Temporal segregation provides evidence for organizational immunity in a complex society.

Lauren E. Quevillon[1,2*], Ephraim M. Hanks[1,3], Shweta Bansal[4,5] and David P. Hughes[1,2,6]

1 Center for Infectious Disease Dynamics, Penn State University, University Park, Pennsylvania, USA, 2 Department of Biology, Penn State University, University Park, Pennsylvania, USA, 3 Department of Statistics, Penn State University, University Park, Pennsylvania, USA, 4 Department of Biology, Georgetown University, Washington, D.C., USA 5 Fogarty International Center, National Institutes of Health, Bethesda, MD, USA 6 Department of Entomology, Penn State University, University Park, Pennsylvania, USA

*** Email: leq103@psu.edu**

Abstract:

High-density living is often associated with high disease risk due to density-dependent epidemic spread. Despite being paragons of high-density living, the social insects have largely decoupled the association with density-dependent epidemics. It is hypothesized that this is accomplished through prophylactic and inducible defenses termed ‘collective immunity’. Here we look for empirical evidence of segregation of Carpenter ant workers that would be likely to encounter infectious agents (i.e. foragers) using a suite of social, spatial, and temporal analyses. Importantly, we do this in the absence of disease to establish whether baseline organizational immunity exists. Behavioural and social network analyses, coupled with spatial movement data, do not provide evidence of segregation of these workers. However, when the temporal ordering of social interactions is taken into account, active and inactive foraging ants are not observed to interact in ways that could lead to the meaningful transfer of disease. Furthermore, resource spread analyses show that such temporal segregation does not appear to impact the colony-wide flow of food originating from these individuals. This study provides an understanding of a complex society’s organization in the absence of disease that will serve as a null model for future studies in which disease is explicitly introduced.

Introduction:

Social insects are paragons of self-organized complex systems[1, 2, 3, 4, 5]. Individuals interact to produce sophisticated colony-level behaviour that is more than, and not necessarily predictable from, the behaviour of the individuals that create it[3]. This emergent behaviour, such as honeybees "democratically" choosing between nest sites[6] or ants creating elaborate living architectures in response to environmental obstacles[7], has likely contributed to the ecological success of the social insects as a whole. Therefore, it remains imperative to understand how behaviours at the scale of the individual and at the scale of the colony dynamically influence each other. Such understanding is salient in the face of perturbation, where changes at the individual level due to disease or predation may have cascading consequences for the entire colony.

Disease is an especially relevant perturbation for social insects because it has been suggested that a significant cost of high-density living is increased disease risk[8, 9, 10, 11, 12, 13]. Though social insect colonies have both higher density and a higher average degree of genetic relatedness than other animal groups[14], they appear to have largely overcome this issue. This is not because they lack infectious agents- social insects are host to a wide array of pathogens and parasites[9, 12, 15, 16]. Rather, they are thought to mitigate intense infection pressures through a series of standing and inducible defenses termed 'social' or 'collective' immunity[17, 18, 19]. These defenses range from the immunological to the behavioural, including how colonies are spatially organized and which tasks are allocated to different workers[19, 20, 21, 22].

The social and spatial segregation of workers most susceptible to infection is often cited as a major mechanism of disease prophylaxis in social insect colonies[19, 23, 24]. However, many of these workers (i.e. foragers) are also responsible for the delivery of food resources into the colony and such segregation could therefore impact resource flow[25]. Understanding how colonies have balanced these opposing demands in both ecological and evolutionary time is important as it will inform how such complex systems operate despite the presence of agents that could perturb them to the point of collapse.

The first step to understanding this balance is to determine if the social and spatial segregation of foragers does indeed occur in the absence of disease. We remain unsure because observing individual behaviour within a realistic colony setting has previously been a formidable task. Recent technological advances have revolutionised the scale and resolution with which we can analyse behaviour[26,27] and advances in network analysis applied to behavioural ecology allow a better linkage of individual- and group-level behaviour[25]. Thus, we are now in a position to investigate the social, spatial, and temporal organization of an experimentally tractable complex system. This will serve as a first step to understanding how such systems can mitigate disease spread while maximizing resource acquisition and flow.

Here we test for the presence of standing organizational immunity through forager segregation in colonies of the black carpenter ant, *Camponotus pennsylvanicus,* using a suite of social, spatial, and temporal analyses. The ant *C. pennsylvanicus* is widespread in the northeastern USA and has evolved to

nest inside dead trees[28]. We mimicked this by maintaining colonies inside wood under complete darkness (video S1).

We first classify ants based on whether they are performing or have previously performed tasks outside the nest (and thus have the theoretical potential for disease exposure). Next, we look at the oral exchange of food (trophallaxis) as the key social interaction of interest because colonies must balance efficient resource flow (food, information) with mitigating disease spread[29]. If social segregation does occur, we would expect to see its signature represented in the trophallaxis interactions between ants that have been outside and those that have remained buffered within the protected confines of the colony[17]. To facilitate comparison of how trophallaxis between ant functional groups could impact potential disease risk, we borrow the concept of 'person-time' used in calculating epidemiological incidence rates[30]. Next, we incorporate individual movement data to assess whether spatial segregation is present in the absence of disease. Finally, we incorporate the time-sensitive ordering of social interactions to understand how observed colony organization serves theoretical resource flow through *C. pennsylvanicus* colonies. Integrating this suite of approaches shows that ant colonies are indeed segregated. Our work serves as a useful null model of a complex social insect system in the absence of perturbation.

