and Franks 1995; Jandt and Dornhaus 2009). Inactive workers also occupy smaller fidelity zones, often near the nest center (near/on the brood pile, Charbonneau et al., 2017), and their placement is an indication that they may represent a reserve workforce specializing in brood care (Charbonneau, Poff, et al., 2017; Charbonneau et al., 2017; Leitner and Dornhaus 2019). Thus, the role of different in-nest locations in colony organization varies, and workers occupying space near or on the brood area often have different roles in the colony than workers occupying space in the periphery or near the nest entrance. We therefore focus much of our analyses on the location of colony members relative to the brood pile or the nest entrance. .

Nest-related collective behaviour has been well studied in the *Temnothorax* genus. Rock-dwelling *Temnothorax* have been the subject of studies involving house-hunting (Franks et al., 2002; Dornhaus et al., 2004; Sasaki et al., 2013; Sasaki et al., 2015), collective nest building (Franks et al., 1992; Franks and Deneubourg 1997; Aleksiev et al., 2007a; Aleksiev et al., 2007b; Aleksiev et al., 2007c; DiRienzo and Dornhaus 2017), life-history strategy and risk-taking

behaviours relating to competition for nest sites (Bengston and Dornhaus 2014; Bengston and Dornhaus 2015; Bengston et al., 2017), among other topics. We used the rock ant *Temnothorax rugatulus* to examine the influence of nest shape on its occupants because they dwell in pre-existing rock-crevices, producing single-chambered nests that are easily replicated and manipulated in a laboratory setting (Charbonneau and Dornhaus 2015). Worker spatial position in *Temnothorax unifasciatus* nests closely predicts the task they perform, such as brood care near the brood pile (Sendova-Franks and Franks 1995), and *T. unifasciatus* workers will return to their spatial positions in relation to one another in new nests (Sendova-Franks and Franks 1994).

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

Methods

Colony collections

We collected 20 colonies of *Temnothorax rugatulus* from rocky semi-steep slopes on the Santa Catalina Mountains (GPS: 32.395, -110.688), USA, Pima County, Arizona from July to October 2017 and February to May 2018. The second half of the colonies (IDs 11-20) were collected

during the colonies' reproductive period, which allowed us to examine alate spatial organization. The first ten colonies had only workers and (non-alate) queens (and brood), whereas seven of the latter ten colonies had alates (i.e. winged queens and males). We found all our colonies in a pine-juniper zone (altitude approximately 2500m) inhabiting granite rock-crevices where entire colonies were collected by aspiration after prying open the nest cavity.

Initial housing and care

We initially placed all colonies in generic artificial nests resembling their natural nest sites in rock crevices. These nests consisted of a 2-mm-thick piece of cardboard (75 mm x 50 mm x 2 mm) sandwiched between 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 side leading to an open nest space (35 mm x 25 mm) (Charbonneau et al., 2015; Appendix, Fig. A1). We gave colonies food and water *ad libitum*, refreshed weekly, for the duration of their housing: water was given through water-filled cotton ball stopped 5 ml plastic tubes, and food was given through 2 ml microcentrifuge tubes of honey water with a concentration of 1/4 teaspoon honey per 50ml water, and 1/8 (approximately 0.075 g) of a fresh-frozen cockroach (*Nauphoeta cinerea*). We kept colonies on a 12:12 h light cycle (8 a.m. to 8 p.m.), constant temperature (approximately 21-24 °C). We placed all artificial nests in open-top plastic containers (11.1 cm x 11.1 cm x 3.3 cm) with walls lined with 'insect-a-slip' (BioQuip 2871A, 'fluon') to prevent escape. We individually marked CO_2 anesthetized workers with four identifying marks with multicolored paints (Testor's Pactra® paint): one each on the head and prosoma, and two on the gaster (Charbonneau and Dornhaus 2015). We marked ants three to five days before the colony was placed into experimental nests.

Experimental setup

We used two nest types, a circle, and a tube-shaped nest, for the experimental phase (Fig. 1). We gave colonies comparable nest densities by scaling the internal area of the nest cavities to colony size (number of workers). We examined the size of a *Temnothorax rugatulus* colony that utilized nearly all of the available nest space in a pre-experimental nest (248 workers, Appendix, Fig. A1) and doubled the area per worker to permit flexible nest space use (0.033 mm^2 per worker) - we termed this the high worker density treatment. We further produced a low worker density treatment by doubling the nest area allocated to each worker compared to that first treatment, producing a density half as dense as the high-density treatment (0.066 mm^2 per worker). These density treatments could result in nature from nest competition: colonies prefer nests that allow lower worker densities (Visscher 2007), but high nest competition could result in populations occupying nests with overall higher density; whereas low nest competition could allow colonies to occupy their preferred nests.

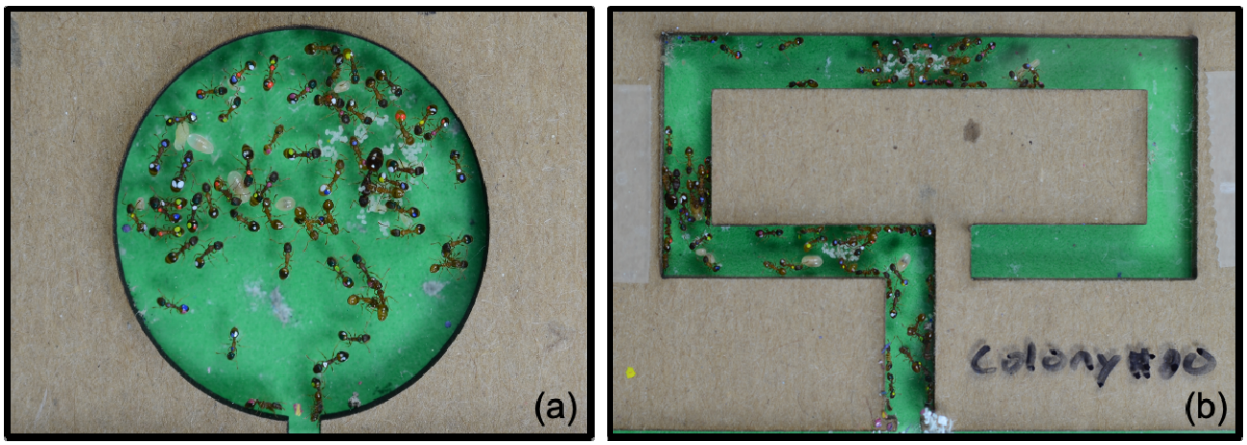


Figure 1. Nest shapes used for this study, a circle (a) and tube (b) nest.

