



A reproductive heir has a central position in multilayer social networks of paper wasps



Nitika Sharma ^{a, b, *} , Raghavendra Gadagkar ^b , Noa Pinter-Wollman ^a

^a Department of Ecology and Evolutionary Biology, University of California Los Angeles, U.S.A.

^b Centre for Ecological Sciences, Indian Institute of Science, Bengaluru, Karnataka, India

ARTICLE INFO

Article history:

Received 8 June 2021

Initial acceptance 12 July 2021

Final acceptance 5 October 2021

MS. number: A21-00356R

Keywords:

dominance
multilayer network
reproductive hierarchy
social insect
social interaction

Reproduction provides direct fitness benefits; therefore, it is important to determine why in some societies certain individuals have disproportionate access to reproductive opportunities. The social interactions that underlie reproductive hierarchies can occur in multiple situations, yet they are rarely studied in unison. The reproductive heir in the social wasp *Ropalidia marginata* is cryptic to human observers until the queen dies or disappears. To determine whether a reproductive heir can be identified through her behaviour, we examined four types of social situations: aggression, spatial overlap, trophallaxis and exchange of solid food. We asked whether accounting for all four social situations in a multilayer network provides more information about the structure of the society than examining each situation on its own or in an aggregate network that does not distinguish between social situations. We found that the reproductive heir had the most social interactions in the multilayer network but not in each of the social situations when considered separately or when all situations were lumped together. Our work demonstrates that multilayer networks can uncover new insights on social organization by explicitly considering the links between multiple situations of social interactions.

© 2021 The Author(s). Published by Elsevier Ltd on behalf of The Association for the Study of Animal Behaviour. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

An animal's social role in a group has important fitness consequences and is determined by many factors. High-ranking individuals may have reproductive benefits (Clutton-Brock, 1998; Nonacs, 2000). The social role, i.e. the part that high-ranking individuals play as members of a group, such as reproductive or foragers, may relate to body size (White et al., 2018), age (Fischer et al., 2008), spatial behaviour (Otani et al., 2020), dominance rank (Alberts, 2019) and other factors. Social interactions are an important mechanism for determining the role of an individual in a group in a wide range of species (Ellis, 1995; Shizuka & McDonald, 2015). Many social animals display aggressive interactions, which lead to dominance hierarchies that dictate access to resources, including reproductive opportunities (Fedigan, 1983; Majolo et al., 2012; Pusey et al., 1997; Stockley & Bro-Jørgensen, 2011). Because the role of individuals in a society can change over time and lead to differences in access to reproductive opportunities, it is important to uncover the mechanisms that underlie which individuals gain these fitness benefits and which do not, because of the resulting population level and evolutionary consequences (McEntire et al., 2021).

Animal social interactions are important for determining the roles that individuals play in a society. Different types of interactions, which occur in different social situations, underlie the formation of social relationships. For example, aggressive interactions may lead to avoidance (Hsu et al., 2006) and affiliative interactions may build social alliances (Berghänel et al., 2011); thus, together they create relationships that form a social structure that relies on multiple situations. Furthermore, interactions in different social situations (such as aggressive and affiliative interactions) may combine nonlinearly, contributing to the emergence of a group's social structure (Finn et al., 2019). The social structure of a group can determine the social roles of group members (Krause et al., 2010; Pinter-Wollman et al., 2014; Sih et al., 2009). The recent development of multilayer network analysis (Kivelä et al., 2014) provides new tools to examine how social interactions in different situations each contribute to the global social structure of a society and uncover the level to which social interactions in different situations integrate to form social dynamics (Finn, 2021; Finn et al., 2019; Hasenjager et al., 2021). Our work utilizes these novel tools to determine how integrating information from multiple social situations can improve our ability to uncover biologically meaningful social structures in complex societies.

* Corresponding author.

E-mail address: nitikasharma@ucla.edu (N. Sharma).

In social insects, there are among-individual differences in the roles that individuals play in the colony (Jandt et al., 2014; Jeanne, 1988) and only one or few individuals gain direct fitness benefits through reproduction (Leadbeater et al., 2011; Saha et al., 2018; Sumner et al., 2010). In some social insects, reproductive individuals can be replaced, leading to a reproductive hierarchy, which can be predicted by factors such as social interactions, age and physiology. For example, social interactions that determine dominance result in the most dominant female becoming the next reproductive (Beshers & Fewell, 2001; Ito & Higashi, 1991). Furthermore, the oldest individual (Cronin & Field, 2007; Strassmann & Meyer, 1983), the biggest individual (Turillazzi & Pardi, 1977), mated females or those with developed ovaries can become the new reproductive (Bhadra & Jordán, 2013; Liebig, 2010; Lommelen et al., 2006; Wheeler, 1986). However, in the paper wasp *Ropalidia marginata*, which we study here, the process of replacing the primary reproductive individual cannot be predicted in the presence of a fertile queen. When a queen is absent (e.g. if it dies), one worker, referred to as the ‘potential queen’, becomes the new queen after an almost 10-fold amplification in her aggression that subsides once her reproductive status is established (Bang & Gadagkar, 2012). The potential queen is a cryptic heir designate who does not arise because of scramble competition and is almost never challenged by nestmates in her brief hyperaggressive phase. The function of this short and unidirectional aggression is not to suppress competition but to accelerate ovarian development (Lamba et al., 2007). Thereafter, the established queen maintains her status as the sole reproductive through pheromonal control of worker reproduction rather than aggression (Bhadra et al., 2010; Mitra et al., 2011; Sumana & Gadagkar, 2003). Because females are multiply mated (polyandry), colonies can be inherited by sisters, daughters, cousins or nieces (serial polygyny), and colonies can be founded by a single foundress or by multiple unrelated foundresses. The reproductive heir in *R. marginata* is cryptic and cannot be identified in the presence of the queen based on her body size, aggressive behaviour, mating status or genetic relatedness (Chakraborty et al., 2018; Gadagkar, 2001). However, workers appear to know the potential queen’s identity and do not challenge her even though her identity is cryptic to us (Bhadra & Gadagkar, 2008). Worker age is a weak predictor of the potential queen (Bang & Gadagkar,

2012), and no links between social interactions and potential queen role have been identified in the three decades this species has been studied. We propose that because social relationships are a culmination of interactions in more than one situation, previous studies have overlooked the influence of social interactions on the role of a potential queen because each social situation was examined separately.

Here we examine whether considering multiple types of social situations improves our ability to detect important individuals in a society. We use new tools from multilayer network analysis to identify the cryptic potential queen in colonies of the wasp *R. marginata*. We integrate information about aggressive interactions, two types of food exchange and overlapping space use to determine the social role of individual wasps. We first hypothesized that there is individual variation among workers in a colony in their likelihood to interact with nestmates. We predicted that variation among individuals would be more apparent when interactions in all social situations were considered synergistically, in a multilayer network, compared to a simple additive aggregation of all interactions. Second, we hypothesized that an examination of multiple social situations would provide more information about specific roles of individuals in a colony compared to examining each social situation separately. We predicted that we would be able to identify the potential queen in the presence of the queen based on her position in the social network only when all social situations were considered in a multilayered structure. We did not expect to identify the potential queen when each social situation (aggression, spatial overlap and food sharing) was considered separately or when they were all aggregated additively into one network in which the social situations could not be distinguished.

METHODS

Study System and Experimental Procedure

To record the behaviour of wasps, we collected six colonies of *R. marginata* (Fig. 1) from different parts of Bangalore in India and allowed them to settle in a vespiary at the Indian Institute of Science, Bangalore. *Ropalidia marginata* is an obligately primitively eusocial tropical paper wasp that builds unenveloped nests

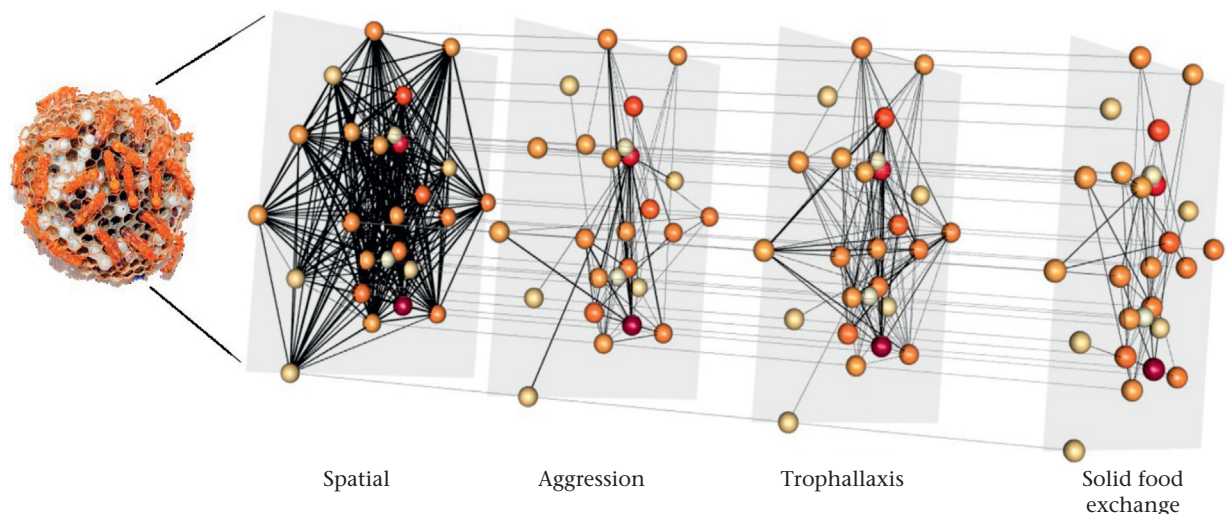


Figure 1. Multilayer network of colony v87 depicting different social situations as different layers. From right to left the situations include solid food exchange, trophallaxis, aggressive behaviour, and spatial overlap. Spheres (nodes) depict wasps, darker node colours indicate wasps with higher outdegree versatility. Black lines connecting nodes in the same layer (within a grey plane) are intralayer edges – line thickness corresponds to edge weight. Grey lines connecting nodes across layers are interlayer edges connecting the same individuals. To the left of the multilayer network is a photo of a colony of the primitively eusocial tropical paper wasp *R. marginata* (photo credit: Thresiamma Varghese).

