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Special Issue: Golden Age of Animal Communication Networks

Higher-order interactions facilitate food dissemination in ant colonies

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Animals interact to communicate information and disseminate resources. Interactions are often examined as dyadic encounters, yet many interactions are higher-ordered, with multiple individuals interacting simultaneously (e.g. an alarm call). Higher-order interactions may expedite the spread of knowledge and resources. Here we examine how the size of higher-order interactions influences the spread of food through trophallaxis, mouth-to-mouth food exchange, among colony members of Argentine ants, *Linepithema humile*. We hypothesized that the size of trophallaxis groups, a higher-order interaction, relates to colony hunger and affects the behaviour of foragers and food recipients. We found that trophallaxis groups were larger when colonies were hungry, resulting in rapid food dissemination to many individuals. However, feeding multiple individuals simultaneously in large higher-order trophallaxis groups resulted in slower food intake by each recipient, because they had to share with others. Thus, we show that when engaging in higher-order interactions in the context of food transfer there is a trade-off between rapid food dissemination throughout a colony and food uptake by each individual worker. Finally, as a colony became satiated, foragers distributed less food more slowly, either because they retrieved less food from outside the nest or because recipients offloaded less food from the foragers. Our findings show the importance of considering the size of higher-order interactions in social systems to account for both system level implications, such as speed of transmission, and the experience of interacting individuals. In the context of communication networks, the size of higher-order interactions may have important implications for trade-offs between the speed and the amount, or quality, of information that is being transmitted, and it may impact the response of individuals to the information they receive.

Social animals interact to communicate information and share resources. Traditionally, the study of interactions among individuals has focused on dyadic interactions between two individuals, e.g. by experimentally observing the response of one individual to another. Network analysis has expanded the examination of social interactions to include interactions among multiple individuals in a single framework (Croft et al., 2008; Pinter-Wollman et al., 2014; Wey et al., 2008). While this expansion has provided novel insights into the causes and consequences of social interactions (Webber & Vander Wal, 2019), interactions in a network are still described and analysed as dyadic events, with each edge in a network representing an interaction between two individuals. Thus, network analysis does not distinguish multiple dyadic interactions from interactions in which multiple individuals interact simultaneously, which we refer to as ‘higher-order interactions’ (Greening et al., 2015; Silk et al., 2022).

Higher-order simultaneous interactions can facilitate efficient information flow and food distribution, i.e. the most amount of information or food transfer in the least amount of time. For example, in the context of animal communication, an alarm call is broadcast to multiple individuals at once, as a higher-order interaction, rather than through multiple one-on-one interactions (McRae, 2020). Broadcasting information to multiple individuals simultaneously expedites the dissemination of information because it reduces the time required for information to reach all group members. It further minimizes the potential for introducing errors through miscopying of a signal over multiple communication events, but interference or attention splitting may introduce errors. Similarly, a tutor can demonstrate the use of tools to multiple individuals simultaneously, spreading information

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faster and potentially more accurately than multiple one-on-one tutoring events (Greening et al., 2015). However, large group sizes can lead to reduced understanding by the recipients of the information (e.g. negative effects of class size on student learning outcomes; Antoniou et al., 2024; Blatchford et al., 2011; Krassel & Heinesen, 2014). The distribution of resources, such as food, to multiple individuals can be faster if shared simultaneously (Hasenjager et al., 2024). However, when sharing a resource that is internalized, consumed, or otherwise depleted, by receivers in a higher-order interaction, the size of the sharing group can impact the amount of resource that each group participant receives. Here we examine how the dynamics of distributing food, a consumable good, change with the number of individuals that interact in higher-order interactions.

In social insect colonies, a small subset of individuals, referred to as foragers, retrieve food from outside the nest to share with their nestmates. Returning foragers transfer the food they collected to nestmates and then decide whether or not to continue foraging, weighing colony hunger against the risks of foraging outside the protected nest (Barbee & Pinter-Wollman, 2022). Much work has been devoted to understanding how foragers decide whether or not to continue foraging. Harvester ant foragers use social information obtained from interactions with other ants inside the nest to decide whether or not to continue foraging (Gordon, 2010; Greene et al., 2013; Pinter-Wollman et al., 2013) and Argentine ant, *Linepithema humile*, foragers rely on the proportion of trophallaxis interactions they experience to decide whether or not to continue foraging (Miller & Pinter-Wollman, 2023). In contrast, carpenter ant foragers use personal information, specifically, the rate at which their crop empties, to decide when to leave the nest for more food (Baltiansky et al., 2023; Greenwald et al., 2018). While much research has been devoted to the decision making of foragers, little is known about how food is distributed within the nest, once it arrives. The process of food dissemination inside the nest is important because it may impact foraging regulation, as well as the ability of a colony to sustain all its workers.

Ant species that consume liquid food often use trophallaxis to share food. Foragers collect liquid food outside the nest, store it in their abdomen and offload it to nestmates by regurgitating the food into the mouth of another ant, a process referred to as ‘trophallaxis’. The receiving ants may consume the food, store it in their abdomen or transfer it to other workers or to larvae (Dussutour & Simpson, 2008). Indeed, workers that are not foragers frequently transfer food to their nestmates (Greenwald et al., 2015; Quque et al., 2021; Sendova-Franks et al., 2010) with an increase in food transfer observed in starved, compared to satiated, colonies (Howard & Tschinkel, 1981; Sendova-Franks et al., 2010). Furthermore, food transfer can slow down as colonies become satiated (Buffin et al., 2009). Observations of trophallaxis in starved colonies have revealed the formation of trophallaxis groups (also referred to as ‘rosettes’; Wallis, 1964), in which a single forager offloads her food to multiple workers simultaneously, in a higher-order interaction. Offloading food to multiple individuals simultaneously in a higher-order interaction can be more efficient than multiple one-on-one interactions, potentially allowing foragers to return to forage outside quickly. However, each food recipient may gain less food per time unit, on average, as trophallaxis group size increases, because the food recipients have to share the offloaded food with more individuals.