Methods:

Ant colony set-up and filming

Two queen-right *C. pennsylvanicus* colonies were collected from field sites in Centre County, central Pennsylvania, U.S.A. in December 2012. Seventy-five worker ants were selected from each colony and were individually labeled. Labels consisted of numbers printed on photo paper that were affixed to the ants' posterior abdomens (gasters) with optically clear nail polish. Following a 5-minute acclimatization period, the labeling was not observed to alter the ants' behaviours, movement or interactions.

The labeled ants and the queen were housed in a nest set-up consisting of a four-chambered wooden nest (total area = 63 cm^2) that was gridded to a resolution of 1cm^2 and covered with a plexiglas top (video S1). The nest was contained within a filming box so that nest conditions were always dark. The nest was separated from a sand-bottomed foraging arena (total area = 144 cm^2) by a 4-m long maze. The length of the maze was observed to create a clear separation between workers allocated to foraging versus internal colony tasks. Inside the foraging arena, ants had *ad libitum* access to water, 20% sucrose solution and a protein source (mealworms).

Each colony was filmed for approximately 30 minutes beginning at 21:00 hrs for 8 consecutive nights in June 2013 using a GoPro Hero2 camera with a modified IR filter (RageCams.com) illuminated under infrared light. Ants cannot detect infrared light, so the set-up was similar to the dark within-nest conditions that they naturally experience.

Video analysis and ant worker classification

For each night of filming, all trophallaxis interactions of each individual ant inside the nest were recorded for a 20-minute observation window, which lead to 401 hours of observation (76 ants x 2 colonies x 0.33 hours x 8 nights). The identities of the individuals interacting, the start and stop time of their interaction, and the location of their interaction within the nest was recorded (nests were divided into 1 cm^2 grids). Additionally, the overall functional classification of every ant during each observation period was recorded (i.e. nest worker, forager, non-active forager, queen). Nest workers were ants that were never observed to leave the nest. Foragers were ants that actively entered or left the nest during the observation. Inactive foragers were ants that had been observed leaving the nest on previous nights, but which did not leave the nest during the current 20-minute period in which they were being analysed.

Trophallaxis count and duration

The number of trophallaxis events and their duration for each individual was recorded as above. To test for differences in mean trophallaxis count and duration as a function of ant functional classification (i.e. forager, inactive forager, nest worker, or queen), two-sided Kruskal-Wallis one-way analysis of variance tests were conducted using the kruskal.test function in R[31]. Mann-Whitney U tests were then used to determine which functional classes had significant differences from one another. Data from all nights of observation were pooled together, but each colony was analysed separately.

Static network analysis and visualization

Unweighted, undirected static network analyses were conducted using the iGraph package implemented in R[31, 32]. Network analyses were aimed at identifying whether key individuals that could serve as brokers or attenuators of food and/or disease flow were associated with a particular ant functional class; metrics analysed included degree, betweeness centrality, closeness centrality, and Burt's constraint[33, 34]. Degree is the number of connections that an individual ant has to other ants, while betweeness centrality captures how important an individual ant is to promoting connection across the entire colony[27]. Closeness centrality is the average social distance of a given ant to all other ants in the colony[33]. Burt's constraint is a more nuanced measure to qualify; it measures the extent to which an ant's interaction partners are redundant and thus identifies which ants could act as brokers of food or disease between 'structural holes' in a network[35].

These metrics were analysed separately for each individual in each colony for each night of observation. To test whether there were differences in these metrics between the various ant functional groups and because these metrics were not normally distributed, a Kruskal-Wallis test was implemented using the kruskal.test function in R.

Trophallaxis networks were visualized using the circular layout in GEPHI[36], in which circles represent individual ants (Fig. S1). Each ant was assigned a position based on its tracking ID that was maintained in all visualizations for all nights; trophallaxis interactions between ants are represented as lines (edges) between the respective circles. The length of the

edges conveys no information, but the width of a given edge is proportional to the number of trophallaxis interactions those ants had during that video observation.

Ant functional group networks

To better understand the functional connectivity of the ant trophallaxis networks, the network graphs were reconstructed by collapsing each ant functional group (foragers, inactive foragers, nest workers, queen) into nodes and representing the total duration of interactions between each group as connections between nodes (edges) (Fig. 2A,B). The width of edges is proportional to the total duration of trophallaxis spent between those two functional groups.

Ant-time calculation

While the total duration of trophallaxis (edge weight) in the ant functional group networks are the same for each group in the interacting pair, this time actually represents variable disease transmission risk due to different numbers of ants in each functional group and variable amounts of time in the nest for the forager class. Thus, to get a more accurate understanding of how trophallaxis duration corresponds to potential transmission risk, we standardised these total trophallaxis durations using the epidemiological concept of 'person-time'[30], hereafter referred to as 'ant-time'.

To calculate ant-time, we took the number of ants in each functional group for each night of observation and multiplied by the total time they were in the nest and therefore available to engage in trophollactic interactions with other ants. The queen, nest workers, and inactive foragers were by definition in the nest for the entire 20-minute observation period each night and accordingly the

calculation of ant-time is a simple product of the number of those ants by the 1,210-second observation window. However, foragers were in the nest for variable amounts of time and so ant-time for the forager class is calculated by summing how much time each individual forager was in the nest for a precise calculation of the time they were available for within-nest interactions. For ant-time formulas, refer to the supporting information..