on buildings or vegetation and have an aseasonal, perennial colony cycle. Level of sociality changes throughout the life of the colony, from early founding stage being solitary or subsocial to obligately eusocial after worker emergence (Gadagkar, 2001). Colonies used in this study were all from the obligately eusocial post worker emergence stage. The colonies we collected ranged in size from 21 to 36 individuals, which represents the range of colony sizes found in nature (average \pm SD = 21.9 ± 22.3 individuals) (Gadagkar, 2001). The vespiary had mesh windows to allow wasps to forage on their natural diet of lepidopteran larvae or spiders but prevent the entrance of large predators, such as *Vespa tropica*. Each wasp was colour-coded with a unique mark, using Testors paints (Testors Corporation, Rockford, IL, U.S.A.), for individual identification. After an initial acclimatization period of approximately 1 week, the colonies were videorecorded using Panasonic HD video cameras for three consecutive days from 0800 to 1800 hours daily. On the fourth day, we removed the queen to identify the potential queen. The potential queen was identified by her hyperaggression towards nestmates. In one of the colonies (v14), the potential queen was absent for 2 of the 3 days of observation before queen removal, leading to scarce information about her interactions; we therefore excluded this colony from our analysis and present data on five colonies. To obtain the spatial use of each wasp, we recorded the x – y coordinates of each individual on the nest every 6 min throughout the 30 h of each video using ImageJ (v1.50i, National Institutes of Health, Bethesda, MD, U.S.A., <http://rsbweb.nih.gov/ij/>).

Ethical Note

This work was conducted in accordance with the ASAB/ABS Guidelines for the use of animals in research. Wasps are invertebrates and so do not require special institutional permissions for experimentation. We handled wasps with extreme care. When translocating colonies into the vespiary we minimized disturbance by moving them at night when wasps are not active and keeping them in dark conditions to reduce agitation. Furthermore, we used nontoxic and quick-drying paint to individually mark the wasps. We did not anaesthetize or capture the wasps for marking; we waited until a wasp was standing still and marked it as quickly as possible, with no apparent disturbance. The study was noninvasive and involved observation of natural colonies that were free to fly in and out of the vespiary and hunt prey freely in their usual tropical habitat in southern India. Wasps were never swatted, even when handlers were stung.

Social Interactions

To quantify the social interactions of each individual, we recorded instances of aggressive encounters, trophallaxis and exchange of solid food. Each colony was videorecorded from 0800 to 1800 hours for three consecutive days amounting to 30 h of video recordings for each colony. We recorded all occurrences of behaviours in 5 min sessions. There were 150 such sessions over the 30 h of video, and sessions were separated by at least 1 min. Thus, all behaviours were sampled at the same rate and at the same sampling effort. Aggressive interactions included pecking, nibbling, biting, attack, chasing and ‘falling fight’, defined as two wasps being entangled and biting each other until they fell to the ground and continued fighting. Aggressive interactions were recorded as directed from the initiator of the aggressive behaviour to the receiver. Trophallaxis interactions were recorded when two individuals touched their mandibles and a droplet of liquid food was exchanged. A solid food exchange interaction was recorded when two individuals

transferred a piece of solid food, from the mandibles of one individual to the mandibles of the other. The direction of these interactions was from the receiver of food to the donor. The weight of an interaction between two individuals for both aggressive and food exchange networks was the number of interactions/h of behavioural observation. Because aggressive and food exchange interactions were infrequent, we used their frequency/h (i.e. the number of events divided by the total hours of observation: number of observation sessions \times 5 min \div 60).

To quantify spatial overlap, we used the proportion of overlap between the core areas of each pair of wasps. This measure is similar to home range overlap (Frère et al., 2010) and is a noninvasive proxy for assessing chemical communication through nonvolatile chemical signals that are rubbed on the nest surface and are common in *R. marginata* (Mittra, 2014; Sumana et al., 2008). A core area was defined as a region of the nest where a wasp spent 50% of her time based on a kernel density estimate calculated with the ‘adehabitat’ R package (Calenge, 2006). The proportion of core area overlap was assigned as the weight of an edge on a directed network that connected individuals in the spatial overlap network, as detailed in Sharma and Gadagkar (2019). Briefly, each edge weight in the spatial overlap network is the proportion of spatial overlap of one wasp’s core area by another wasp. The direction of these edges was assigned based on the denominator of this proportion: consider two wasps with respective core areas A and B, the area $A \cap B$ is the overlap and the value $A \cap B / A$ would give the weight of the edge that is directed from A to B. Similarly, $A \cap B / B$ would give the weight of the edge that is directed from B to A. For example, if wasp A has a core area of 0.4 cm^2 and wasp B has a core area of 0.1 cm^2 and the overlap between the two areas is 0.05, then the weight of the edge that is directed from A to B is 0.125 ($0.05/0.4$) and the weight of the edge directed from B to A is 0.5 ($0.05/0.1$). The difference in these two edge weights shows that a greater proportion of B’s space on the nest is shared with A as compared to A’s space on the nest that is shared with B. The spatial overlap between pairs of wasps was not correlated with interactions in other social situations ($r < 0.2$; Appendix, Fig. A1).

To examine all social interactions, without distinction of social situations, as is often done in other systems (Bhadra & Jordán, 2013; De Domenico et al., 2014; Holme & Saramäki, 2012; Liu et al., 2018; Wasserman & Faust, 1994), we created an aggregate network of the four types of interactions. To create the aggregate network, we summed the weights of all the edges in all four networks between each pair of individuals. For example, if individuals A and B were connected with an edge weight of 2 in the aggression situation and an edge weight of 3 in spatial overlap but not connected in the other two situations, the edge connecting A and B in the aggregate network would have a weight of 5.

In the multilayer network, each of the four social situations (aggression, trophallaxis, solid food exchange, spatial overlap) was depicted as a weighted and directed layer. Intralayer connections were as detailed above for each of the social situations. Interlayer edges in the multilayer network link the same individual across layers, with no variation in the weights of the interlayer edges (Fig. 1). In our analysis, we considered the weight of these interlayer edges as zero to avoid exaggerating the degree calculations based on multiple intraindividual edges of wasps across social situations.

Quantifying Social Network Structure

To determine whether the social network structure of *R. marginata* colonies can provide information about the role of different individuals in the society, we examined the degree distribution of both the multilayer and the aggregate interaction

Table 1
Glossary of network types and measures

	Description
Network type	
Spatial overlap	Proportion of core area (50% utilization distribution) of one individual overlapped by the core area of another individual
Aggression	Number of aggressive behaviours/h (pecking/ nibbling/ biting/ attack/ chasing/ falling fight) directed towards another individual
Trophallaxis	Number of exchanges of liquid food/h
Solid food exchange	Number of exchanges of solid food/h
Aggregate network	A network in which each interaction between two individuals is the sum of all interactions between them in the four social situations described above
Multilayer network	Collection of networks that form layers that are connected through interlayer edges. In this study, each layer represents a different social situation (Fig. 1)
Network measures	
Outdegree	Number of unique individuals interacted with, indicating the number of individuals one might socially influence
Outdegree versatility	In a multilayer network, sum of the number of unique individuals interacted with in each layer
Outstrength	Sum of weights of all outgoing interactions, indicating the magnitude of potential social influence on others
Outstrength versatility	In a multilayer network, sum of the weights of all outgoing interactions in all layers
Degree distribution	The probability density of the number of unique individuals each individual interacted with, used to quantify the spread of social interactions across all individuals

networks (Table 1, Fig. 2). To allow comparison of degree distributions across colonies, we standardized the degree distributions by dividing the degrees by the number of wasps in each colony. Degree is the number of unique individuals that a wasp interacts with and so the shape of the degree distribution provides information on the uniformity or variation in interaction patterns. The shape of the degree distribution of a network can reveal whether interactions are distributed uniformly across wasps or whether some wasps interact with more individuals than others. Skewness quantifies the shape of a distribution, with skewness values close to 0 indicating more uniform distributions. To compute the skewness of the degree distribution, we used the ‘moments’ package in R (Komsta & Novomestky, 2015). We quantified variation in the standardized degree distributions of the multilayer and the aggregate networks as the interquartile range (IQR).

Measures of Individual Centrality

Many centrality measures can be used to quantify the role of individuals in a society (Freeman, 1978; Wey et al., 2008), and versatility is a common measure of centrality in multilayer networks (De Domenico et al., 2015). To determine whether the potential queen can be identified in the presence of the queen based on her position in the social network, we examined biologically appropriate centrality measures, including outdegree and outstrength, of all wasps in the multilayer aggregate and in each situation-specific single-layer network.