We examined the effect of higher-order group size on food dissemination in starved colonies of Argentine ants, a species in which trophallaxis plays an important role in food dissemination (Markin, 1970). Furthermore, Argentine ants are a highly invasive species (Suarez et al., 2001) that disrupts natural ecological communities (Suarez et al., 1998), and a better understanding of their biology may assist control efforts. We hypothesized that trophallaxis group size is affected by collective colony hunger and that it affects both the behaviour of the food recipients and the behaviour of the foragers as they offload food. Specifically, we predicted that (1) the size of a trophallaxis group would increase with colony starvation, to maximize the number of unique food recipients. However, we predicted that (2) disseminating food to multiple individuals simultaneously would result in slower food intake by recipients because they have to share the consumable good with more individuals. We further predicted that (3) food recipients would join another trophallaxis event sooner if they participated in a larger trophallaxis group, because they would potentially receive smaller amounts of food when participating in larger trophallaxis groups. Finally, as the colony becomes satiated, we expected that (4) foragers would retrieve less food and/or that recipients would offload less food from foragers. In both cases, we expected that the amount of food a forager offloaded in the nest would decrease with colony satiation and that the time taken by a forager to offload food would increase with colony satiation.

METHODS

Animal Collection and Maintenance

Ant colonies used in the present study are the same as in Miller and Pinter-Wollman (2023), but in the present study, we collected different data from these colonies and address different biological questions using unique data and data analysis. We collected workers and queens of Argentine ants from the University of California Los Angeles (UCLA) Botanical Gardens and outside Temescal Canyon State Park, Los Angeles. We collected ants from both foraging trails and the nest interior by flooding the nest entrance and aspirating escaping individuals. Because *L. humile* are naturally polygynous (i.e. have multiple queens in a nest) and groups of workers with queens naturally disperse from the nest to establish new colonies (Hee et al., 2000), we formed eight biologically meaningful experimental groups of workers with queens, which we refer to here as ‘colonies’. Each equally sized experimental colony consisted of 200 workers, approximately 50 brood and one queen, which were placed in plastic boxes (20 × 9.7 × 9.8 cm) with Fluon-coated walls to prevent escape. Ants were provided with a nest made of Plexiglas with moistened plaster on the floor (Fig. 1a, b) and foil covering the top to create a dark interior. As a water source, we provided a vial filled with water and plugged with a cotton ball. We stored the colonies in 25 °C and 50% humidity on a 14:10 h light:dark cycle. Before we began to observe food dissemination, we starved the colonies for 14 days, while providing them with ad libitum water to prevent dehydration. By the time the experiments began, colonies lacked brood because the brood died or was eaten. The lack of brood in the experimental colonies likely had no, or negligible, impact on our results because we examined the transfer of carbohydrate-rich food, which is consumed by workers, and not protein-rich food, which is consumed by brood (Markin, 1970).

Recording Food Transmission

To record food dissemination inside the nest, we fed the starved colonies with fluorescently dyed sugar water and imaged the ants’ activity inside the nest (Fig. 1a–c) following the protocols established by Greenwald et al. (2015). We provided the starved colonies with a glass vial containing 1 ml of 1 M sucrose solution with 5 mg of fluorescein (FITC-F1300, Invitrogen, ThermoFisher Scientific, Waltham, MA, U.S.A.; absorption 490 nm/emission 514 nm),