Proportion of time-budget engaged in trophallaxis

Having calculated the ant-time for each class for each night, the percentage of each functional group's total time-budget engaged in trophallaxis can be calculated. The total duration of trophallaxis (edge weight) between two ant types provides the numerator and this is the same for both functional groups in the dyadic interaction being considered. However, the ant-time denominator varies for each type and thus the same total trophallaxis duration represents different total percentages of each group's total time-budget available for interaction. The percentage time-budget for each dyadic interaction (for example, forager-forager, forager-inactive, forager-nest, forager-queen) is presented in Fig. 3. For % time-budget engaged in trophallaxis percentages, refer to Table S3.

Ant functional network motifs

Understanding the functional connectivity between foragers, inactive foragers, nest workers and the queen is important for a broader understanding of how resource and disease flow is accomplished in Carpenter ant societies. Comparing networks in which individual ants are modeled as nodes (Fig. S1) is less useful than comparing ant functional groups as nodes (Fig. 2) because the

identity of the individual ant in the society is less important than the functional role they are performing. To facilitate comparison of these functional networks across nights and between colonies, we categorised the empirically observed network motifs. Network motifs are the different patterns of connection that can occur between nodes. The full range of possible network motifs for a 4-node (i.e. forager, inactive forager, nest, and queen) network with varying numbers of connections (i.e. 1-6 edges) and the network motifs that were empirically observed are given in figure 2C.

Spatial movement analysis

Five known forager ants, five randomly selected nest workers, and the queen were chosen from each colony for additional spatial movement analysis. The wooden nest in which ants were housed was gridded to a resolution of $1cm^2$, and the cell locations where the majority of the ant's body was located as well as the time stamp when it was in that location were recorded for each observation period. The residence time spent in each cell was recorded to determine nest spatial use. This data was used to fit a continuous-time discrete space random walk model (see model description, SI) for ant movement behaviour[37, 38], with the goal of identifying the relative spatial promiscuity of foragers vs. nest workers and movement behaviour (location and speed) around the queen.

Resource spread analysis

Interactions from the static networks were analysed with the additional inclusion of interaction time-stamps. Temporal networks were constructed using the package 'timeordered'[39] implemented in R. To understand how the pattern and

timing of social interactions converge to impact the flow of food or disease, a resource-spread analysis was conducted using the *spreadanalysis* function in the 'timeordered' package. Given the temporal networks actually observed, the *spreadanalysis* function computes the fraction of the network reached over time when a theoretical resource is seeded from an ant randomly selected by the function. For each colony and night of observation, the fraction of the network reached in 100-second intervals was computed for 20 ants randomly selected by the function. The mean fraction of the networked reached at each interval as a function of ant functional classification was computed for each colony for each night.

Results:

Individual trophallaxis count and duration

There was a significant difference in the number of distinct trophallaxis events between ant functional groups in one colony (colony 1: $p<0.0002$, two-sided Kruskal-Wallis test). Inactive foragers engaged in more trophallaxis events than did either nest workers or the queen, ($p<0.0005$ [nest workers] and $p<0.003$ [queen] for colony 1, two-sided Mann-Whitney test, Table S1) but there was no significant difference between active and inactive foragers. In colony 2, foragers had significantly more trophallaxis interactions than did nest workers ($p<0.04$). The duration of these trophallaxis events was not statistically different between functional groups in either colony.

Static network analysis

Static, undirected networks for each colony for each night of observation are presented in Fig. S1. We tested for differences in network metrics aggregated over all nights between the different groups (foragers, inactive foragers, nest workers, and queen). Foragers and inactive foragers have a higher mean degree centrality (number of unique individuals interacted with) compared to both nest workers and the queen in both colonies ($p<0.05$, Mann-Whitney test, Table S2), but they are not significantly different than each other. In colony 1, the closeness centrality of active and inactive foragers was significantly lower than that of nest workers and the queen, as well as between nest workers and the queen ($p<0.05$, two-sided Mann-Whitney, Table S2C), which indicates that in that colony foragers and inactive foragers are more socially distant on average than other colony members. There were no significant differences in closeness centrality between ant types in colony 2. The Burt's constraint (redundancy of contacts) of the queen was significantly higher than inactive foragers and nest workers in colony 1 ($p<0.009$ and $p<0.02$ respectively, two-sided Mann-Whitney test), but not significantly different in colony 2. While the queen had a median degree of 1, the identity of the individual she interacted with was not consistent across all nights for both colonies.

Ant functional group networks

Of the 59 possible ant functional group network motifs, only 4 motifs were empirically observed in the 16 nights of observation across both colonies (Fig. 2C). Of these, 2 motifs in particular (forager-inactive-nest closed triad and inactive-nest-queen) accounted for ~87% of all observed motifs.

Percent time-budget calculations

The percent time-budget each functional class engaged in trophallaxis with all other ant functional classes is given in Table S2. Taking the ant-time of each functional class into account provides a more nuanced view of ant group interactions. While interactions with the queen represent a small fraction of the total trophallaxis happening in the nest, this was found to represent about 4-5% of her entire time budget in both colonies. This represents a greater percentage of time at risk than would be expected if just the duration of her trophallaxis events out of the whole were to be considered. In colony 2, foragers spent between 5-10% of their time within the nest engaging in trophallaxis.