Outdegree

Total number of edges pointing out of a node. This measure depicts the number of individuals that a wasp initiated interactions with; therefore, it quantifies how many other wasps an individual might have influenced. In the aggressive layer, the outdegree of a wasp is the number of individuals that received aggression from her. In food exchange interactions, outdegree is the number of individuals that a wasp received food from. In the spatial overlap network, outdegree is the number of individuals that a wasp's core area was overlapped by. In multilayer networks, the sum of outgoing edges of an individual in different social situations is often referred to as versatility (De Domenico et al., 2015), but here we refer to it as outdegree versatility. In the aggregate network, outdegree is the number of outgoing edges to nestmates, irrespective of social situation (Table 1).

Outdegree in aggregate networks is calculated after all social situations are combined into a single network. In this case, if two individuals interact in more than one social situation, their edge will be counted only once in the aggregate network outdegree. In the multilayer network, each interaction in each social situation is treated as a unique interaction and contributes to the multilayer outdegree versatility. So, if two individuals interact in two different social situations, the connection between them will be counted twice for the multilayer outdegree versatility. For example, if wasp X interacted with wasps A, B and C in social situation 1 and with wasps B, C and D in social situation 2, its outdegree centrality in the aggregate network would be four. The outdegree versatility in the multilayer for individual X would be six because it would sum the

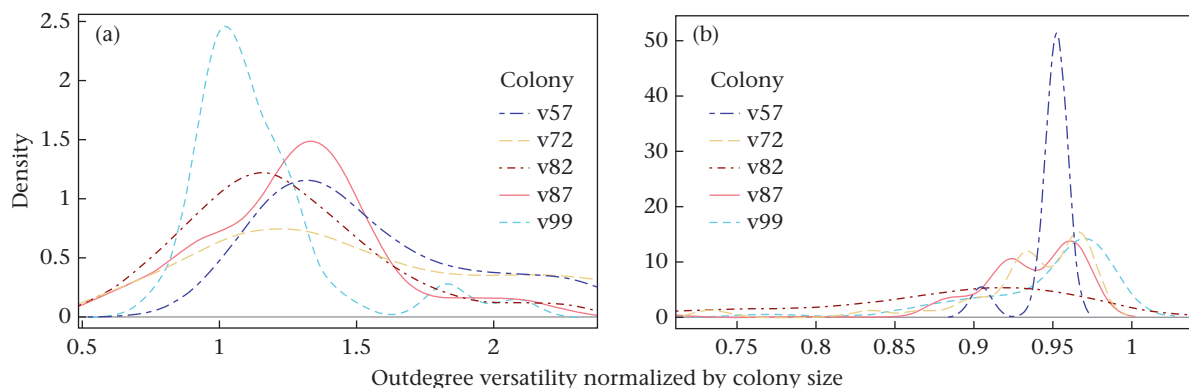


Figure 2. Distributions of outdegree for the (a) multilayer and (b) aggregated networks. Each of five colonies is depicted in a different colour and line type. The outdegree (X axis) was standardized by the total number of wasps in each layer for (a) and for the number of wasps in the colony for (b).

unique individuals in each social situation, i.e. counting interactions with B and C twice – once for each social situation in which X interacted with them.

Outstrength

Outstrength is the intensity of interactions that a node initiates, quantified as the sum of weights of all its outgoing edges. This measure provides information on how much of a behaviour an individual initiates; therefore, it quantifies how much influence an individual exerts on others. For aggression networks, outstrength of a wasp is the number of times it initiated an aggressive interaction towards its nestmates. Outstrength in the food exchange networks is the number of times a wasp received food. Outstrength in the spatial overlap network indicates the extent of a wasp's core area overlapped by other wasps. Both in the multilayer and aggregate network, outstrength is the sum of all outgoing edge weights of a node. We refer to outstrength in the multilayer network as outstrength versatility (Table 1).

The outstrength in the aggregate network is calculated as the sum of edge weights for each node after all social situations are combined into a single network. Prior to combining them into a single network, the weights of the edges in each layer are standardized by the largest edge weight in each layer. In the multilayer networks, the outstrength is first calculated for each node in each layer, based on the standardized edge weights. Then, all outstrengths for each node from all layers are summed to get the multilayer outstrength for each node. Overall, the magnitude of outstrength for each node in both the multilayer and aggregate networks is the same. However, the method in which they are computed – first aggregating and then computing outstrength (aggregate) or first computing outstrengths and then summing them (multilayer) – results in different outputs in the reference models described below.

To determine whether the potential queen can be identified based on her network centrality (outdegree or outstrength) in the presence of the queen, we compared these network measures of the potential queen to those expected by chance. To create a chance reference distribution that preserves the observed network structure but shuffles the position of each individual in the network, we ran 1000 simulations in which we permuted node identities on the observed network, i.e. only node identities (IDs) were shuffled, not edges (Hobson et al., 2021). For the multilayer network, we shuffled

node IDs only within a layer and not across layers to account for the absence of interactions by some wasps in certain social situations. For each permuted network, we calculated the versatility/centrality measures of the potential queen. If the observed network measure fell outside the 95% confidence interval of the distribution of the measure from the 1000 permuted networks, we concluded that the observed network measure of the potential queen was not a result of chance alone. All analyses were conducted in R v.3.6 (R Core Team, 2013); see Supplementary materials for code.

RESULTS

Do Individuals Differ in Their Likelihood to Interact?

There was variation among individuals in their likelihood to interact when all situations were considered in a multilayer network. The outdegree distribution of the multilayer networks was positively skewed for all colonies, with skewness ranging from 0.33 to 1.92 (Fig. 2a). Thus, most individuals interacted with few individuals and few individuals interacted with a large number of nestmates. In contrast, the outdegree distribution of the aggregated networks was negatively skewed, with skewness ranging from -1.43 to -2.76 (Fig. 2b), meaning that most individuals interacted with many other individuals in the colony. The variance in outdegree, measured as the interquartile range (IQR; low IQR indicates low variation), was consistently greater in multilayer networks compared to aggregate networks for all five colonies (v57: $IQR_{mul} = 11, IQR_{agg} = 0$; v82: $IQR_{mul} = 10, IQR_{agg} = 3$; v87: $IQR_{mul} = 11, IQR_{agg} = 1$; v72: $IQR_{mul} = 29, IQR_{agg} = 1$; and v99: $IQR_{mul} = 8.5, IQR_{agg} = 2$). For more information on the summary statistics of the aggregate and multilayer networks see Appendix, Tables A2–A4A.

Can the Potential Queen be Identified in the Presence of the Queen Based on Her Interactions in the Social Network?

The centrality of the potential queen before the queen was removed was high according to outstrength and outdegree versatility in the multilayer networks (Fig. 3, Appendix, Figs A2–A5). The potential queen either had the highest or second-highest outdegree versatility in a multilayer network before the queen was removed (Fig. 3a), and this position was significantly higher than expected at

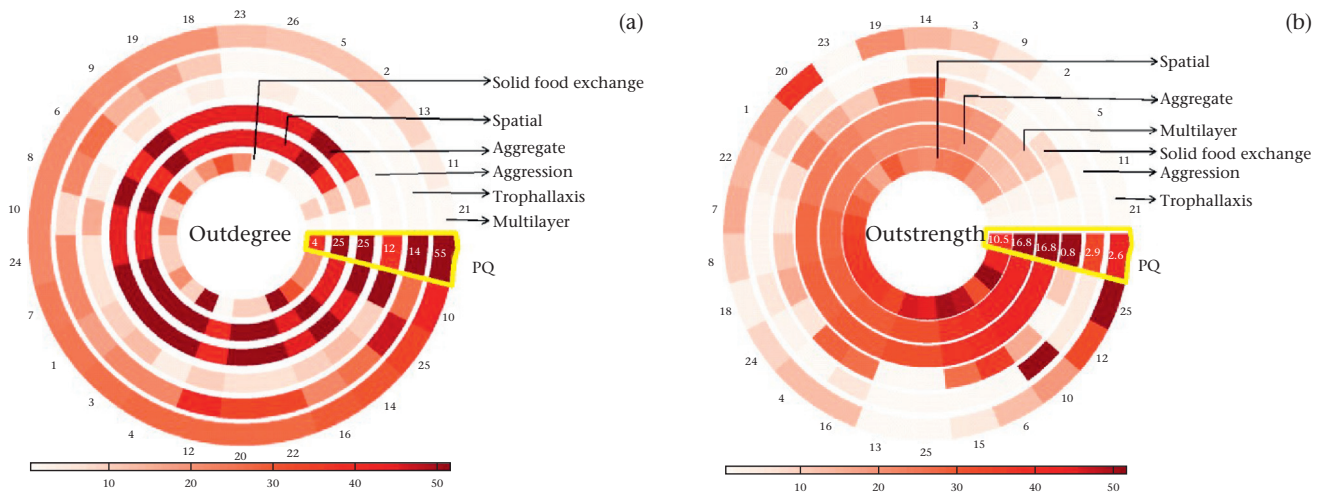


Figure 3. Annular representation of the (a) outdegree and (b) outstrength centrality or versatility of individuals in colony v87. Darker colours indicate a higher rank of outdegree or outstrength while lighter shades of red indicate lower ranks. Each slice in a ring denotes an individual and the potential queen (PQ) is highlighted with a yellow outline. Results for the other four colonies are given in the Appendix (Figs A2–A5).

random (Fig. 4a). However, the potential queen was not the most central in the single-layer spatial network, nor did she show aggression to the most individuals or receive food from significantly more wasps than any other individual. The potential queen was not the most central individual in the aggregate network – she did not have the highest outdegree centrality in the aggregate network (Fig. 3a), and her position was not significantly different from chance expectation (Fig. 4b). The potential queen's occupation of a highly connected position in the multilayer network in all five colonies was not by chance. According to the randomization test, the observed outdegree versatility of the potential queen in all colonies was significantly greater than her expected outdegree versatility based on the reference models in which network structure was maintained and node IDs were shuffled (Fig. 4a). In contrast, the observed outdegree of the potential queen in the aggregate network and in the single-layer networks (spatial

overlap, aggression, trophallaxis, solid food exchange) did not significantly differ from the outdegree expected by chance in any of the colonies (Fig. 4b–f). In the trophallaxis network, the potential queen appeared to consistently have the largest number of partners, but her central position could still be explained by chance according to the randomization test, i.e. her observed outdegree in the trophallaxis layer did not fall beyond the 95% CI of the randomized outdegrees (Fig. 4e).