Experimental timeline

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

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

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

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

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

Photo collection

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

Photo analysis

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

Nest sections and colony member densities

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

Scaled distances in the nest

Distance to the nest entrance: We calculated the shortest linear distance *within the available nest cavity* from each colony member to the nest entrance (Appendix, Fig. A3). Specifically:

In the circle nest, we calculated each colony member's distance to the nest entrance (per a scaled reference x and y coordinate) using the formula: sqrt((colony member x - entrance x)^2 + (colony member y - entrance y)^2) (Appendix, Fig. A3a).

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

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

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

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

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

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

Site fidelity

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

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

Fidelity and occurrence zone size: We determined fidelity and occurrence zone sizes adding the occupied 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).

Random walk simulations

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

Data processing

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 at 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).

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

Worker site fidelity and distances in the nest: We used linear mixed effects models to test the effect of either worker mean scaled distance to the nest entrance and nest shape, or the effect of

worker mean scaled distance to the brood center and nest shape on site fidelity. We assessed how nest density influenced these relationships through the interaction terms between nest distances and nest density, and the three-way interaction between nest distances, nest shape, and nest density. We lastly used an ANOVA to test whether workers had spatial fidelity in relation to the brood center across nest shapes.

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

Results

Colony distributions

Nest shape influenced how colony members were distributed across the nest

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

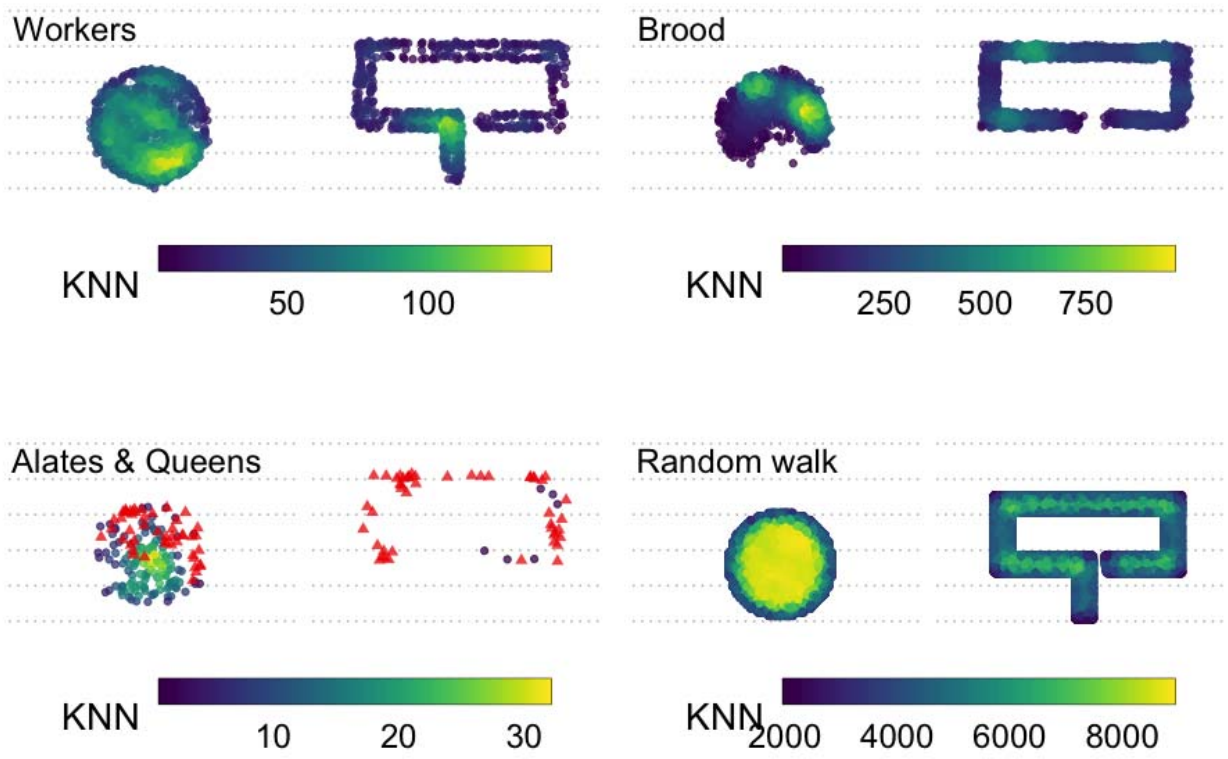


Figure 2. Illustration of colony distribution in nests for one example colony (no. 11). Shown are the distributions of workers (top-left), brood and queens (top-right, queens are red triangles), alates (bottom-left), and the low density Netlogo random walk simulated workers (bottom-right). Distributions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