Ant movement and spatial analysis

The average spatial usage of foragers, nest workers, and the queen is given in Fig. 4. Foragers occupied a greater proportion of the nest than did either non-foraging nest workers or the queen. The queen was largely immobile in both colonies, though in one colony (colony 1), the queen spent some time in three of the four chambers of the nest.

Results of our movement analysis show that in colony 2 nest workers are more mobile (have higher movement rates) than foraging ants while the latter are in the nest ($p<0.01$, two-sided T-test). This result does not hold in colony 1, and the overall effect size is small. There was no evidence of directional queen avoidance by foragers or nest ants in either colony, but there was evidence in both colonies that foraging ants move faster than nest ants when near the queen than when in another chamber ($p<0.01$, two-sided T-test).

Resource spread analysis

Social network data has traditionally been analysed as a time-aggregated or static graph (Fig. S1) in which the timing and order of interactions is ignored. However, such timing and order is crucially important for dynamic flow processes, such as disease transfer[29]. Based on the timing of interactions, returning foragers were never actually observed to interact in a way necessary for disease transmission to the queen (i.e. after a forager has returned from a trip outside the nest). Spread analysis indicates that such temporal segregation does not appear to inhibit the flow of a theoretical food source, with no apparent differences between the mean network fractions reached when seeded by different ant types in either colony. Food generated from active and inactive foragers in colony 1 was able to reach a higher mean fraction of the network than food generated from either nest workers or the queen (Fig. S2). In colony 2, food seeded from a forager initially reached a higher mean fraction of the network, but this converged to ~25% by the end of the 20 minutes regardless of what functional class the food was seeded from.

Discussion:

Social immunity theory predicts that interaction between foragers, which are the ants presumed to have increased pathogen exposure as a consequence of leaving the nest, and other classes (i.e. nest workers) should be minimized[17]. In addition, foragers are predicted to maximize their contact with the same few individuals[17, 24]. Our behavioural analyses show that active foragers engage in

more trophallaxis interactions than nest workers (approximately 2 additional interactions, Fig. 1A,B). Static social network analyses complement these findings; in addition to engaging in more trophallaxis events (higher degree), foragers exchange food with a greater number of unique individuals (SI Table 2), indicating that their contact redundancy is lower than predicted.

Re-analyzing the static network data though ant functional group networks (Fig. 2A,B), in which trophallaxis connections are represented between functional groups rather than individuals, allows broader patterns of colony organization to emerge. Importantly, it also facilitates network comparison across nights and colonies and likely reflects a level of analysis more biologically relevant to ant colony functioning. Two network motifs predominate, accounting for 87% of those observed: inactive-nest-queen and forager-inactive-nest closed triad (Fig. 2C); these represent only two out of 59 possible patterns of functional group connection. From these motifs, it appears that inactive foragers play an important role as brokers of trophallaxis in Carpenter ant colonies, and that active foragers are interacting with nest workers more than expected.

This becomes even clearer when ant-time standardization is taken into account (Table S3). While nest worker interactions account for a large amount of colony trophallaxis, when this is corrected for by their higher abundance, active and inactive foragers spend a greater percentage of their total in-nest time budget actually engaged in trophallaxis. This suggests that under conditions in which perturbation is not present through disease or resource competition,

organizational immunity is not accomplished through social segregation of potentially exposed members.

In addition to the social position of foragers within the colony, we were also interested in their spatial usage. Analysis of nest spatial usage showed that foragers are spatially promiscuous relative to both nest workers and the queen (Fig. 4). While the queen's lack of movement synchronizes well with predictions from social immunity (i.e. to be secluded) the expansive movement of the foragers is counterintuitive. It is reasonable to assume that foragers should avoid internal areas of the nest[17, 19] but we did not observe this (Figure 4). However, we found evidence in both colonies that foragers could be modulating their speed in response to their social environment (Table S4). When foraging ants were in the same chamber as the queen, they moved faster than their nest worker counterparts. Such speed modulation could potentially reduce the impact of pathogen transmission to which foragers may have been exposed by moving faster near the most important individuals (i.e. queen, younger workers). This has not been previously considered as a mechanism to mitigate disease spread within the nest. Future studies that specifically address whether different ant classes alter their speed in response to different social environments inside the nest and what, if any, biological impact this has for potential disease transmission are needed.

The static network analyses of colony social organization and the spatial promiscuity of foragers reveal an ant society that may not be particularly well suited to the prevention of disease transmission. This would appear counter to

the verbal models of social immunity where intuition, without within-nest behavioural data, has suggested a strong segregation between worker types, especially foragers [17]. In considering social immunity, however, time has been a neglected component[19]. In our data when the timing and order of trophallaxis interactions are taken into account, foragers and the queen never interact in a way that could lead to the biologically meaningful transfer of disease (i.e. after a forager has come back into the nest after a foraging trip, carrying some pathogen that might transfer to the queen via either oral food exchange or prolonged physical contact). Thus, the timing of social interactions provides evidence for nuanced, standing behavioural prophylaxis within *C. pennsylvanicus* colonies.