The potential queen had the largest outstrength versatility in the multilayer network of four of the five colonies and the second largest in the fifth colony, indicating that the potential queen's interactions were not only numerous but also intense (Fig. 3b, Appendix, Figs A2–A5). The outstrength of the multilayer and aggregate networks was identical because it was the sum of weights of all edges that emerged from a node. However, the randomization tests resulted in different outcomes because of the

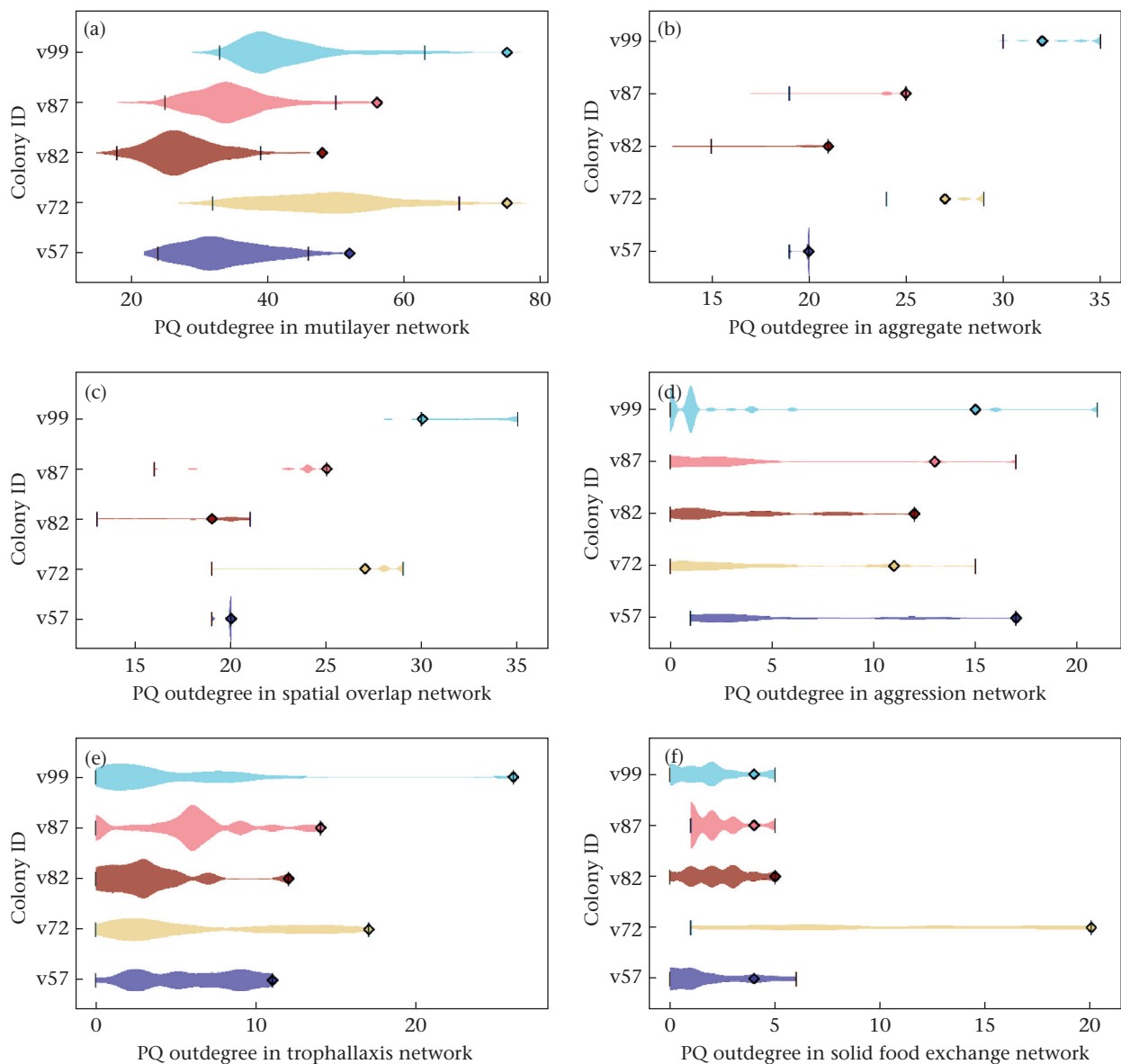


Figure 4. Observed and randomized outdegrees of the potential queen (PQ) in the (a) multilayer, (b) aggregate, (c) spatial overlap, (d) aggression, (e) trophallaxis and (f) solid food exchange networks. Observed outdegree values are depicted as diamonds. Distributions depict simulated outdegree based on 1000 runs in which we randomized node identities (IDs) while maintaining the network structure (edges). Vertical black lines indicate 95% quantiles of the randomized distribution. Each row within a plot corresponds to one of the five colonies.

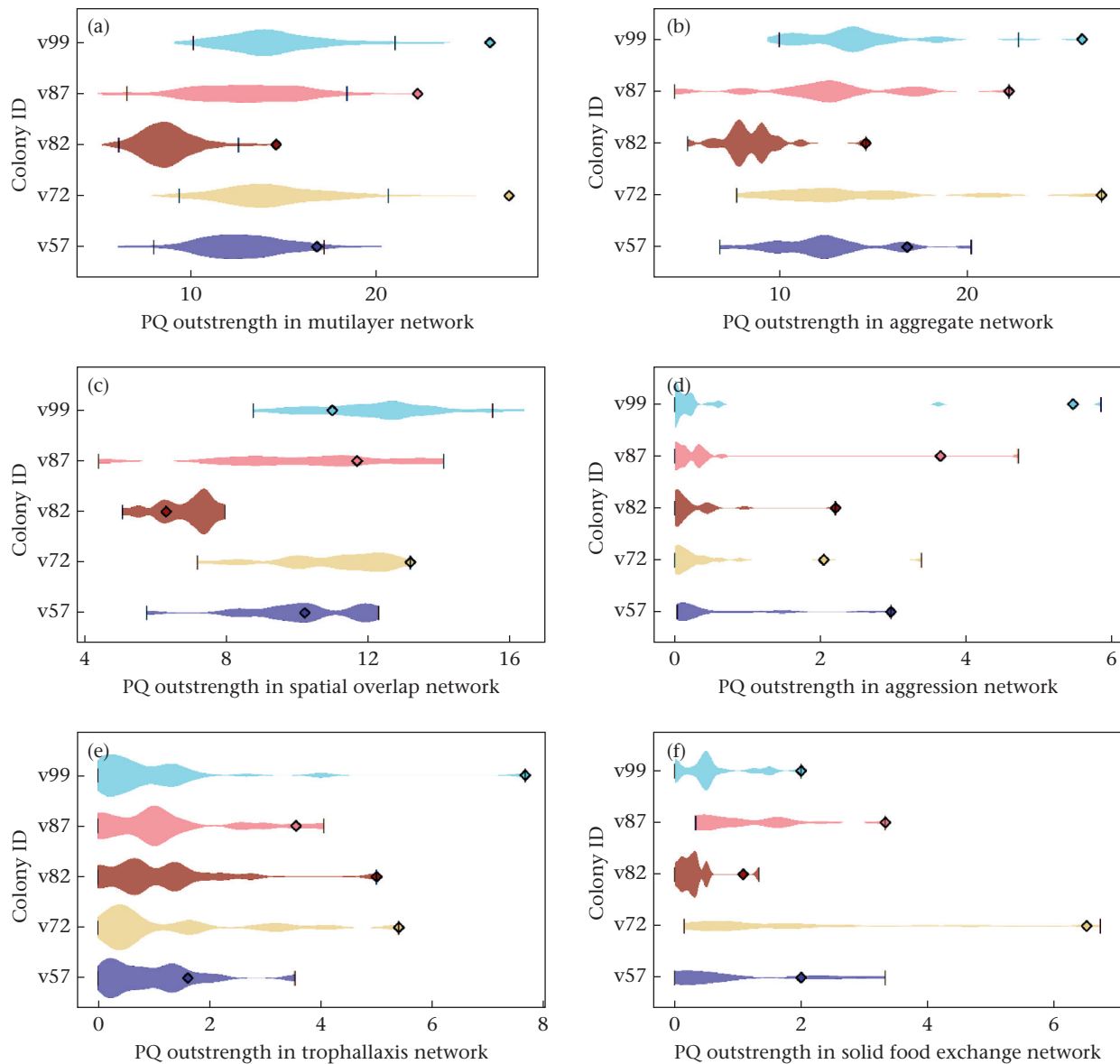


Figure 5. Observed and simulated outstrengths of the potential queen (PQ) in the (a) multilayer, (b) aggregate, (c) spatial overlap, (d) aggression, (e) trophallaxis and (f) solid food exchange networks. Observed outstrength values are depicted as diamonds. Distributions depict simulated outstrength based on 1000 runs in which we randomized node identities (IDs) while maintaining the network structure (edges). Vertical black lines indicate 95% quantiles of the randomized distribution. Each row within a plot corresponds to one of the five colonies.

way in which outstrength was computed for the two different networks, as detailed in the Methods. Randomization tests show that the outstrength versatility of the potential queen was significantly greater than expected by chance alone for the multilayer networks for four of the five colonies (Fig. 5a). However, in the aggregate network, the outstrength of the potential queen was significantly greater than random only in one colony (Fig. 5b). Furthermore, the potential queen's outstrength was not different than expected by chance in any of the single-layer networks (Fig. 5c–f).