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

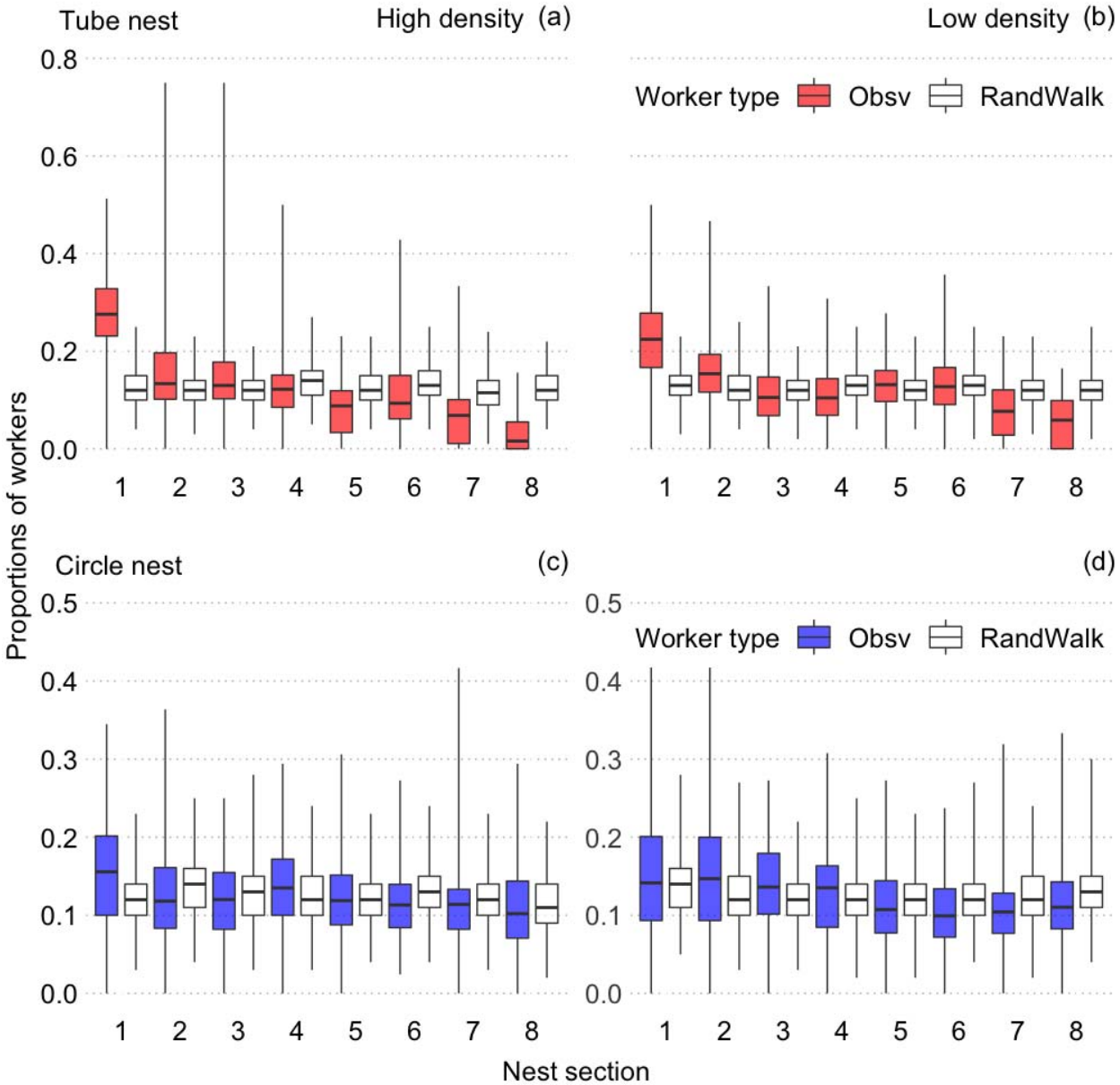
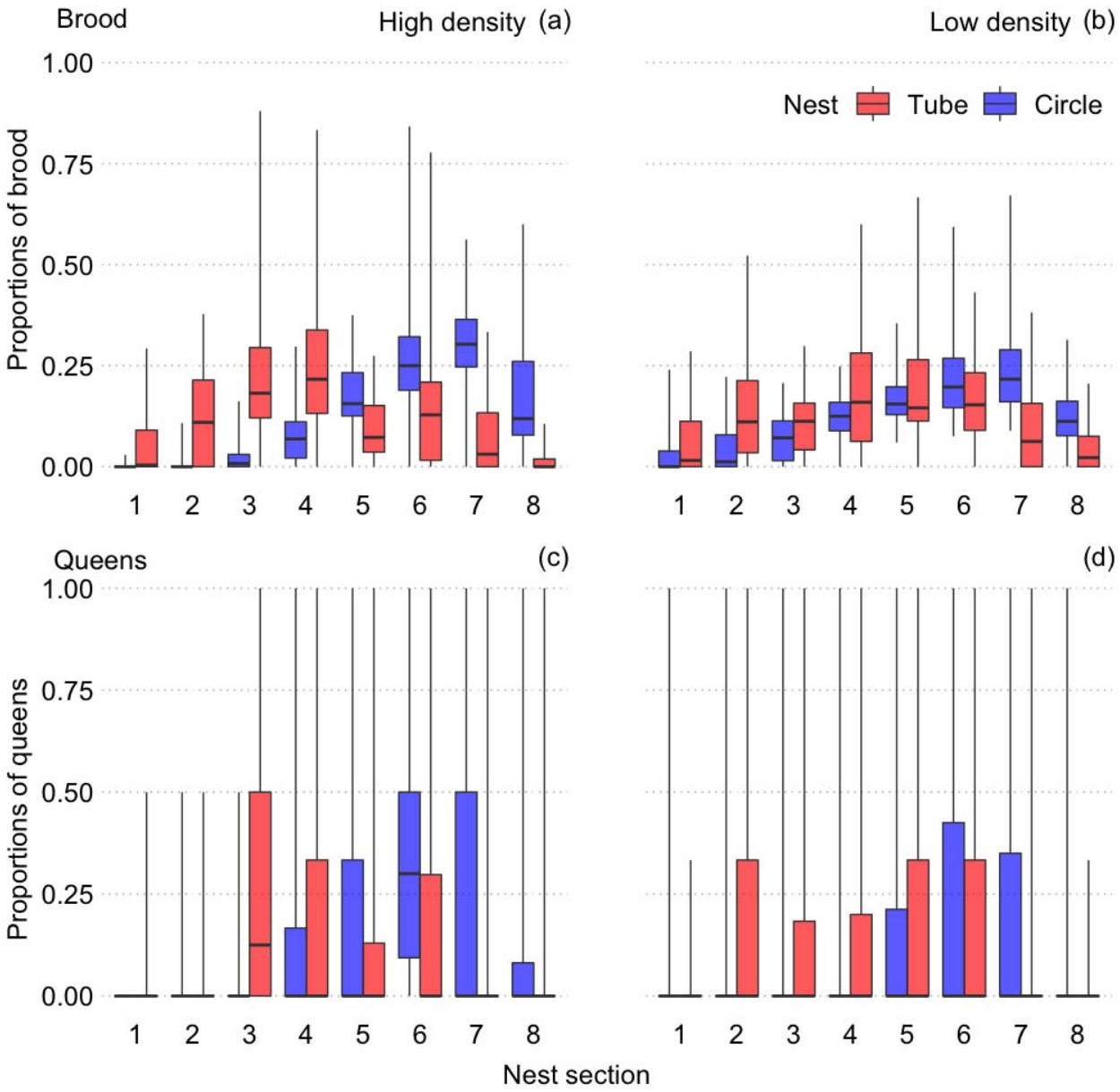


Figure 3. Workers tend to be clustered near the entrance in both nest types, but this is more pronounced in the tube nest: shown here are the worker ('Obsv', solid colors) and Netlogo simulated random walk result ('RandWalk', white) proportions of workers in each nest section of the tube (a, b; red) and circle (c, d; blue) nests (each section has the same area across both nest types, but is scaled with colony size). These figures also show the high (a, c) and low (b, d) nest density treatment. Here, and in all following boxplots, boxes represent first and third quartiles

(75% of the data), the bar within boxes is the median, and whiskers are the data range. Sample size was 3192 worker proportions in nest sections across 20 colonies.



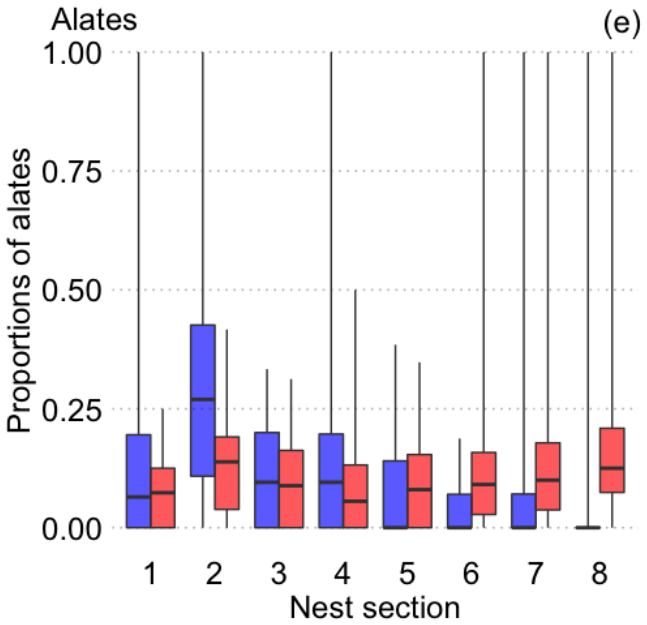


Figure 4. Brood and queens, but not alates, are generally found further from the entrance than workers in both nest types shown here are the difference in brood (a, b), queen (c, d) and alate (e) proportions across tube (red) and circle (blue) nest sections. The brood and queen densities are represented in both the high (a, c) and low (b, d) nest density treatments. Note that here, and below, alates were only present in the low nest density treatment. Sample sizes for colony members in nest sections: brood = 3184, queen = 3192, alates = 456.