Infectious agents are not the only pressure that ant colonies face. Since trophallaxis interactions are a conduit for both disease and food, there is a fundamental tradeoff in optimizing food flow while minimizing disease spread, both of which are brought into the colony by foragers[12]. This tradeoff is of interest because it could impact colony functioning even in the absence of disease; the temporal segregation of foragers could prolong the time it takes for incoming food to reach other colony members. Resource spread analysis (Fig. S2) provides an understanding of the flow tradeoff that could result from temporal segregation of foraging ants by analyzing the spread of a theoretical resource through the empirically observed temporal networks. Interestingly, in both colonies the mean fraction of the network reached a high of 20-25% after 20 minutes. In colony 1, this was achieved in food originating from active and inactive foragers, showing that their temporal segregation did not appear to impact the potential flow of food.

In colony 2, food saturated at this percentage regardless of what ant type it originated from.

Our study also highlights the need to consider an ant's behavioural past when considering its present and future role in colony functioning and introducing disease risk. Foragers who have just returned from outside the nest are clearly capable of introducing potential pathogens into the colony. What remains unclear is for how long they remain capable of transmitting disease- at what point does a potentially infected incoming forager transition to inactive forager? While the classification of inactive forager used here was defined by the methods of the study, there are clear behavioural differences between these ants and nest workers who have never been observed to leave the nest. Had they been included within the nest worker categorisation, this would have obscured the network and spatial signals observed. We advocate that future studies of disease transmission in social insect societies follow individuals over a time period that will capture past exposure, and thus the continuum of foraging behaviour and pathogen risk.

Conclusion:

Through the incorporation of dyadic- and network-level social interactions, individual movement data, and the timing of social interactions, we now have a clearer insight into how disease prophylaxis could be accomplished in *C. pennsylvanicus* ant societies. The standing organizational immunity in an ant colony, exemplified by the Carpenter ant colonies studied here, appears to be more nuanced than previously imagined. The timing of social interactions may

provide an additional layer of disease prophylaxis in social insect societies. This adds evidence for the growing argument that temporal information and meaningful behavioural interactions should be included into social network analyses if we are to make biologically accurate conclusions[25].

Analyzing Carpenter ant colony organization and functioning in the absence of disease and environmental heterogeneity provides a useful null model; we are now primed to study how these complex systems react to perturbation. Future experiments in which laboratory infections are combined with integrated social, spatial, and temporal approaches will further inform how social insect colony organization and individual behaviour dynamically interact to reduce disease transmission. Social insects are host to a range of both generalist and specialist parasites[12], some of which can change the behaviour of the infected host (ie. *Ophiocordyceps*) or potentially alter the interactions between healthy and infected nest mates (ie. *Beauveria, Metarhizium*), and some of which cause no discernible change in behaviour. Such studies will also afford us the ability to synchronize theoretical predictions about disease transmission in societies from agent-based and SIR modeling approaches[40, 41] with empirical data from experiments in which disease can actually be introduced.

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Additional Information

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Author contributions
L.E.Q. and D.P.H. designed the study. L.E.Q. collected the data. L.E.Q. and D.P.H. conducted the behavioural and network analyses with the expertise of S.B. E.M.H. designed the spatial movement model. L.E.Q. and D.P.H. wrote the manuscript; all authors contributed equally to its editing.

Competing financial interests
The authors declare no competing financial interests.

Figures and tables:

Figure 1: Trophallaxis count and duration.
a) Trophallaxis count and b) duration as a function of ant behavioural classification. Asterisks represent statistically significant differences between groups ($p<0.05$, two-sided Mann-Whitney U test). Black lines represent the median values and whiskers represent 95% confidence intervals.

Figure 2: Ant functional networks.
a) Representative ant functional group network showing trophollactic interactions within and between ant functional groups groups. The total duration of all interactions between groups is given on the edge, and the percentage of the ant group's total time budget is also given. b) All ant functional group networks for both colonies over all nights. c) All possible functional group interaction patterns (network motifs); blue shading indicates which motifs were actually observed over the eight nights of observation for both colonies and the number in the brackets indicates how many times that particular motif was observed.

Figure 3: Trophallaxis as percentage of total ant-time budget.
Boxplots showing the mean percentage of total time budget for each dyadic interaction between ant functional groups over all eight nights for each colony, error bars are +/- sd.

Figure 4: Segregated Use of Nest Space.
Aggregated residence times in ant-days for queens, active foragers, and non-foraging ants from colonies 1 and 2.