DISCUSSION

The multilayer network analysis allowed us to identify a highly central and influential individual, the potential queen, who could

not be identified based on any of four social situations separately. We were able to identify the potential queen in the presence of a queen based on her social interactions only when considering the four social situations together in a multilayer network (Figs 3, 4a and 5a) that had high individual variation in interactions (Fig. 2a). However, we were not able to identify the potential queen when collapsing the social situations into one aggregate network (Figs 3, 4b and 5b) that had little variation among individuals in interactions (Fig. 2b), nor when each situation was examined separately (Figs 3, 4c–f, 5c–f). The potential queen's number of partners (outdegree versatility; Fig. 4a) and the intensity of her interactions (outstrength versatility; Fig. 5a) in the multilayer network were significantly greater than expected by chance alone. In contrast, the potential queen's number of partners and her interaction strength were not significantly different from chance in any single social

situation or in the aggregate networks of the five colonies (Figs 4b–f, 5b–f). Although the magnitude of outstrength was the same in the multilayer and aggregate networks, the potential queen's outstrength was significantly higher than expected by chance in four of five colonies in the multilayer network but only for one colony in the aggregate networks. This difference arose from the way in which the randomizations were implemented for each type of networks – shuffling IDs within each of the four layers in the multilayer network versus shuffling all IDs in the aggregate network. The probability of the potential queen having an outstrength greater than expected by chance in the nearly saturated aggregate network was low due to limited variation among individuals in the number of interactions in this network. In contrast, the multilayer network preserved high individual variation in interactions.

The multilayer network approach outperformed the aggregate network because it retained more information on individual variation in interactions. The multilayer network degree distributions were right-skewed, with few highly interactive individuals and most individuals with few interactions (Fig. 2a). However, the degree distributions of the aggregate networks were left-skewed (Fig. 2b), with most individuals interacting with almost all others because all social situations were collapsed together, rather than kept separate as in the multilayer network. High variation in interactions allowed us to identify particular individuals that differed from others, a task that is not possible when there is little variation in interactions. Right-skewed degree distributions, like the ones we observed in the multilayer network, have been documented in sparse or short-term networks (e.g. ants: Pinter-Wollman et al., 2011; birds: Ryder et al., 2008; lions, *Panthera leo*: Craft et al., 2009). Left-skewed degree distributions, similar to those we observed in the aggregate networks, have been seen in proximity networks of nonterritorial animals (e.g. Grévy's zebra, *Equus grevyi*, and onager, *Equus hemionus*: Sundaresan et al., 2007; Tasmanian devil, *Sarcophilus harrisi*: Hamede et al., 2009). Interaction distributions become less right-skewed (i.e. more uniformly distributed across individuals) with increasing observation durations and with number of social situations examined. However, in this study, all behaviours were recorded at the same effort. Furthermore, in *R. marginata*, there are certain interactions among particular pairs of wasps that have never been recorded, no matter how long the animals were observed, because those behaviours do not occur (Chandrashekara & Gadagkar, 1991). A multilayer network provides a way to maintain information about individual variation in interactions when many social situations are examined, allowing opportunities to identify individuals with important social roles.

Each social situation provided slightly different information, which when assembled together in a multilayer network, revealed new information that could not be obtained from each layer separately. The potential queen could not be identified if any one of the four social situations was missing (Appendix, Figs A6–A13). Removing the trophallactic and aggressive interactions from the multilayer networks had an especially high impact on the ability to detect the potential queen (Appendix, Figs A8–A11), suggesting that these two social situations are particularly important. It is possible that trophallactic interactions had a substantial influence on our ability to detect the potential queen because the potential queen had many trophallactic partners (Fig. 3a). In contrast, queens have significantly fewer trophallaxis partners than other individuals in the colony, possibly to avoid transmission of infectious

agents through food exchange (Sharma & Gadagkar, 2019). Interactions in the aggression situation may be important for identifying the potential queen due to the role of such interactions in ovarian development in *R. marginata* (Lamba et al., 2007). Previous work showed that spatial overlap – akin to home range overlap – with the queen's core use area is not sufficient for identifying the potential queen (Sharma & Gadagkar, 2019). While examining the spatial network on its own did not provide predictive information about the identity of the potential queen (Figs 3, 4c and 5c), spatial overlap with many nestmates may facilitate chemical communication among individuals (Mitra, 2014; Sumana et al., 2008) even when they do not interact directly in situations like aggression and food exchange. Solid food exchange interactions were the sparsest of all situations and may indicate rare, yet unique, and therefore important, interactions between individuals in the colony (Appendix, Figs A12a, A13a, Table A1).

Our work resolves the long-standing mystery of who the potential queen is, in colonies of *R. marginata*. Previous work on this species was unable to predict who would become a potential queen before the queen was removed based on the wasps' social interactions, including aggressive interactions and food exchange (Bhadra & Jordán, 2013; Bhadra et al., 2009), or spatial overlap (Sharma & Gadagkar, 2019). Our method of identifying the potential queen by combining multiple social situations in a multilayer framework may provide further understanding of how queens of *R. marginata* monopolize reproduction through social behaviour (Gadagkar, 2001; Sumana & Gadagkar, 2003). In a sister species, *Ropalidia cyathiformis*, the potential queen possibly signals her status as an unchallenged heir through aggression and can thus be identified in the presence of the queen (Bhadra & Jordán, 2013). It seems as though the potential queen in *R. marginata* colonies uses a combination of multiple social situations to signal her heir status. Further studies are needed to uncover how workers in colonies of *R. marginata* integrate information from multiple social situations to identify the potential queen as the successor of the queen. Our findings emphasize the need for studying behaviour in multiple situations to improve our understanding of complex societies.

Our work shows that examining social interactions in a manner that accounts explicitly for more than one social situation can provide important information about a social system that cannot be revealed otherwise. This novel development is important for the study of social behaviour because animals rarely interact with one another in only one social situation, yet studies of social behaviour often overlook this complexity. The development and use of new analytic tools to identify prominent individuals in a society may help uncover important social roles that have thus far been overlooked, despite their substantial impact on the ecology and evolution of social systems.

Data and Code Access

The raw data and R codes supporting this study have been provided as [supplementary material](#).

Funding

This work was supported by grants to R.G. from the Ministry of Environment, Forests and Climate Change, Government of India, Department of Science and Technology (including DST-FIST Program), the Science and Engineering Research Board (SERB), Department of Science and Technology, Department of

Biotechnology (including DBT-IISc Partnership Program) and Council of Scientific and Industrial Research. We further acknowledge support to N.P.W. from the U.S. National Institutes of Health (NIH grant GM115509) and the U.S. National Science Foundation (NSF grant 2015662).

Author Contributions

N.S., N.P.W. and R.G. designed the study. N.S. collected and extracted the data, conducted the analysis, and wrote the first manuscript draft. N.S. and N.P.W. designed the simulations and outlined the manuscript. N.P.W. heavily edited various versions of the manuscript. All authors commented on the manuscript.

Declarations of Interest

None.

Acknowledgments

We thank David Fisher and Alice Schwarze for help with troubleshooting during MuxViz set-up and members of the Pinter-Wollman lab, Julie Miller, Natalie Lemanski, Sean O'Fallon, Ana Rubio, Gabby Najm, Eva Horna Lowell, Alejandra Gamboa, Amanda Klingler, Bryce Barbee and Collette White, for valuable comments on the manuscript. We also thank the two referees whose comments improved the manuscript.

Supplementary Material

Supplementary material associated with this article is available, in the online version, at <https://doi.org/10.1016/j.anbehav.2021.12.011>.