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

Represented by a three-way interaction between nest shape, nest density, and nest section, brood were distributed more evenly at the low density treatment in both nest shapes, and they were found closer to the back of the circle nest at high nest density when compared to the tube nest shape (Brood: $\beta + SE = 0.004 + 0.002$, $t_{3170} = 2.513$, $P = 0.012$; Figs. 4a-b, Appendix, Table A2). In contrast, there was no significant three-way interaction between nest shape, density, and section in workers ($\beta + SE = -0.002 + 0.001$, $t_{3178} = -1.695$, $P = 0.090$; Fig. 3, Appendix, Table A1), or queens ($\beta + SE = 0.001 + 0.004$, $t_{3178} = 0.308$, $P = 0.758$, Figs. 4c-d, Appendix, Table

A3). Therefore, brood care may be distributed across more nest space in lower densities, but mobile colony members occupy nest space similarly across densities.

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

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

Distances from specific points in the nest

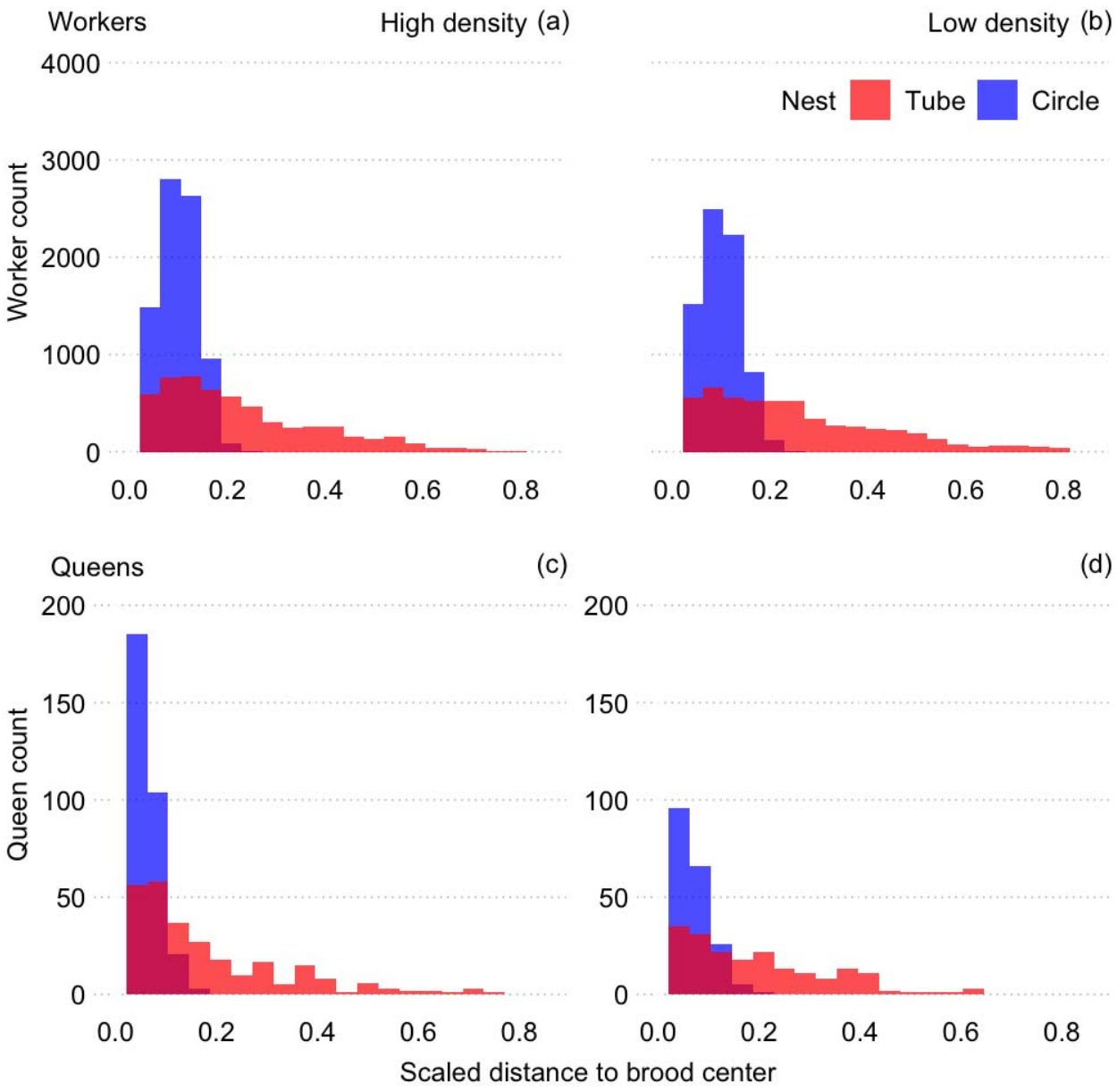
Nest shape affected colony member distance to the nest entrance

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

Nest shape affected mobile colony member distance to the brood center

All mobile colony members, i.e. workers, queens, and alates, were also farther from the brood center in the tube nest shape: (Workers: $\beta + SE = 0.103 + 0.002$, $t_{26669.530} = 45.530$, $P < 0.001$;

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


Workers
High density (a)
Low density (b)
Nest
Tube
Circle
Worker count
4000
3000
2000
1000
0
0.0
0.2
0.4
0.6
0.8
Queens
(c)
(d)
Queen count
200
150
100
50
0
Scaled distance to brood center