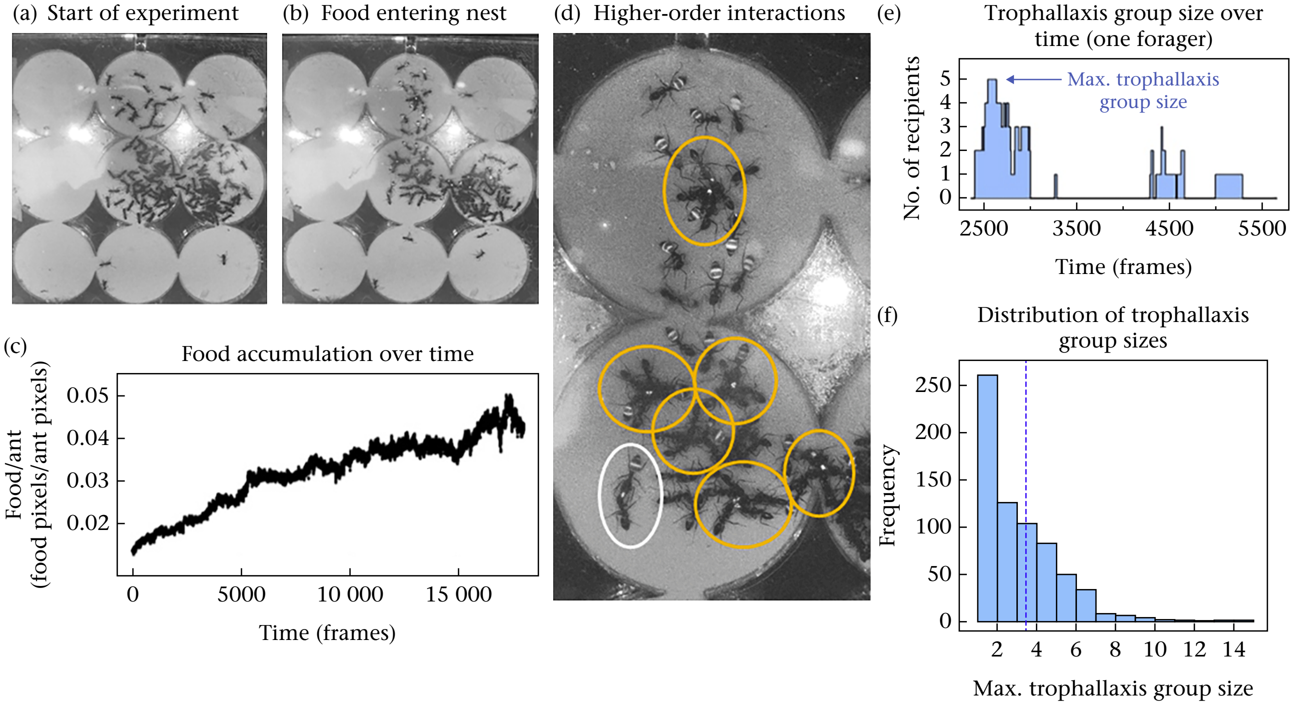

Figure 1. Food transfer and higher-order trophallaxis interactions. At the start of the experiment (a) ants are empty, no fluorescent food is visible. As foragers bring food to the nest (b) the fluorescent food becomes visible in the ants' abdomens (see also magnification in (d)). Throughout the experiment (c) food accumulates in the nest, measured as the number of fluorescing pixels in the nest over time. When food is entering the nest (d) (magnification of two nest chambers from (b)) higher-order trophallaxis interactions (yellow ovals) are formed. One-on-one interactions (white oval) become more frequent as colonies become satiated. (e) Food recipients (*Y* axis) join and leave trophallaxis events with a forager over time (*X* axis). Trophallaxis joining dynamics are shown for a single forager return event, the size of the maximal trophallaxis group for this forager return event is indicated with an arrow, and the integrated experience of the forager is the area under the curve (blue). (f) The distribution of maximum trophallaxis group sizes in all observed forager return events is right-skewed, and the mean is indicated by a vertical dashed line.

instead of the glass vile with water that they had been given earlier. We began recording colony behaviour within 1 min of introducing the food using a CMOS colour camera (FLIR Chameleon 3 3.2 MP Color USB3 Vision, Sony IMX265) fitted with a Tamron lens (M118FM08, 8 mm, 1/1.8 inch) and a 540 nm Semrock filter to capture the fluorescein isothiocyanate (FITC) emission. We used Royal Blue Luxeon Rebel Color LED lights (peak wavelength = 447.5 nm) to illuminate the nest and excite the FITC in the food. The camera was positioned above the nest and recorded at 10 frames/s for 20 min using the MicroManager image capture software (Edelstein et al., 2014). All images were captured through the clear lid of the box to prevent escape and avoid changes to the airflow, and the foil coving the nest was removed 20 min before starting the experiment to allow ants to acclimate to the lighting.

Forager Behaviour

To examine food dissemination from foragers to workers inside the nest we tracked manually the behaviour of ants from the eight experimental colonies using a custom MATLAB (Mathworks Inc., Natick, MA, U.S.A.) script. The script allowed observers to record the time and position of behavioural events by placing the computer mouse cursor on the location of the behaviour and clicking a particular key on the computer keyboard to record the time (frame number) and location of the event. Each event was coded with a different key. To ensure interobserver reliability, all trackers were thoroughly trained, their work was spot-checked by two observers (J.M. and Himadree Radadia) and any ambiguities were reported to and resolved by J.M. To track trophallaxis group size over time, we recorded all trophallaxis events in which each returning forager participated. The events we recorded included the start and end of every trophallaxis for each forager and the joining and departure of each recipient ant. A trophallaxis event was defined as ants touching mouthparts for more than 0.5 s. We only recorded an interaction as a trophallaxis event if it lasted 0.5 s or longer (Fig. 1d), to exclude very brief interactions that would not be sufficiently long to engage in food exchange (for information on other types of interactions in this system, see Miller & Pinter-Wollman, 2023). Based on these data, we computed the number of ants participating in each trophallaxis event throughout its duration. Because food recipients joined and left a trophallaxis event after it started and before it ended (Fig. 1e), we quantified maximum trophallaxis group size as the largest number of ants that participated in the trophallaxis event simultaneously (in Fig. 1e, this number would be 5 for the first trophallaxis event). To quantify how much food a forager offloaded during its time in the nest, we integrated the number of ants in trophallaxis over time, for the entire duration of a forager in the nest (blue area under the curve in Fig. 1e). We assumed that all foragers returned with approximately the same amount of food because *L. humile* workers are monomorphic and Greenwald et al. (2015) showed a linear relationship between a forager's body size and the amount of fluorescent food in its abdomen. We followed each forager from the frame it entered the nest until it either left the nest for another foraging trip, or until the end of the recording, whichever came first. Because ants were not individually marked and we did not record the food outside the nest, a forager that visited the food more than once was recorded as a new forager every time it returned to the nest. Thus, we recorded forager return events rather than individually distinct foragers, but this had no impact on our ability to answer the questions of this study. We tracked all foragers that returned to the nest until 1500 frames before the end of the video, or until the colony was full (i.e. when all ants were fluorescing), whichever came first. We did not track foragers that returned to the nest when less than 1500 frames remained in the video because that would not leave enough time to track all their interactions inside the nest.