References

- Alberts, S. C. (2019). Social influences on survival and reproduction: Insights from a long-term study of wild baboons. *Journal of Animal Ecology*, 88(1), 47–66. <https://doi.org/10.1111/1365-2656.12887>
- Bang, A., & Gadagkar, R. (2012). Reproductive queue without overt conflict in the primitively eusocial wasp *Ropalidia marginata*. *Proceedings of the National Academy of Sciences of the United States of America*, 109(36), 14494–14499. <https://doi.org/10.1073/pnas.1212698109>
- Berghänel, A., Ostner, J., Schröder, U., & Schülke, O. (2011). Social bonds predict future cooperation in male Barbary macaques, *Macaca sylvanus*. *Animal Behaviour*, 81(6), 1109–1116. <https://doi.org/10.1016/j.anbehav.2011.02.009>
- Beshers, S. N., & Fewell, J. H. (2001). Models of division of labor in social insects. *Annual Review of Entomology*, 46, 413–440.
- Bhadra, A., & Gadagkar, R. (2008). We know that the wasps 'know': Cryptic successors to the queen in *Ropalidia marginata*. *Biology Letters*, 4(6), 634–637. <https://doi.org/10.1098/rsbl.2008.0455>
- Bhadra, A., & Jordán, F. (2013). Cryptic successors unrevealed even by network analysis: A comparative study of two paper wasp species. *Network Biology*, 3(2), 54–66. <https://doi.org/10.0000/issn-2220-8879-networkbiology-2013-v3-0005>
- Bhadra, A., Jordán, F., Sumana, A., Deshpande, S. A., & Gadagkar, R. (2009). A comparative social network analysis of wasp colonies and classrooms: Linking network structure to functioning. *Ecological Complexity*, 6(1), 48–55. <https://doi.org/10.1016/j.ecocom.2008.10.004>
- Bhadra, A., Mitra, A., Deshpande, S. A., Chandrasekhar, K., Naik, D. G., Hefetz, A., & Gadagkar, R. (2010). Regulation of reproduction in the primitively eusocial wasp *Ropalidia marginata*: On the trail of the queen pheromone. *Journal of Chemical Ecology*, 36(4), 424–431. <https://doi.org/10.1007/s10886-010-9770-x>
- Calenge, C. (2006). The package 'adehabitat' for the R software: A tool for the analysis of space and habitat use by animals. *Ecological Modelling*, 197(3–4), 516–519. <https://doi.org/10.1016/j.ecolmodel.2006.03.017>
- Chakraborty, S., Shukla, S. P., Arunkumar, K. P., Nagaraju, J., & Gadagkar, R. (2018). Genetic relatedness does not predict the queen's successors in the primitively eusocial wasp, *Ropalidia marginata*. *Journal of Genetics*, 97(2), 429–438. <https://doi.org/10.1007/s12041-018-0926-8>
- Chandrashekhara, K., & Gadagkar, R. (1991). Behavioural castes, dominance and division of labour in a primitively eusocial wasp. *Ethology*, 87(3–4), 269–283. <https://doi.org/10.1111/j.1439-0310.1991.tb00252.x>
- Clutton-Brock, T. H. (1998). Reproductive skew, concessions and limited control. *Science*, 13(7), 288–292.
- Craft, M. E., Volz, E., Packer, C., & Meyers, L. A. (2009). Distinguishing epidemic waves from disease spillover in a wildlife population. *Proceedings of the Royal Society B: Biological Sciences*, 276(1663), 1777–1785. <https://doi.org/10.1098/rspb.2008.1636>
- Cronin, A. L., & Field, J. (2007). Social aggression in an age-dependent dominance hierarchy. *Behaviour*, 144(7), 753–765. <https://doi.org/10.1163/156853907781476436>
- De Domenico, M., Solé-Ribalta, A., Cozzo, E., Kivela, M., Moreno, Y., Porter, M. A., et al. (2014). Mathematical formulation of multilayer networks. *Physical Review X*, 3(4), 1–15. <https://doi.org/10.1103/PhysRevX.3.041022>
- De Domenico, M., Solé-Ribalta, A., Omodei, E., Gómez, S., & Arenas, A. (2015). Ranking in interconnected multilayer networks reveals versatile nodes. *Nature Communications*, 6, 6868. <https://doi.org/10.1038/ncomms7868>
- Ellis, L. (1995). Dominance and reproductive success among nonhuman animals: A cross-species comparison. *Ethology and Sociobiology*, 16, 257–333. [https://doi.org/10.1016/0162-3095\(95\)00050-U](https://doi.org/10.1016/0162-3095(95)00050-U)
- Fedigan, L. M. (1983). Dominance and reproductive success in primates. *American Journal of Physical Anthropology*, 26(S1), 91–129. <https://doi.org/10.1002/ajpa.1330260506>
- Finn, K. R. (2021). Multilayer network analyses as a toolkit for measuring social structure. *Current Zoology*, 67(1), 81–99. <https://doi.org/10.1093/cz/zoaa079>
- Finn, K. R., Silk, M. J., Porter, M. A., & Pinter-Wollman, N. (2019). The use of multilayer network analysis in animal behaviour. *Animal Behaviour*, 149, 7–22. <https://doi.org/10.1016/j.anbehav.2018.12.016>
- Fischer, K., Perlick, J., & Galetz, T. (2008). Residual reproductive value and male mating success: Older males do better. *Proceedings of the Royal Society B: Biological Sciences*, 275(1642), 1517–1524. <https://doi.org/10.1098/rspb.2007.1455>
- Freeman, L. C. (1978). Centrality in social networks conceptual clarification. *Social Networks*, 1(3), 215–239. [https://doi.org/10.1016/0378-8733\(78\)90021-7](https://doi.org/10.1016/0378-8733(78)90021-7)
- Frère, C. H., Krützen, M., Mann, J., Watson-Capps, J. J., Tsai, Y. J., Patterson, E. M., et al. (2010). Home range overlap, matrilineal and biparental kinship drive female associations in bottlenose dolphins. *Animal Behaviour*, 80(3), 481–486. <https://doi.org/10.1016/j.anbehav.2010.06.007>
- Gadagkar, R. (2001). *The social biology of Ropalidia marginata: Toward understanding the evolution of eusociality*. Cambridge, MA: Harvard University Press. <https://doi.org/10.1007/bf02715908>
- Hamede, R. K., Bashford, J., McCallum, H., & Jones, M. (2009). Contact networks in a wild Tasmanian devil (*Sarcophilus harrisii*) population: Using social network analysis to reveal seasonal variability in social behaviour and its implications for transmission of devil facial tumour disease. *Ecology Letters*, 12(11), 1147–1157. <https://doi.org/10.1111/j.1461-0248.2009.01370.x>
- Hasenjager, M. J., Silk, M., & Fisher, D. N. (2021). Multilayer network analysis: New opportunities and challenges for studying animal social systems. *Current Zoology*, 67(1), 45–48. <https://doi.org/10.1093/cz/zoab006>
- Hobson, E. A., Silk, M. J., Fefferman, N. H., Larremore, D. B., Rombach, P., Shai, S., & Pinter-Wollman, N. (2021). A guide to choosing and implementing reference models for social network analysis. *Biological Reviews*, 96(6), 2716–2734. <https://doi.org/10.1111/brv.12775>
- Holme, P., & Saramäki, J. (2012). Temporal networks. *Physics Reports*, 519(3), 97–125. <https://doi.org/10.1016/j.physrep.2012.03.001>
- Hsu, Y., Earley, R. L., & Wolf, L. L. (2006). Modulation of aggressive behaviour by fighting experience: Mechanisms and contest outcomes. *Biological Reviews*, 81(1), 33–74. <https://doi.org/10.1017/S146479310500686X>
- Ito, F., & Higashi, S. (1991). A linear dominance hierarchy regulating reproduction and polyethism of the queenless ant *Pachycondyla sublaevis*. *Naturwissenschaften*, 78(2), 80–82. <https://doi.org/10.1007/BF01206263>
- Jandt, J. M., Bengston, S., Pinter-Wollman, N., Pruitt, J. N., Raine, N. E., Dornhaus, A., & Sih, A. (2014). Behavioural syndromes and social insects: Personality at multiple levels. *Biological Reviews*, 89(1), 48–67. <https://doi.org/10.1111/brv.12042>
- Jeanne, R. L. (1988). *Interindividual behavioral variability in social insects*. Boulder, CO: Westview Press.
- Kivela, M., Arenas, A., Barthelemy, M., Gleeson, J. P., Moreno, Y., & Porter, M. A. (2014). Multilayer networks. *Journal of Complex Networks*, 2(3), 203–271. <https://doi.org/10.1093/comnet/cnu016>
- Komsta, L., & Novomestky, F. (2015). Moments, cumulants, skewness, kurtosis and related tests (R package Version 14). <http://www.r-project.org>. <http://www.komsta.net/>
- Krause, J., James, R., & Croft, D. P. (2010). Personality in the context of social networks. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1560), 4099–4106. <https://doi.org/10.1098/rstb.2010.0216>