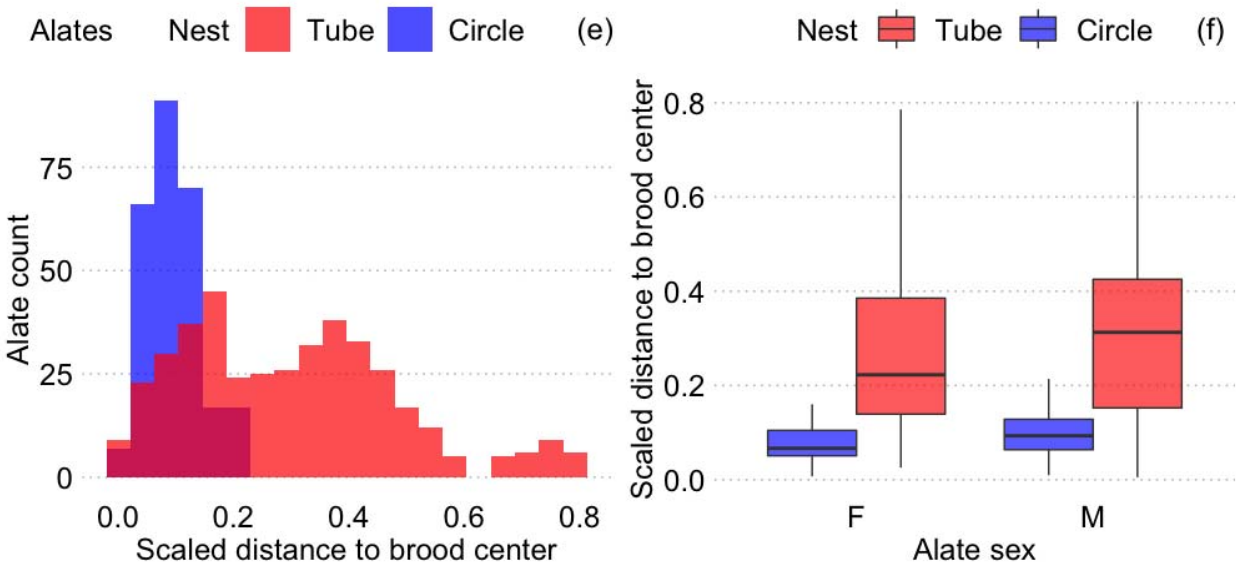


Figure 5. Worker and queen distributions center near the center of the brood distribution; but in the tube nest, the distances of both from the brood center are much larger. The peak of alate distributions is away from the brood center. Shown on the x-axis are the actual distances from the brood center (see Methods), scaled only by setting the farthest point of the tube nest as a distance of 1 (instead of dividing the nest into sections as in Figs. 3-4). This implies that distances given here are also scaled with colony size. Panels show workers (a, b), queens (c, d), and alates (e, f). The difference between male and female alates is shown in (f), denoted by letters A for females and B for males. Differences in distributions are additionally shown across high (a, c) and low (b, d) nest densities. Sample sizes for colony member distances from the brood center: workers = 26733, queens = 1080, alates = 676.

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

Workers and brood were found farther from the nest entrance in both nest shapes at low nest density, but this was more pronounced in the tube nest shape (Workers: Figs. 3a-b, Appendix, Figs. A8a-b, Table A5), (Brood: Figs. 4a-b, Appendix, Figs. A9a-b, Table A6). In contrast, there

was no effect of nest density on queen distances from the entrance in each nest (Queens: Figs. 4c-d, Appendix, Figs. A9c-d, Table A7). These results may imply that at low density, colonies do not prioritize being near the entrance, perhaps because of short travel times.

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

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

Small differences between colonies in distance relationships, particularly in alates

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

Worker site fidelity

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

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

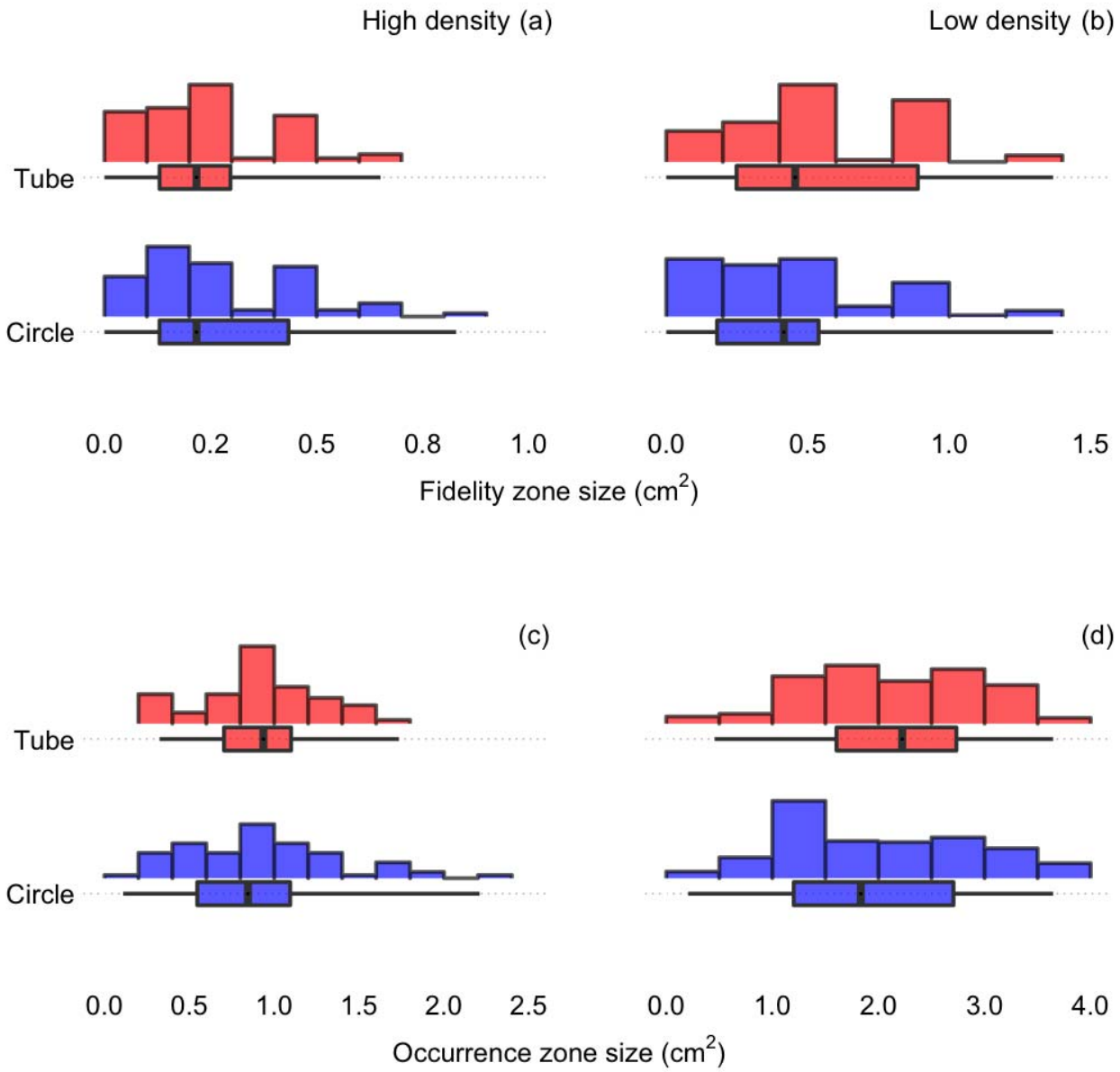


Figure 6. Comparison of worker fidelity and occurrence zones sizes (cm^2) in the circle (blue) and tube (red) nests for both the high (a, c) and low (b, d) nest density treatments. Fidelity zone sizes are the same in each nest shape (β + SE = -0.022 + 0.044, $t_{377.968}$ = -0.494, P = 0.622, Appendix, Table A14) and across density treatments (β + SE = 0.148 + 0.082, $t_{19.161}$ = 1.799, P = 0.088, Appendix, Table A14). Occurrence zone sizes are the same in each nest shape (β + SE = -0.023 + 0.071, $t_{366.580}$ = -0.324, P = 0.746, Appendix, Table A15) but are larger in lower nest density (β