Figure 1

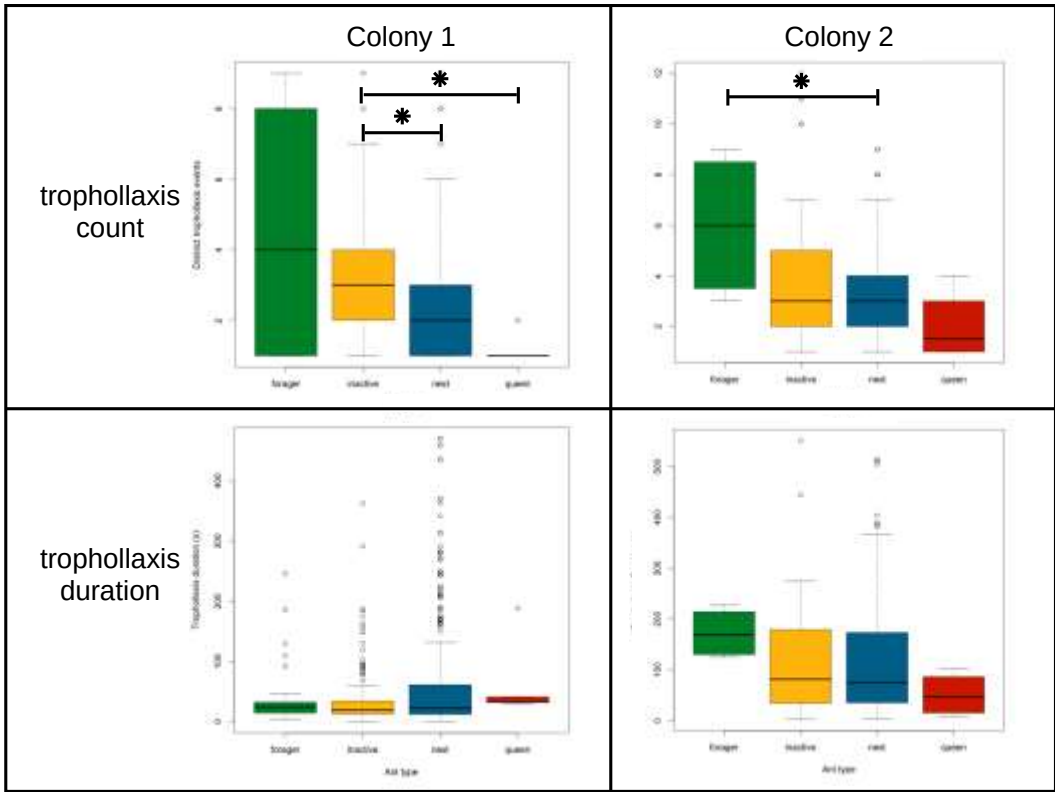

Colony 1
Colony 2
trophollaxis count
trophollaxis duration
*
*
*

Figure 2A

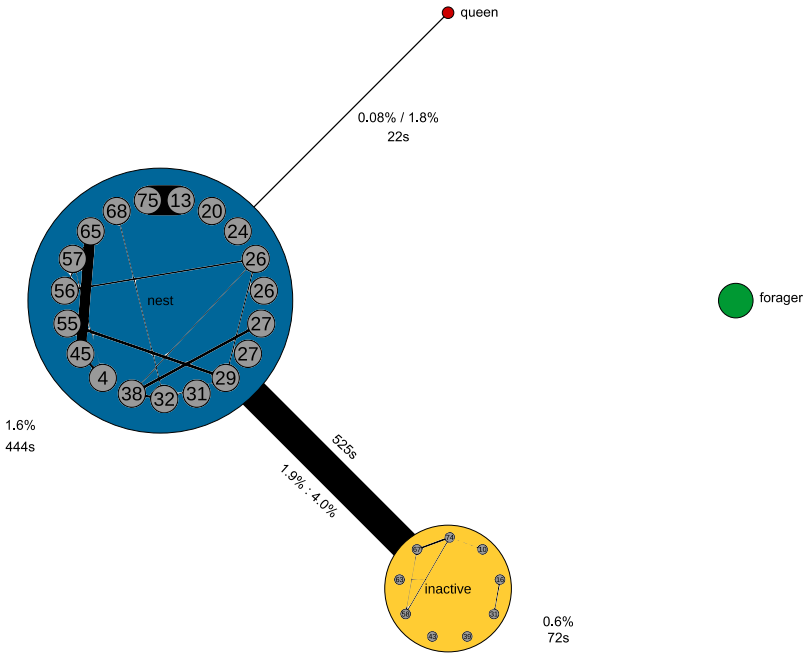


Figure 2B

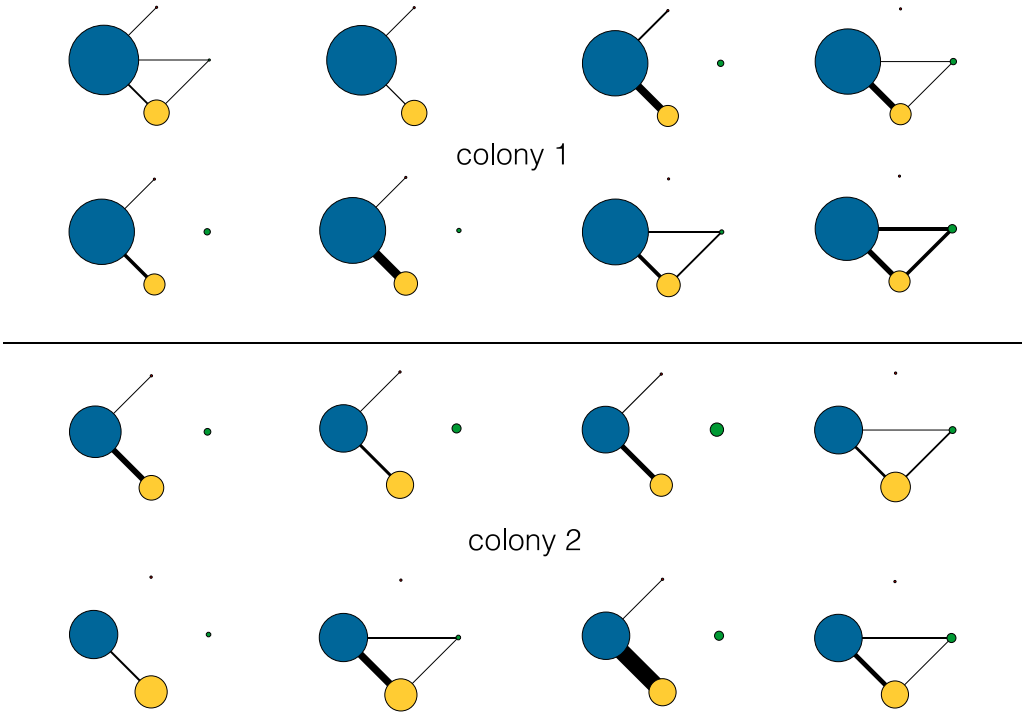


Figure 2C:

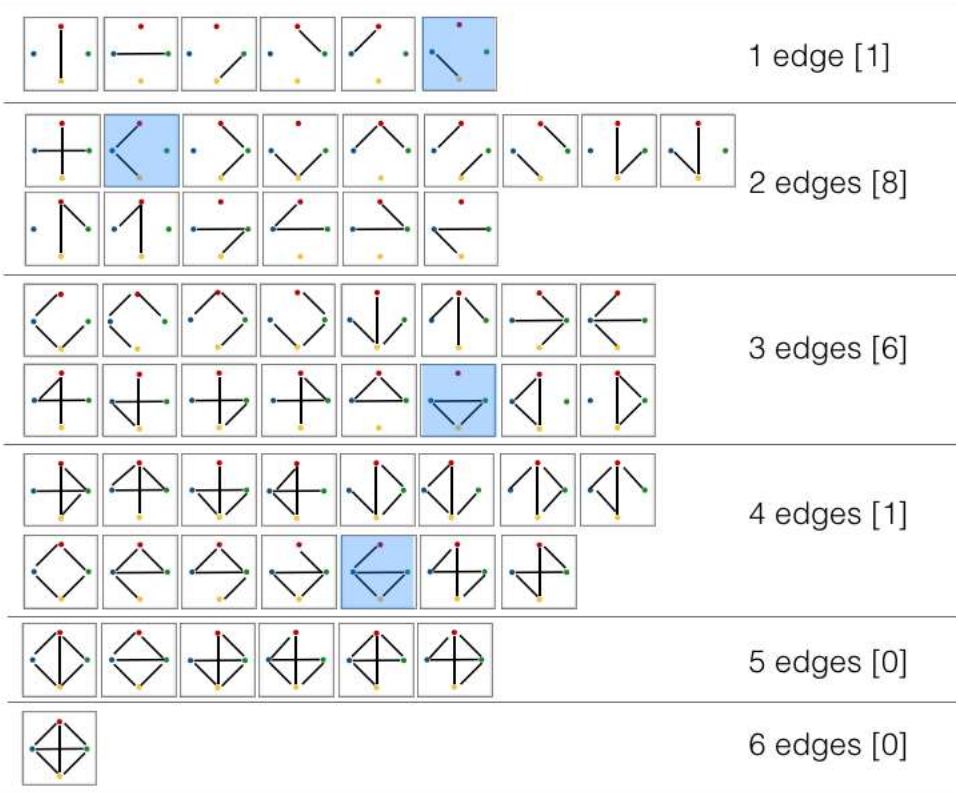


Figure 3

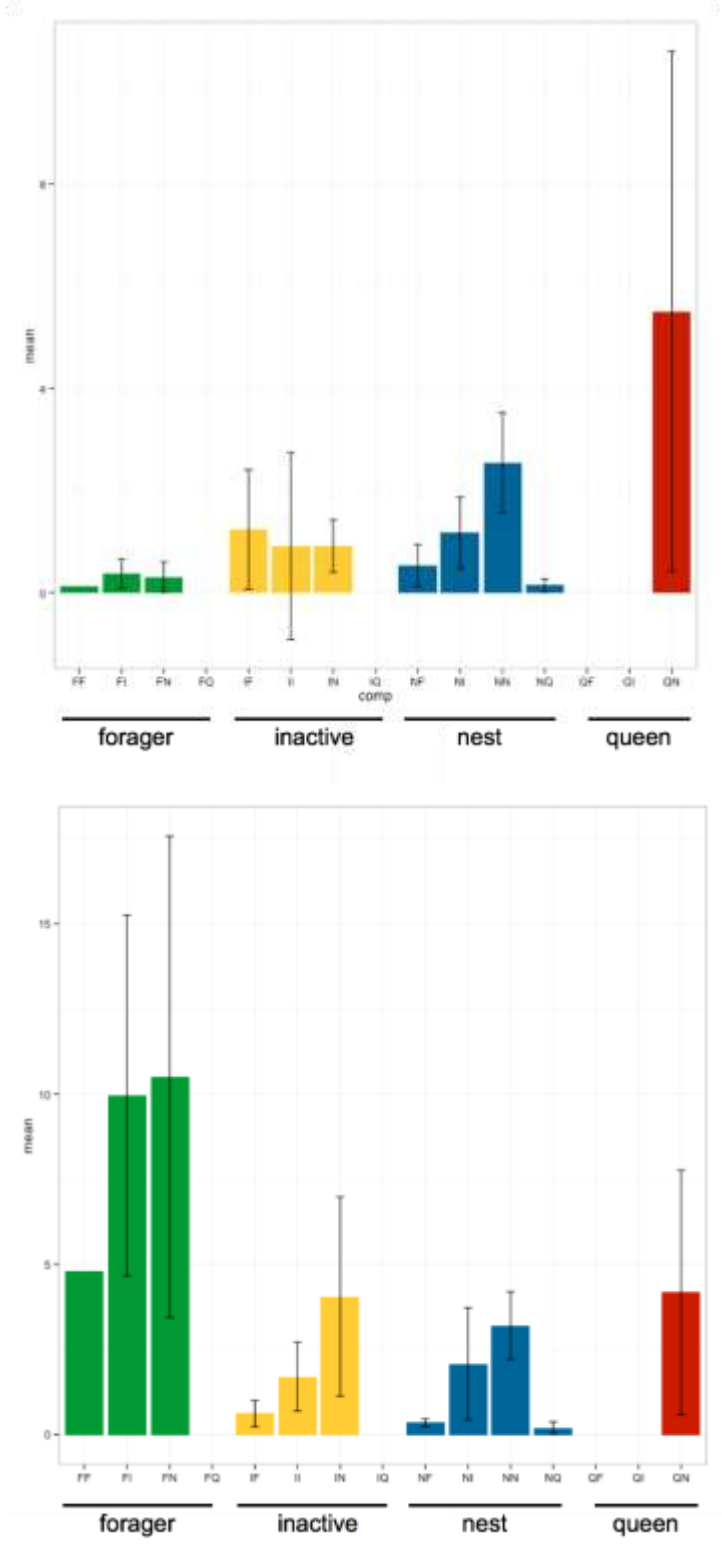
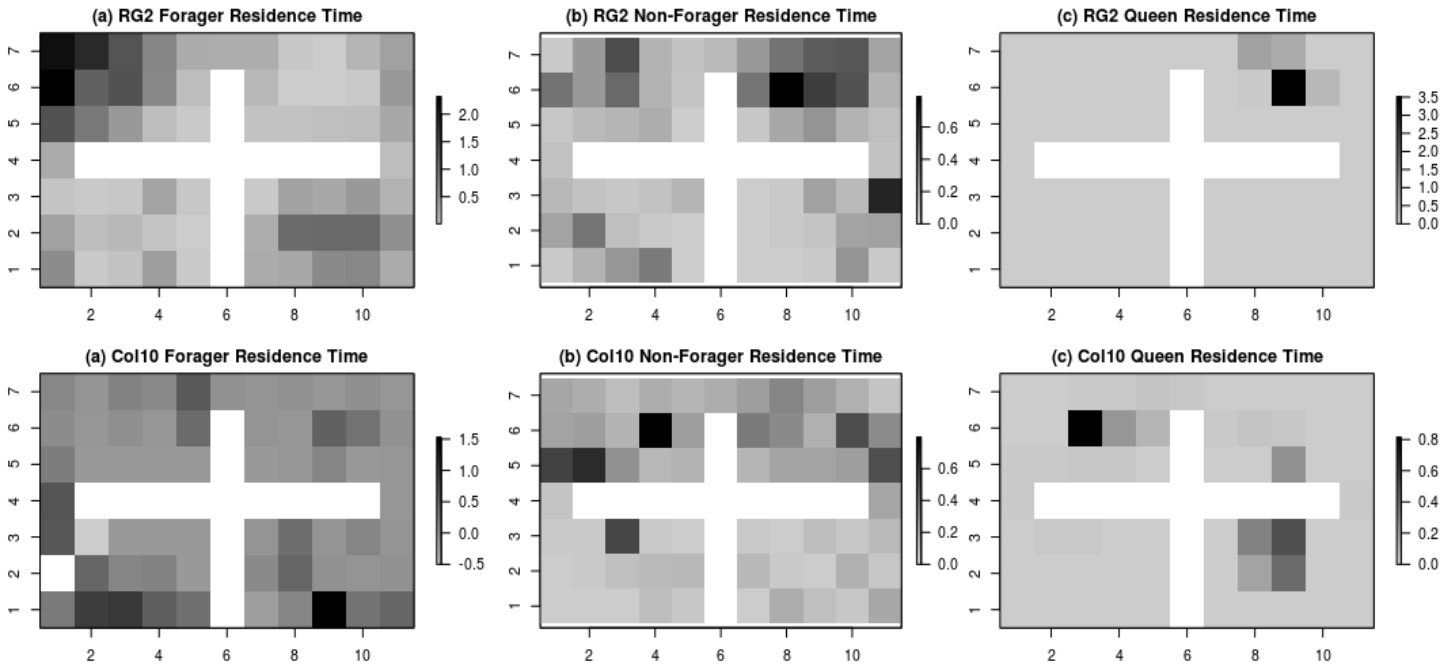

mean
comp
FF
FI
FN
FQ
IF
II
IN
IQ
NF
NI
NN
NQ
QF
QI
QN
forager
inactive
nest
queen

Figure 4:


(a) RG2 Forager Residence Time
(b) RG2 Non-Forager Residence Time
(c) RG2 Queen Residence Time
(a) Col10 Forager Residence Time
(b) Col10 Non-Forager Residence Time
(c) Col10 Queen Residence Time