- Lamba, S., Kazi, Y. C., Deshpande, S., Natesh, M., Bhadra, A., & Gadagkar, R. (2007). A possible novel function of dominance behaviour in queen-less colonies of the primitively eusocial wasp *Ropalidia marginata*. *Behavioural Processes*, 74(3), 351–356. <https://doi.org/10.1016/j.beproc.2006.12.003>
- Leadbeater, E., Carruthers, J. M., Green, J. P., Rosser, N. S., & Field, J. (2011). Nest inheritance is the missing source of direct fitness in a primitively eusocial insect. *Science*, 333(6044), 874–876. <https://doi.org/10.1126/science.1205140>
- Liebig, J. (2010). Hydrocarbon profiles indicate fertility and dominance status in ant, bee, and wasp colonies. In G. J. Blomquist, & A.-G. Bagnères (Eds.), *Insect hydrocarbons: Biology, biochemistry, and chemical ecology* (pp. 254–281). Cambridge, U.K.: Cambridge University Press.
- Liu, Y., Safavi, T., Dighe, A., & Koutra, D. (2018). Graph summarization methods and applications: A survey. *ACM Computing Surveys*, 51(3), 62. <https://doi.org/10.1145/3186727>
- Lommelen, E., Johnson, C. A., Drijfhout, F. P., Billen, J., Wenseleers, T., & Gobin, B. (2006). Cuticular hydrocarbons provide reliable cues of fertility in the ant *Gnamptogenys striatula*. *Journal of Chemical Ecology*, 32(9), 2023–2034. <https://doi.org/10.1007/s10886-006-9126-8>
- Majolo, B., Lehmann, J., De Bortoli Vizoli, A., & Schino, G. (2012). Fitness-related benefits of dominance in primates. *American Journal of Physical Anthropology*, 147(4), 652–660. <https://doi.org/10.1002/ajpa.22031>
- McEntire, D. K., Gage, M., Gawne, R., Hadfield, G. M., Hulshof, C., Johnson, A. M., et al. (2021). Understanding drivers of variation and predicting variability across levels of biological organization. *Integrative and Comparative Biology*. <https://doi.org/10.1093/icb/ibab160>
- Mitra, A. (2014). Queen pheromone and monopoly of reproduction by the queen in the social wasp *Ropalidia marginata*. *Proceedings of the Indian National Science Academy*, 80(5), 1025–1044. <https://doi.org/10.16943/ptinsa/2014/v80i5/47971>
- Mitra, A., Saha, P., Chaouideer, M. E., Bhadra, A., & Gadagkar, R. (2011). Chemical communication in *Ropalidia marginata*: Dufour's gland contains queen signal that is perceived across colonies and does not contain colony signal. *Journal of Insect Physiology*, 57(2), 280–284. <https://doi.org/10.1016/j.jinsphys.2010.11.014>
- Nonacs, P. (2000). Measuring and using skew in the study of social behavior and evolution. *The American Naturalist*, 156(6), 577–589. <https://doi.org/10.1086/316995>
- Otani, Y., Sawada, A., & Hanya, G. (2020). Spatial position-associated mating strategies employed by male Japanese macaques (*Macaca fuscata yakui*) in Yakushima. *Primates*, 61(3), 415–426. <https://doi.org/10.1007/s10329-020-00792-8>
- Pinter-Wollman, N., Hobson, E. A., Smith, J. E., Edelman, A. J., Shizuka, D., De Silva, S., Waters, J. S., Prager, S. D., Sasaki, T., Wittemyer, G., Fewell, J., & McDonald, D. B. (2014). The dynamics of animal social networks: Analytical, conceptual, and theoretical advances. *Behavioral Ecology*, 25(2), 242–255. <https://doi.org/10.1093/beheco/art047>
- Pinter-Wollman, N., Wollman, R., Guetz, A., Holmes, S., & Gordon, D. M. (2011). The effect of individual variation on the structure and function of interaction networks in harvester ants. *Journal of The Royal Society Interface*, 8(64), 1562–1573. <https://doi.org/10.1098/rsif.2011.0059>
- Pusey, A., Williams, J., & Goodall, J. (1997). The influence of dominance rank on the reproductive success of female chimpanzees. *Science*, 277(5327), 828–831.
- R Core Team. (2013). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Ryder, T. B., McDonald, D. B., Blake, J. G., Parker, P. G., & Loiselle, B. A. (2008). Social networks in the lek-mating wire-tailed manakin (*Pipra filicauda*). *Proceedings of the Royal Society B: Biological Sciences*, 275(1641), 1367–1374. <https://doi.org/10.1098/rspb.2008.0205>
- Saha, P., Nandi, A. K., Unnikrishnan, S., Shilpa, M. C., Shukla, S. P., Mandal, S., Mitra, A., & Gadagkar, R. (2018). A route to direct fitness: Natural and experimentally induced queen succession in the tropical primitively eusocial wasp *Ropalidia marginata*. *Journal of Insect Behavior*, 31(1), 54–65. <https://doi.org/10.1007/s10905-017-9657-6>
- Sharma, N., & Gadagkar, R. (2019). A place for everything and everything in its place: Spatial organization of individuals on nests of the primitively eusocial wasp *Ropalidia marginata*. *Proceedings of the Royal Society B: Biological Sciences*, 286(1911). <https://doi.org/10.1098/rspb.2019.1212>, 20191212.
- Shizuka, D., & McDonald, D. B. (2015). The network motif architecture of dominance hierarchies. *Journal of The Royal Society Interface*, 12(105), 20150080. <https://doi.org/10.1098/rsif.2015.0080>
- Sih, A., Hanser, S. F., & McHugh, K. A. (2009). Social network theory: New insights and issues for behavioral ecologists. *Behavioral Ecology and Sociobiology*, 63(7), 975–988. <https://doi.org/10.1007/s00265-009-0725-6>
- Stockley, P., & Bro-Jørgensen, J. (2011). Female competition and its evolutionary consequences in mammals. *Biological Reviews*, 86(2), 341–366. <https://doi.org/10.1111/j.1469-185X.2010.00149.x>
- Strassmann, J. E., & Meyer, D. C. (1983). Gerontocracy in the social wasp, *Polistes exclamans*. *Animal Behaviour*, 31(2), 431–438. [https://doi.org/10.1016/S0003-3472\(83\)80063-3](https://doi.org/10.1016/S0003-3472(83)80063-3)
- Sumana, A., Deshpande, S. A., Bhadra, A., & Gadagkar, R. (2008). Workers of the primitively eusocial wasp *Ropalidia marginata* do not perceive their queen across a wire mesh partition. *Journal of Ethology*, 26(2), 207–212. <https://doi.org/10.1007/s10164-007-0049-9>
- Sumana, A., & Gadagkar, R. (2003). *Ropalidia marginata* – a primitively eusocial wasp society headed by behaviourally non-dominant queens. *Current Science*, 84(11), 1464–1468.
- Sumner, S., Kelstrup, H., & Fanelli, D. (2010). Reproductive constraints, direct fitness and indirect fitness benefits explain helping behaviour in the primitively eusocial wasp, *Polistes canadensis*. *Proceedings of the Royal Society B: Biological Sciences*, 277(1688), 1721–1728. <https://doi.org/10.1098/rspb.2009.2289>
- Sundaresan, S. R., Fischhoff, I. R., Dushoff, J., & Rubenstein, D. I. (2007). Network metrics reveal differences in social organization between two fission–fusion species, Grevy's zebra and onager. *Oecologia*, 151(1), 140–149. <https://doi.org/10.1007/s00442-006-0553-6>
- Turillazzi, S., & Pardi, L. (1977). Body size and hierarchy in polygynic nests of *Polistes gallicus* (L.) (Hymenoptera Vespidae). *Monitore Zoologico Italiano - Italian Journal of Zoology*, 11(1–2), 101–112. <https://doi.org/10.1080/00269786.1977.10736294>
- Wasserman, S., & Faust, K. (1994). *Social network analysis: Methods and applications*. Cambridge, U.K.: Cambridge University Press.
- Wey, T., Blumstein, D. T., Shen, W., & Jordán, F. (2008). Social network analysis of animal behaviour: A promising tool for the study of sociality. *Animal Behaviour*, 75(2), 333–344. <https://doi.org/10.1016/j.anbehav.2007.06.020>
- Wheeler, D. E. (1986). Developmental and physiological determinants of caste in social Hymenoptera: Evolutionary implications. *The American Naturalist*, 128(1), 13–34. <https://doi.org/10.1086/284536>
- White, K. N., Rothermel, B. B., Zamudio, K. R., & Tuberville, T. D. (2018). Male body size predicts reproductive success but not within-clutch paternity patterns in gopher tortoises (*Gopherus polyphemus*). *Journal of Heredity*, 109(7), 791–801. <https://doi.org/10.1093/jhered/esy036/5056032>

Appendix

Table A1

Colony sizes and reduction in information quality measured as the difference between information quality of spatial overlap layer and aggression layer for the five colonies

Colony	Colony size	Reduction in information quality
v57	21	0.73
v72	30	0.85
v82	22	0.83
v87	26	0.87
v99	36	0.92

Table A2

Network densities of the four social situations in each of the five colonies

Colony	Spatial overlap	Aggression	Trophallaxis	Solid food exchange
v57	1	0.27	0.30	0.11
v72	1	0.15	0.26	0.38
v82	1	0.17	0.16	0.13
v87	1	0.13	0.26	0.10
v99	1	0.08	0.14	0.10

Table A3

Outstrength (mean and SD) of all interacting dyads in each of the five colonies

Layer	Colony									
	v57	v82	v87	v72	v99					
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Spatial	0.455	0.175	0.355	0.241	0.387	0.201	0.397	0.206	0.365	0.202
Aggression	0.389	0.513	0.295	0.495	0.204	0.202	0.232	0.321	0.235	0.22
Trophallaxis	0.257	0.23	0.116	0.062	0.15	0.114	0.145	0.112	0.165	0.115
Solid food exchange	0.17	0.068	0.141	0.149	0.127	0.062	0.195	0.172	0.105	0.054

Table A4
Information quality of the four social situations comprising the multilayer networks for the five colonies