Recipient Behaviour

To determine the effect of trophallaxis group size on the behaviour of recipients, we selected recordings from three of the eight experimental colonies for further analysis. To control for colony hunger, we tracked food recipients that interacted with foragers that entered the nest early in the experiment (based on data we obtained about foragers, as detailed above). We focused on trophallaxis events with foragers whose first trophallaxis groups attained their maximum size between frames 3000 and 5000 to ensure that recipients were all approximately equally hungry (i.e. all three colonies tracked were similarly deprived of food). Before frame 3000, only a few foragers returned to the nest because most foragers were still collecting food outside the nest, and after frame 5000, the number of fed ants inside the nest began to increase. To minimize the difference in the amount of food available at the trophallaxis groups that we studied, we ensured that the forager was completely full when trophallaxis began by only tracking trophallaxis recipients that participated in the first trophallaxis group of a forager after it entered the nest, i.e. before a forager offloaded any of its food. To sample extreme values of group sizes, we tracked food recipients that participated in trophallaxis events in which the maximum group size was one or two recipients, or events with a maximum group size of seven or more recipients. All food recipients in each selected trophallaxis event were tracked manually to record the (1) time (frame) at which each recipient joined the trophallaxis event, (2) time (frame) at which each recipient left the trophallaxis event and (3) time (frame) at which each recipient either left the nest or joined another trophallaxis event after leaving the focal trophallaxis event. Once a recipient left the nest or joined another trophallaxis event, we stopped tracking it. Because ants joined and left trophallaxis events (Fig. 1e), we quantified the group size that a recipient perceived (‘perceived group size’) as the average number of ants over time that participated in the trophallaxis event while the focal recipient was part of the trophallaxis group.

Data Analysis

To determine whether trophallaxis group size was related to colony satiation, we examined the relationship between trophallaxis group size and time. We used a generalized linear mixed model (GLMM) with a Poisson distribution because the maximum group size data were count data (Fig. 1f). Maximum trophallaxis group size was the response variable and the time at which a forager entered the nest, a proxy for colony satiation (Miller & Pinter-Wollman, 2023), was the explanatory variable. We implemented the statistical model with the ‘glmer ()’ function from the *R* package ‘lme 4’ (Bates et al., 2007). We included colony ID as a random effect to account for variation among colonies. Here, and in subsequent models with a random effect, we report the variance explained by the random effect as the difference between the conditional and marginal R^2, obtained with the function ‘r2_nakagawa ()’ from the *R* package ‘performance’ (Lüdecke et al., 2021).

To determine whether the time that recipients spent in a trophallaxis event was related to the size of the trophallaxis group they experienced, we used a GLMM with a negative binomial link function to account for overdispersion, implemented with the function ‘glmmTMB()’ from the ‘glmmTMB’ *R* package (Brooks et al., 2017). Duration in a trophallaxis group was the response variable and perceived group size was the explanatory variable, with colony ID as a random effect. To determine whether latency to join a subsequent trophallaxis event was related to the size of the trophallactic group a recipient just left, we used a linear model in which the latency to join the next trophallaxis event was the response variable and perceived group size and colony ID were the explanatory variables. Similarly, to determine whether the size of the trophallaxis group impacted the latency of a recipient to leave the nest, we ran a linear model in which latency to leave the nest was the response variable and perceived group size and colony ID were the explanatory variables. We included colony ID as a fixed effect rather than as a random effect in these two models to account for lack of singularity when running a mixed effects model. We used the ‘lm ()’ function and report the results using the ‘Anova ()’ function from the ‘car’ *R* package (Fox & Weisberg, 2019).

To determine whether colony satiation impacted the amount of food that foragers offloaded in the nest, we used a GLMM with a negative binomial link function to account for overdispersion, implemented with the function ‘glmmTMB()’ from the glmmTMB *R* package (Brooks et al., 2017). The response variable was the integrated number of ants a forager engaged in trophallaxis with over time and the explanatory variable was the time at which the forager entered the nest, with a random effect of colony ID. Finally, to determine whether colony satiation impacted the time a forager spent in the nest, we use an LMM in which the response variable was the time a forager spent in the nest and the explanatory variable was the time at which the forager entered the nest, with a random effect of colony ID, implemented with the ‘lmer ()’ function from the *R* package ‘lme 4’ (Bates et al., 2007).

All model assumptions were tested and visualized with the *R* package ‘performance’ (Lüdecke et al., 2021) and statistical model predictions were plotted using the *R* packages ‘sjPlot’ (Lüdecke, 2025) and ‘ggplot 2’ (Wickham, 2016). Data were analysed using *R* version 4.2.2 (R Core Team, 2014).

Ethical Note

Our work was conducted in accordance with the ASAB/ABS Guidelines for the use of animals in research. Ants are invertebrates and do not require special institutional permissions for experimentation. We handled ants with extreme care to minimize mortality and we did not use any invasive methods. Ants were starved before the experiments to motivate foraging behaviour, and the starvation period was chosen to prevent queen mortality or above-average mortality of adult workers. After the experiments, we kept the ants in the laboratory and provided them with food ad libitum until they died naturally.