+ SE = 0.808 + 0.337, $t_{16.682}$ = 2.398, P = 0.029, Appendix, Table A15). Histograms represent the data distribution; boxplots are the first and third quartiles where the black bar is the median and the whiskers extend to the data range. Please see the methods for details on how scaled worker fidelity and occurrence zone size calculations, which here were multiplied by the total area of the nest to achieve unscaled zone sizes. The sample size was 383 marked ants across 19 colonies.

Worker identity explained some worker site fidelity variation

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

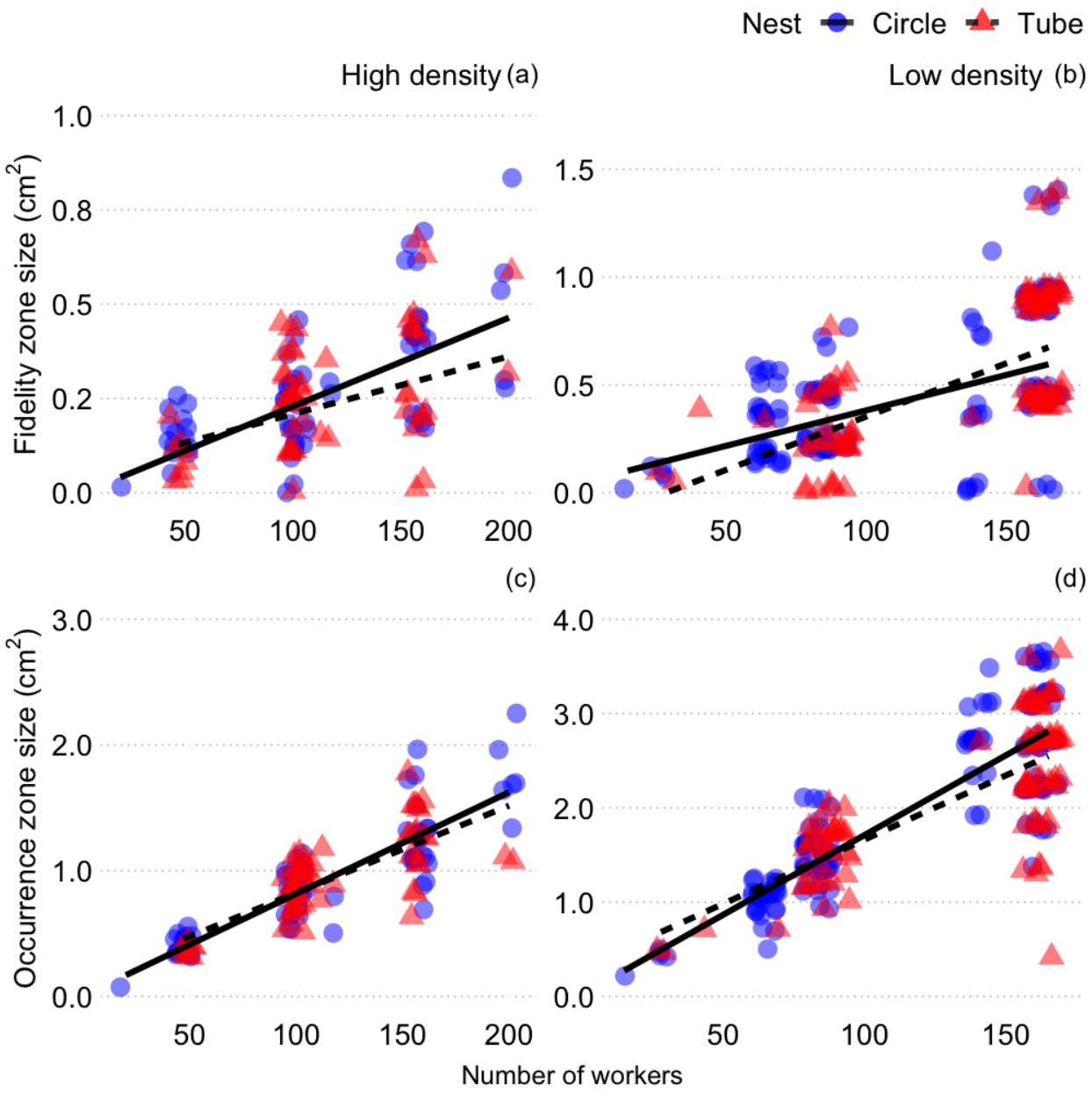


Figure 7. Relationship between true worker fidelity and occurrence zones sizes (cm^2) and colony size in the circle (blue) and tube (red) nests for both the high (a, c) and low (b, d) nest density treatments. Points are jittered on the x and y axis. Lines show significant linear relationships, but note that the relationships in (a, c) and (b, d) are not significantly different (Appendix, Tables A16-17). The sample size was 383 marked ants across 19 colonies.

Worker site fidelity and distances in the nest

No consistent relationship between worker site fidelity and distance to the nest entrance

We saw that both worker spatial fidelity and occurrence zones had inconsistent relationships with that worker's distance to the nest entrance both across nest shapes and nest densities (β + SE = 6.710 + 3.339, t_{375} = 2.010, P = 0.045; Appendix, Figs. A10a-b, Table A18), (β + SE = -14.610 + 4.870, $t_{374.994}$ = -3.000, P = 0.003; Appendix, Figs. A10c-d, Table A19) - e.g. fidelity zones: the relationship in the circle nest was positive in high density but negative in low density, both of which were the opposite of the tube nest relationships in their respective nest densities (Figs. Appendix, Figs. A10a-b, Table A18). Therefore, we did not find a consistent relationship between worker site fidelity and distance to the nest entrance.

Distance to the brood center did not influence spatial fidelity zones

There was no relationship between worker spatial fidelity zones and distance from the brood center (β + SE = 1.517 + 4.715, $t_{360.029}$ = 0.322, P = 0.748; Figs. 8a-b, Appendix, Table A20), but occurrence zones had an inconsistent relationship both across nest shapes and nest densities (β + SE = 18.951 + 6.889, $t_{374.920}$ = 2.751, P = 0.006; Figs. 8c-d, Appendix, Table A21). We therefore do not find that workers in different areas of the nest have differently sized spatial fidelity zones.

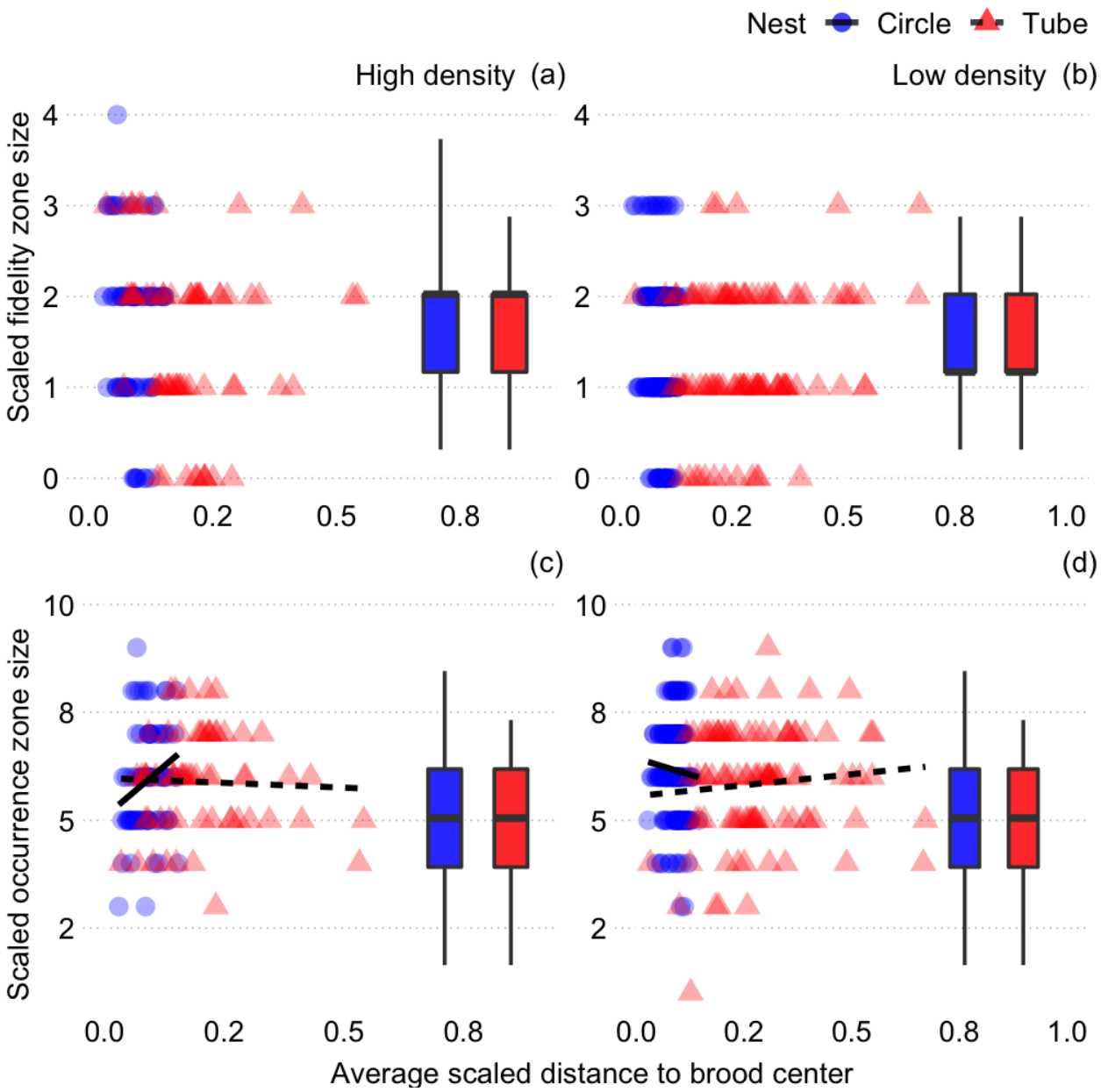


Figure 8. The relationship between scaled individual worker fidelity (a, b) and occurrence (c, d) zone sizes (scaled relative to total nest size, which was related to colony size) and average scaled distance to the brood pile in the circle (blue circles, solid lines) and tube (red triangles, dashed lines) nest shapes. Lines represent significant linear relationships. These differences are additionally compared across the high (a, c) and low (b, d) nest density treatments. There were

no significant differences in any scaled zone sizes across nest shapes or density treatments (Appendix, Tables A20-21). The sample size was 383 marked ants across 19 colonies.

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

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

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

In models of the relationship between worker site fidelity and distance to the entrance, we saw that the variation explained by our random effect (ColonyID) was 3.7% for fidelity zone size (marginal $R^2 = 0.045$, conditional $R^2 = 0.082$, Appendix, Table A18) and 6.2% for occurrence zone size (marginal $R^2 = 0.041$, conditional $R^2 = 0.103$, Appendix, Table A19). In models of the relationship between worker site fidelity and distance to the brood center, we saw that the variation explained by our random effects was 3.1% for fidelity zone size (marginal $R^2 = 0.036$, conditional $R^2 = 0.067$, Appendix, Table A20) and 8.0% for occurrence zone size (marginal $R^2 = 0.038$, conditional $R^2 = 0.118$, Appendix, Table A21).

Random walk simulations

Empirical worker distributions differed from those predicted by a random walk

We saw that empirical and random walk simulated distributions were different (Appendix, Tables A23-26). Specifically, simulated agents were more evenly distributed, while real (observed) workers were found closer to the nest entrance (NestSection^2 * Nest * WorkerType: β + 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 significantly different from the outcome that would result from a random walk.

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

We saw that the nest section with the highest proportion of workers (peak worker distribution) was closer to the entrance in the tube nest than the circle nest, whereas simulated peaks were equally likely to be in any nest section (NestSection^2 * WorkerType * Nest: β + SE = -0.133 + 0.041, z_{35168} = -3.262, P = 0.001; Appendix, Fig. A11, Appendix, Table A25).

Discussion

Available nest shape influences the spatial organization of colonies in the rock-dwelling ant *Temnothorax rugatulus*. Overall, ants preferred the areas in their nest that were closer to the entrance and moved closer to the entrance over the 10 days of our measurements. But in the tube-shaped nest, we found that workers, brood, queens, and alates were on average located further from the nest entrance (Figs. 3-4, Appendix, Figs. A8-9) and further from the brood pile compared to the circle nest (Fig. 5), presumably because the circle nest did not allow such longer distances, but maybe also because of a lack of available space closer to these points in the tube nest. Supporting this second interpretation, workers, brood, and queens were found closer to the nest entrance *relative to the maximum available distance* in that nest type in the tube-shaped

nest. None of these patterns are explained by a null model of ants moving around randomly in the same nest shapes (Fig. 3, Appendix, Figs. A8, A11). Nest shape alone could therefore significantly impact the distances between individuals and biologically relevant points in the nest, and thus how ants can interact with each other and their nest space.