RESULTS

Trophallaxis group size decreased with colony satiation. The maximum number of ants in a trophallaxis group decreased with the time at which a forager entered the nest ($N = 680$ forager return events), which we used as a proxy for colony satiation (GLMM: estimate ± SE = -0.0002 ± 0.00001, $Z = -16.74$, $P < 0.0001$; Fig. 2, Supplementary Table S1). The random effect colony ID accounted for 7.84% of the variance in the model, $R^2_{\text{marginal}} = 0.32$.

Food recipients spent more time in a trophallaxis event as trophallaxis group size increased. We found a significant positive relationship between a recipient's duration in a trophallaxis event ($N = 78$ recipient trophallaxis events) and the average number of co-recipients that were present in the trophallaxis event while the focal recipient was part of the trophallaxis (GLMM: estimate ±

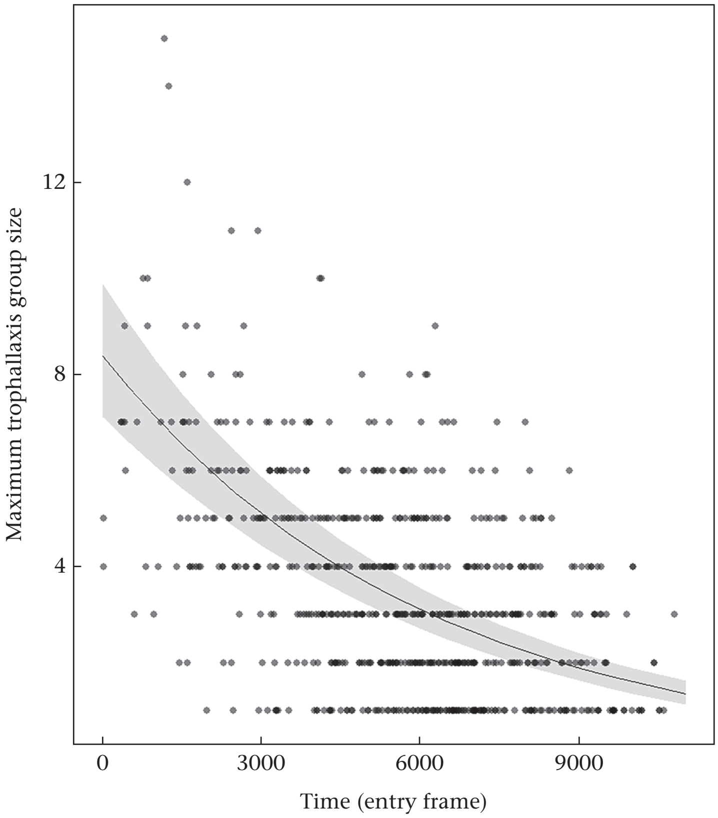

Figure 2. Trophallaxis group size over time. The maximum size of a trophallaxis group during forager return events (points, $N = 680$) relative to the time at which the forager returned to (entered) the nest, a proxy for colony satiation. Here and in all following plots, the line is the fit of the statistical model to the data and the shaded area is the standard error.

SE = 0.46 ± 0.08, $Z = 5.355$, $P < 0.0001$; Fig. 3, Supplementary Table S1). The random effect colony ID accounted for 15.7% of the variance in the model, $R^2_{\text{marginal}} = 0.39$.

We did not detect an effect of trophallaxis group size on the latency of food recipients to join a subsequent trophallaxis group or leave the nest. Latency to join the next trophallaxis event ($N =$ 58 trophallaxis events after which the recipient joined another trophallaxis event) was not significantly related to the average number of ants in the current trophallactic event (LM: sum of squares = 1909, $F_1 = 0.021$, $P = 0.885$; Fig. 4a, Supplementary Table S2) or to colony ID (LM: sum of squares = 16573, $F_2 =$ 0.092, $P = 0.912$; Supplementary Table S2). Furthermore, we did not find a significant relationship between latency to leave the nest ($N = 20$ trophallaxis events after which the recipient left the nest) and the number of co-recipients an ant experienced in the trophallaxis event (LM: sum of squares = 3736, $F_1 = 0.113$, $P =$ 0.741; Fig. 4b; Supplementary Table S2) or colony ID (LM: sum of squares = 44932, $F_2 = 0.679$, $P = 0.521$; Supplementary Table S2).

The amount of food that foragers offloaded to nestmates decreased with colony satiation. The integrated experience of foragers ($N = 680$ forager return events) significantly decreased with the time at which a forager entered the nest (GLMM: estimate ± SE = -0.0001 ± 0.00001, $Z = -8.48$, $P < 0.0001$; Fig. 5, Supplementary Table S1). The random effect colony ID accounted for 3.45% of the variance in the model, $R^2_{\text{marginal}} = 0.093$.

Finally, foragers stayed in the nest for longer as the colony became satiated. We found a significant positive relationship between forager duration in the nest ($N = 680$ forager return events) and the time at which the forager entered the nest, a proxy for colony satiation (LMM: estimate ± SE = 0.118 ± 0.032, $t = 3.698$, $P = 0.0002$; Fig. 6, Supplementary Table S1). The random effect colony ID accounted for 3.86% of the variance in the model, $R^2_{\text{marginal}} = 0.023$.

DISCUSSION

In support of our hypotheses, the size of higher-order interactions was impacted by colony hunger and it impacted the behaviour of both food recipients and foragers. When the colony was hungry, there were larger trophallaxis groups (Fig. 2), facilitating the spread of food to multiple unique individuals. The rate of food intake by recipients was slower when participating in large groups, as evident by the longer duration that food recipients spent in larger trophallaxis groups (Fig. 3), possibly because recipients had to share food with more individuals. Interestingly, and in contrast with our predictions, the size of a trophallactic group did not impact how quickly recipients joined the next trophallaxis group or left the nest (Fig. 4), suggesting that spending more time in a larger group allowed for sufficient food transfer, despite the potentially slower transmission. As the colony became satiated, foragers offloaded less food (integration went down; Fig. 5) and spent more time in the nest (Fig. 6). It is possible that the decrease in size of the trophallaxis groups over time increased the time it took foragers to offload food, resulting in spending more time in the nest, while potentially increasing the amount of food received by each recipient in the trophallaxis group.

Trophallaxis group size decreased over time as colonies became satiated. When colonies are hungry, they engage in more dyadic interactions than when they are fed (Guo et al., 2024; Sendova-Franks et al., 2010). Here we extend this observation to show that the size of higher-order interactions relates to colony hunger. Work on other ant species has found similar trends.

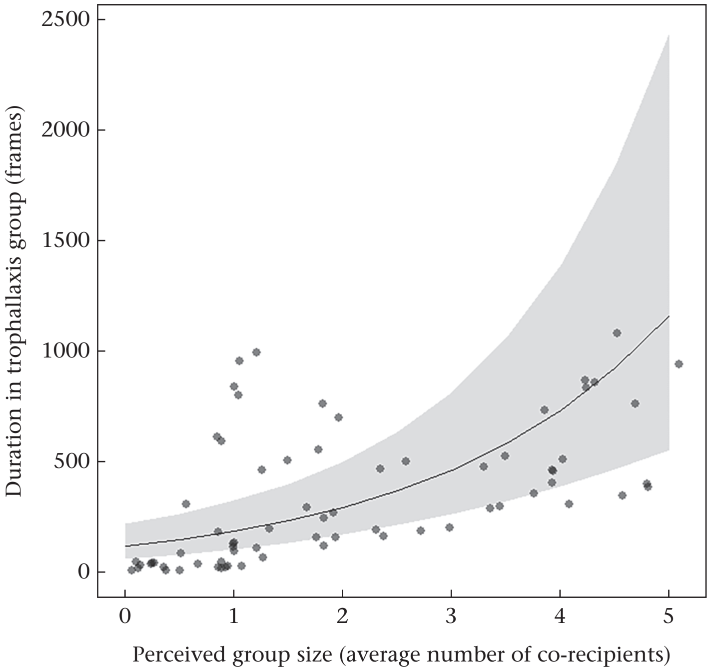

Figure 3. Duration in trophallaxis group relative to group size. For each food recipient (points, $N = 78$), the time it spent in a trophallaxis group relative to the size of the group that it experienced (i.e. the average number of co-recipients).

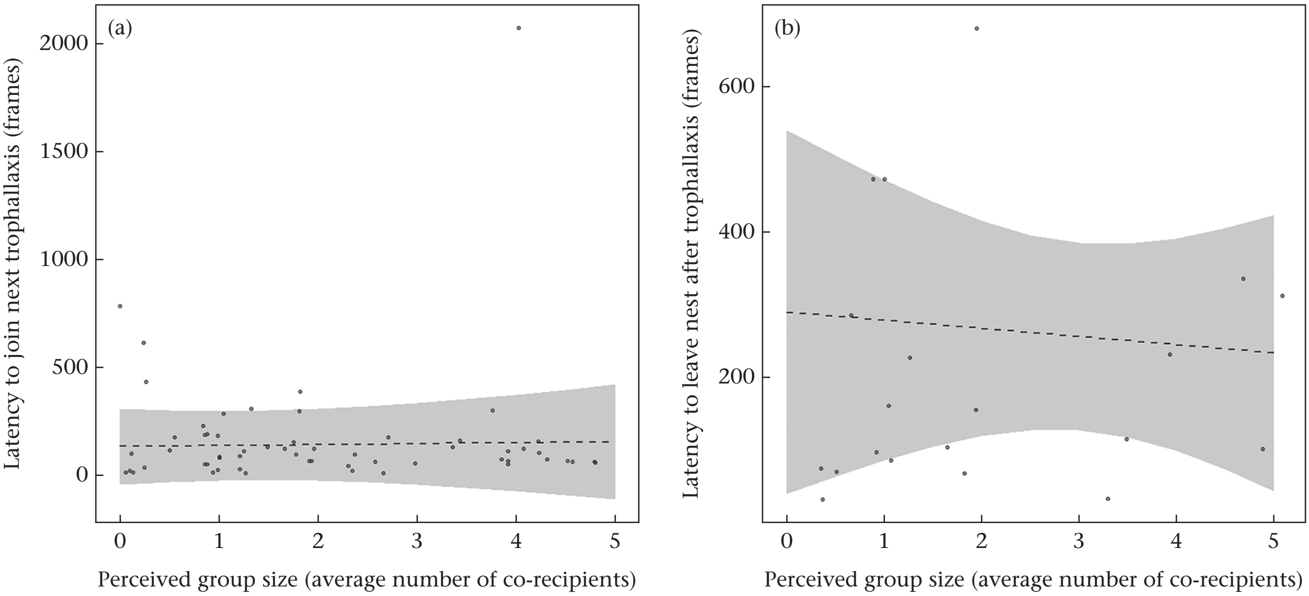

Figure 4. Trophallaxis group size relative to a food recipient's (points) latency to (a) join the next trophallaxis ($N = 58$) or (b) leave the nest ($N = 20$). Dashed lines indicate that the relationship was not statistically significant.