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

regulate their zone size even against constraints imposed by nest shape, to maintain a desired association between workers and their tasks. This bears further investigation.

Our study confirms that the spatial distribution of colony members in ant nests is not identical to one that would be produced by ‘random movement’. We do not propose that it is realistic that ants move randomly; 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; Shiwakoti 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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Appendix

Table A1. Proportions of workers in each nest section related to nest shape and physical properties. Here and below: Sections (NestSect) begin at the nest entrance (1) and complete at the back of the nest (8) and is a quadratic term. Density is the amount of area allocated for each worker (high = 0.033 cm^2, low = 0.066 cm^2 x 2), Day is the observation day along the experimental timeline. Corner is the presence of corners in the nest section. The random effect ColonyID is colony identification. Asterisks denote interactions, and bold *P* values indicate significance. Linear mixed effects model: PropWorker ~ poly(NestSect, degree = 2, raw = TRUE) * Nest * Density + Day + Corner + (1 | ColonyID)

Table A2. Proportions of brood in each nest section related to nest shape and physical properties. Linear mixed effects: PropBrood ~ poly(NestSect, degree = 2, raw = TRUE) * Nest * Density + Day + Corner + (1 | ColonyID)

Table A3. Proportions of queens in each nest section related to nest shape and physical properties. Linear mixed effects: PropQueens ~ poly(NestSect, degree = 2, raw = TRUE) * Nest * Density + Day + Corner + (1 | ColonyID)

Table A4. Proportions of alates in each nest section related to nest shape and physical properties. Linear mixed effects: PropAlates ~ poly(NestSect, degree = 2, raw = TRUE) * Nest + Day + Corner + (1 | ColonyID)

Table A5. Relationships between workers scaled distance to the nest entrance and nest shape and physical properties. Distance to the entrance is scaled such that 1 is the back of the tube nest shape. Linear mixed effects: ScaledDist ~ Nest * Density + Day + Corner + (1 | ColonyID)

Table A6. Relationships between brood scaled distance to the nest entrance and nest shape and physical properties. Linear mixed effects: ScaledDist ~ Nest * Density + Day + Corner + (1 | ColonyID)

Table A7. Relationships between queens scaled distance to the nest entrance and nest shape and physical properties. Linear mixed effects: ScaledDist ~ Nest * Density + Day + Corner + (1 | ColonyID)

Table A8. Relationships between workers scaled distance to the nest entrance and nest shape, nest physical properties, and alate sex. Sex is male or female alates, and ratio is the proportion of male alates over all alate types in the observation. Linear mixed effects: ScaledDist ~ Nest + Sex + Ratio + Day + Corner + (1 | ColonyID)

Table A9. Relationships between workers scaled distance to the brood center and nest shape and physical properties. The brood center is a colony's brood center in each observation. Distance to the brood center is the absolute value of the difference between the scaled distances of the brood center from each worker to the entrance and is scaled such that 1 is the back of the tube nest. Linear mixed effects: ToBrood ~ Nest * Density + Day + Corner + (1 | ColonyID)

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

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

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

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

Table A14. The relationship between true worker spatial fidelity zone size (cm^2) and nest shape. Here and below the ratio between the total number of occupied zones with >15% observations (fidelity zone size) and the twenty-four possible zones is multiplied by nest area to produce true fidelity zone size. Linear mixed effects: SFZ_Area ~ Nest * Density + (1 | ColonyID)

Table A15. The relationship between true worker occurrence zone size (cm^2) and nest shape. Here and below the ratio between the total number of occupied zones (occurrence zone size) and the twenty-four possible zones is multiplied by nest area to produce true occurrence zone size. Linear mixed effects: Occur_Area ~ Nest * Density + (1 | ColonyID)

Table A16. The relationship between true worker spatial fidelity zone size (cm^2) and colony size (worker.number). Linear mixed effects: SFZ_Area ~ Number.workers * Nest * Density + (1 | ColonyID)

Table A17. The relationship between true worker occurrence zone size (cm^2) and colony size (worker.number). Linear mixed effects: Occur_Area ~ Number.workers * Nest * Density + (1 | ColonyID)

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

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

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

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

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

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

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

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

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

Figure A1. The reference photo used to determine the nest area allocated to each worker in a colony. The colony contains 248 individuals, determined with the image analysis software Fiji. The internal area is approximately 4.11mm^2, producing 0.017 mm^2 for each worker, which was doubled to 0.033 mm^2 to promote more flexible space usage. This value represents the high nest density treatment and was doubled to produce the low nest density treatment: 0.066 mm^2 for each worker in a colony

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

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

Figure A4. The criteria used to determine if a colony member required an alternative linear distance to the nest entrance near the corner formed from the nest entrance tunnel opening into the nest (black solid lines). The red dot represents a colony member, and the black dot represents the entrance. The dashed lines represent the hypotenuses between the colony member and the corner (red) and the corner and the entrance (black). The arcs represent angles, and the black square represents 90°. The solid red polygon indicates nest space that would require this type of

alternative calculation of distance to the nest entrance. Please see the methods for a full description.

Figure A5. Visual example of the shortest distance from an example worker (red dots) to an example brood center (white dots) in the circle (a) and tube (b) nests.

Figure A6. The twenty-four equal area zones used to calculate worker site fidelity in the circle (a) and tube (b) nests.

Figure A7. The relationship between the number of observations that contributed to the sizes of worker fidelity (a, c) and occurrence (b, d) zones. Zones are scaled (a, b) and unscaled (cm^2) (c, d). Points are individual workers and are jittered in height and width. The sample size was 383 marked ants across 19 colonies.

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

Figure A9. The differences in brood (a, b), queen (c, d), and alate (e, f) scaled distances from the nest entrance. Differences in these distributions are also shown across the low (a, c) and high (b,

d) nest density treatments. The significant difference between each alate sex's scaled distances to the nest entrance is shown in (f), denoted by an A for females and B for males (Appendix, Table A8). Sample sizes for colony member distances from the entrance: workers = 30247, brood = 59459, queens = 1178, alates = 1006.

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

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

Figures A12-31. The densities of workers, brood, queens (red triangles), and, where present, alates in the circle (left) and tube (right) nest shapes for all experimental colonies. Densities of Netlogo simulated workers are also shown in the high (colonies 1-10) or low (colonies 11-20)

nest density treatments. Each point is colored by the number of k-nearest neighbors within a radius that's standardized by the data range, where the sub-figure legends are increasing nearest neighbor values from left to right. Note that alates are not always present in both nest shapes.