Wallis (1964) observed more trophallaxis ‘rosettes’ in starved colonies of *Formica fusca* compared to satiated colonies, and Howard and Tschinkel (1980) found that trophallaxis groups in *Solenopsis invicta* were largest when colonies were starved and that trophallaxis group size decreased over time. Hungry ants most likely crowd, or ‘mob’, returning foragers to obtain food, and as they become full, they are less likely to join trophallaxis events. As food spreads throughout the nest, the nonforaging workers are no longer hungry, or have no more space in their abdomens to store food, and are less likely to engage in trophallaxis events. It is possible that the crowding of returning foragers by hungry ants increases the efficiency of food transmission to all colony members. Theoretical work, inspired by ant trophallaxis behaviour, has shown that in supply chains, the dissemination of goods becomes more efficient when there are higher-order interactions compared to supply chains with no higher-order interactions (Hasenjager et al., 2024). Thus, higher-order interactions may play an important role in effectively disseminating food throughout an ant colony to reach all colony members, which is mediated by the size of the higher-order trophallactic groups. Whether effective food transfer depends on the speed of food offloading by the forager, food uptake by

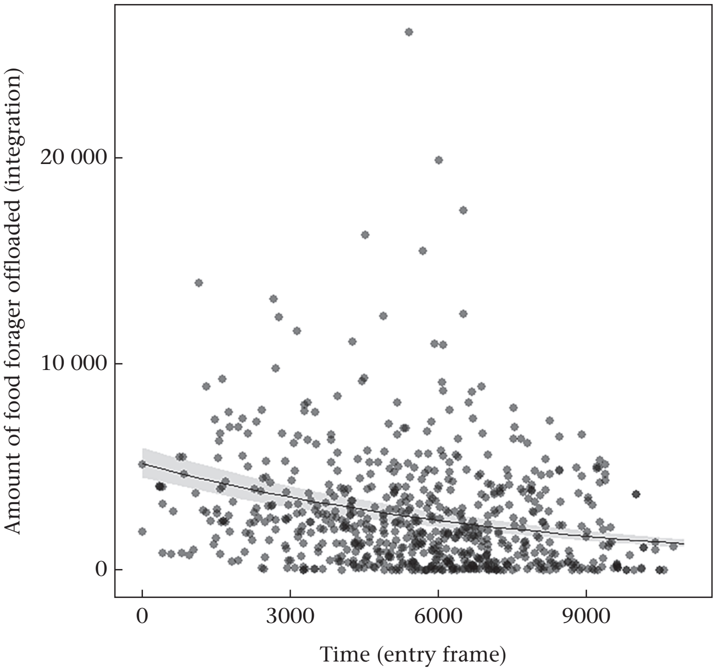

Figure 5. Food offloaded over time. For each forager return event tracked ($N = 680$ points), the integrated number of ants in trophallaxis groups throughout the forager's duration in the nest (Fig. 1e) relative to the time at which the forager entered the nest, a proxy for colony satiation.

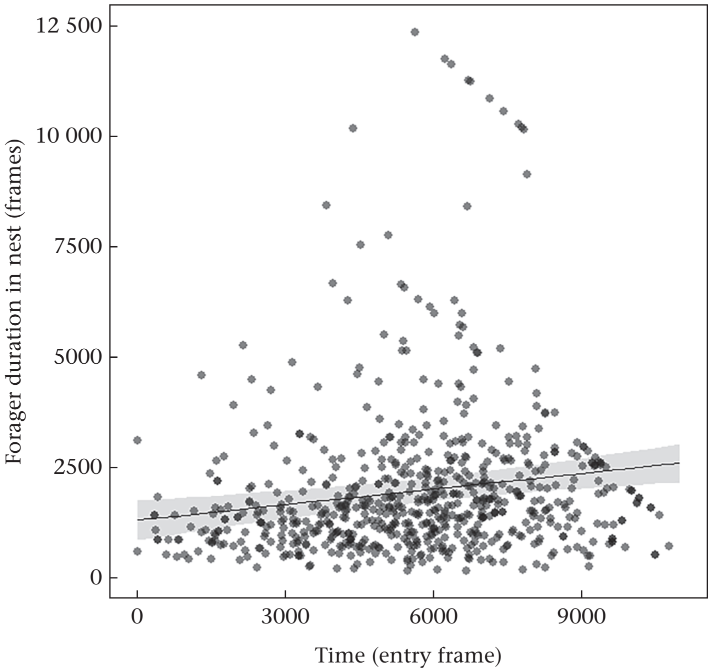

Figure 6. Forager duration in nest over time. The time a forager (points) spent in the nest relative to the time at which it returned to the nest ($N = 680$ forager return events), a proxy for colony satiation.

recipients or the way in which food is spread throughout the colony is a question for future research.

The number of hungry ants that can be fed simultaneously is likely limited by the physical constraints on the number of ants that can engage in a trophallaxis simultaneously. Furthermore, the amount of food a forager retrieves can limit the size of a trophallaxis group. However, because it takes time to offload food,; the physical constraints on the number of ants that can engage in a trophallaxis simultaneously is the more likely driver of maximum trophallactic group size. Because trophallaxis events occur in an approximately two-dimensional plane, the size, dimensions and geometric shape of an ant can limit the number of individuals that can gather around a returning forager. In *S. invicta*, trophallactic groups contain up to eight ants, with a mean of four ants (Howard & Tschinkel, 1980), while the largest trophallactic group in *F. fusca* was four ants (Wallis, 1964). In our study, across all eight colonies, the largest trophallaxis group observed had 15 individuals, but the mean of the maximum group size was 3.45 individuals (Fig. 1f), and the largest average group size a recipient perceived did not exceed five individuals in the three colonies in which we examined food receivers (Figs 3–4). Thus, it seems as though ant species with smaller bodies (e.g. *S. invicta* and *L. humile*) can reach larger trophallactic group sizes compared to ant species with larger bodies (*F. fusca*). Body size influences other collective outcomes in ants, such as the width of nest tunnels (O'Fallon et al., 2022), due to physical needs and constraints. Future comparative work examining the physical constraints on trophallactic group size and the relationship between body size and maximal trophallactic group size may shed light on the ability of different ant species to distribute food effectively to all colony members. Uncovering how ant body size mediates food dissemination dynamics within the nest may reveal important evolutionary constraints on collective foraging.

Food receivers spent more time in a trophallactic event if there were more individuals in the higher-order interaction. If we assume that all ants take up food from a returning forager at a similar rate, it seems as though food receivers spend more time in large trophallaxis groups to receive similar amounts of food as they would have received if they had to share it with fewer nestmates. Indeed, trophallaxis group size did not affect the receivers' latency to join a subsequent trophallaxis event, suggesting that receivers left a trophallaxis group sufficiently satiated, regardless of the size of the higher-order trophallaxis in which they participated. Thus, while higher-order trophallaxis events might increase the global efficiency of food dissemination at the colony level, it seems to decrease food uptake efficiency by individual recipient ants, because food recipients had to spend more time to obtain the same amount of food when engaged in larger trophallaxis groups. In ants, natural selection acts at the level of the colony because workers are sterile and only queens produce reproductive individuals, which are cared for by the sterile workers, and then leave the nest to establish new colonies. Therefore, it is likely that natural selection has favoured food dissemination mechanisms that prioritize group efficiency over individual efficiency.

The amount of food that foragers offloaded decreased with time. Based on the data collected in this study, this decrease could be explained either by foragers retrieving less food, or by receivers decreasing their engagement in trophallaxis. Foragers of Argentine ants use trophallaxis interactions with nestmates to decide whether or not to continue foraging for food (Miller & Pinter-Wollman, 2023). Thus, it is possible that, as a colony becomes satiated, the decrease in trophallaxis group size leads to fewer trophallaxis interactions for foragers, resulting in negative feedback that slows the colony's foraging activity or results in foragers bringing less food on each foraging trip. In natural settings, Argentine ant colonies adjust their collective foraging in response to environmental conditions, such as food availability (Page et al., 2018), by allocating exploratory individuals to unfamiliar locations (Madrzyk & Pinter-Wollman, 2023). These studies focus on foraging responses outside the nest. Our work in laboratory-based colonies expands our understanding of the feedback mechanisms that operate inside the nest to further adjust foraging dynamics. As trophallaxis group size decreased with colony satiation, foragers would decrease offloading rate and therefore need to spend more time in the nest to offload the food they retrieved, as the colony became satiated. If food receivers are driving the size of trophallaxis groups, then as they become satiated, they would be less likely to engage in trophallaxis, regardless of the amount of food foragers retrieve. Future work on larger groups, at longer timescales and in steady-state conditions might uncover whether foragers or food recipients determine the size of higher-order interactions, and thus the dynamics and efficacy of food retrieval and dissemination to all nestmates.

The ability to disseminate resources and communicate information effectively is important for all social systems. Trophallaxis can impact ants' ability to communicate information about nest identity because ants distinguish nestmates from non-nestmates based on cuticular hydrocarbons (CHC) (Lahav et al., 1999), and Dahbi et al. (1999) showed that trophallaxis affects the spread of CHCs, ensuring CHC uniformity throughout the colony. It is important for all ants in a colony to have the same CHC so they can identify each other as nestmates and be able to distinguish nestmates from non-nestmates. Thus, the ability of ants to communicate their colony membership relies on the uniform spread of goods throughout the colony via trophallaxis, and higher-order interactions can mediate such uniform spread. Trophallaxis is further used to exchange small proteins and hormones and thus can impact colony development (LeBoeuf et al., 2016). Exchange of food further provides important information about the nutritional value of food items, and potentially, information about the food source. Thus, the size of a higher-order interaction group can impact communication about food availability and quality. Furthermore, higher-order interactions facilitate rapid flow of resources and information. Expediting transmission dynamics can be critical in communication networks because the larger the size of the higher-order group, the faster the spread of the information that is being communicated. However, there might be a trade-off between the speed of information spread and its accuracy, if information is lost or degraded in higher-order interactions. In conclusion, examining higher-order interactions in the golden age of communication networks can uncover important dynamics of information flow that might be hidden when studying only dyadic interactions.

Author Contributions

Emily Surrell: Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Julie S. Miller:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Nina H. Fefferman:** Writing – review & editing, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Noa Pinter-Wollman:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Data Availability

Data and code are available as Supplementary Material.

Declaration of Interest

None.

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Supplementary Material

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