Colony	Spatial overlap	Aggression	Trophallaxis	Solid food exchange
v57	0.16	0.17	0.11	0
v82	0.15	0.15	0.07	0
v87	0.17	0.18	0.19	0
v72	0.16	0.16	0.07	0
v99	0.24	0.19	0.16	0

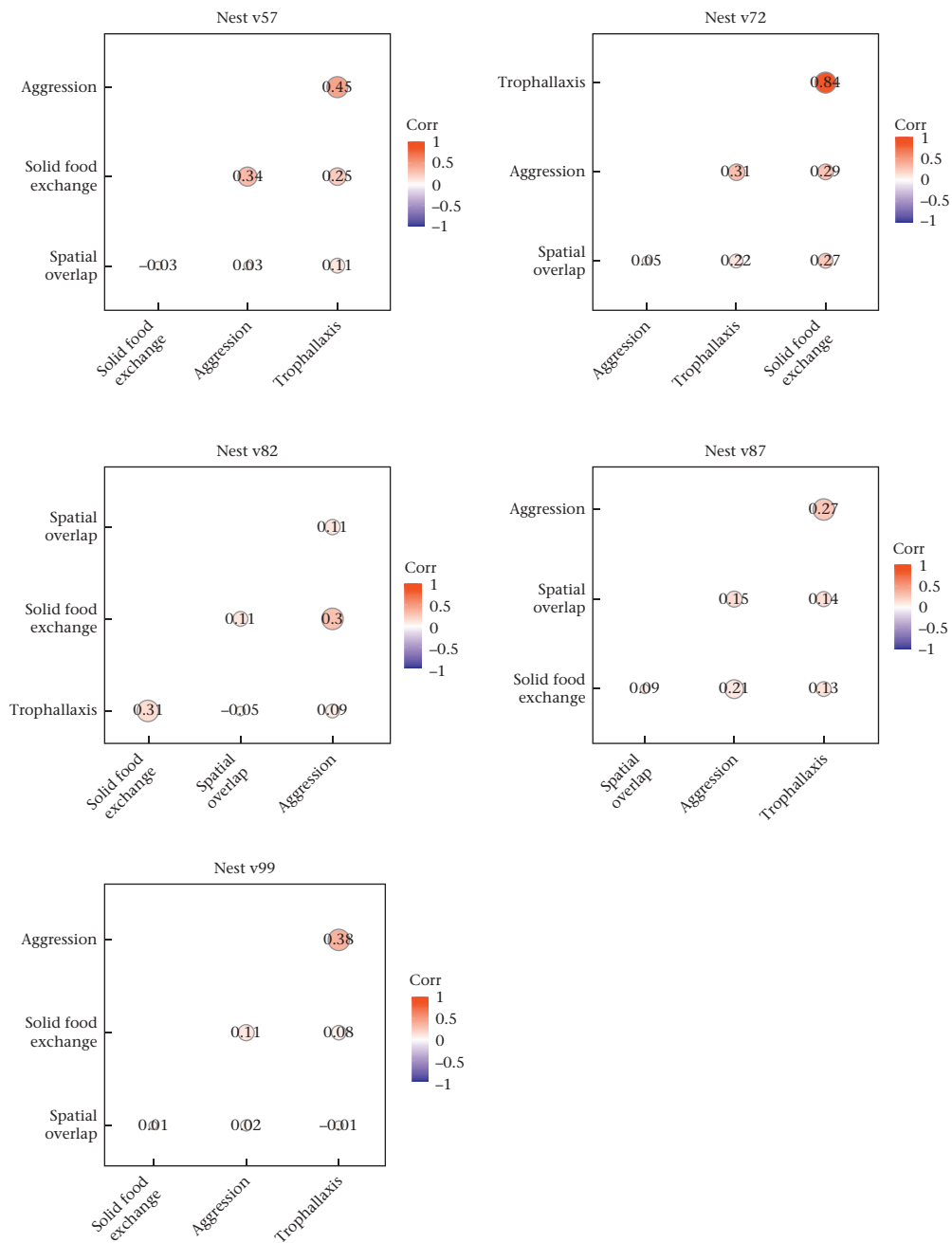


Figure A1. Correlation matrices of all layers within each colony.

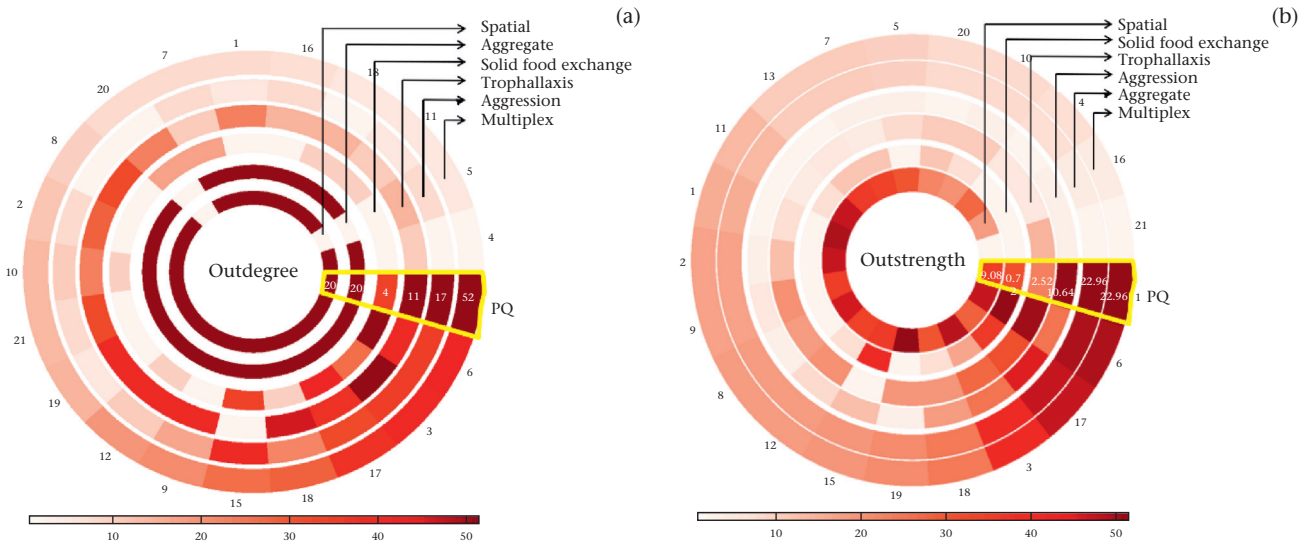


Figure A2. Annular representation of the (a) outdegree and (b) outstrength of individuals in colony v57. Darker colours indicate a higher rank of outdegree or outstrength while lighter shades of red indicate lower ranks. Each slice in a ring denotes an individual and the potential queen (PQ) is highlighted with a yellow outline.

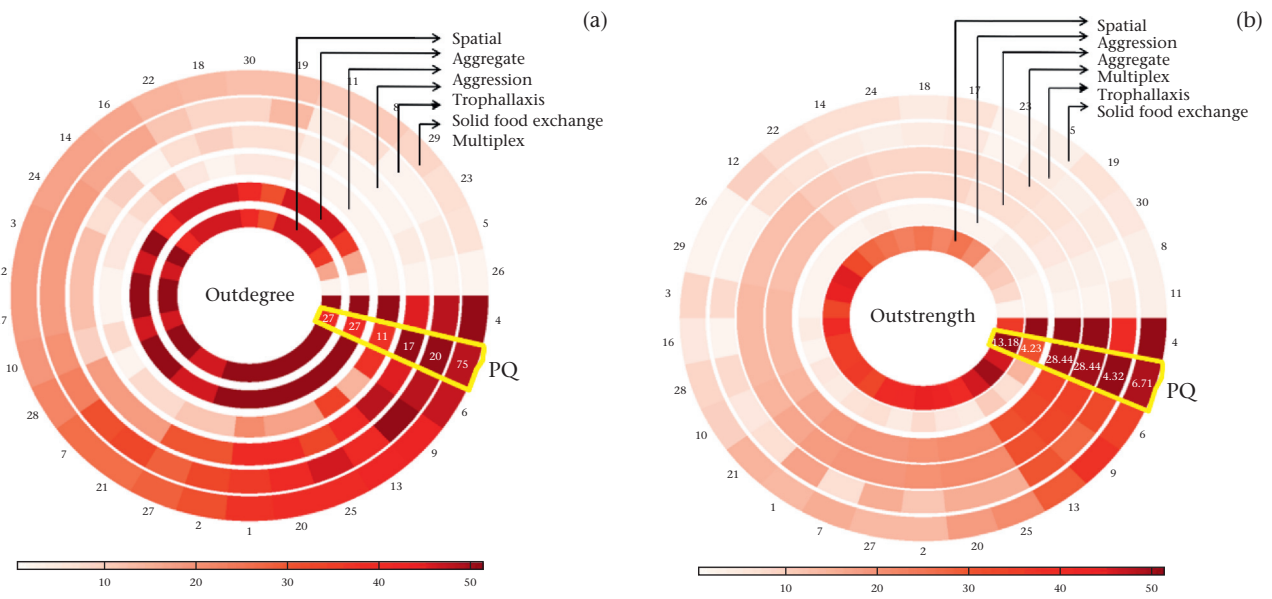


Figure A3. Annular representation of the (a) outdegree and (b) outstrength of individuals in colony v72. Details as in Fig. A2.

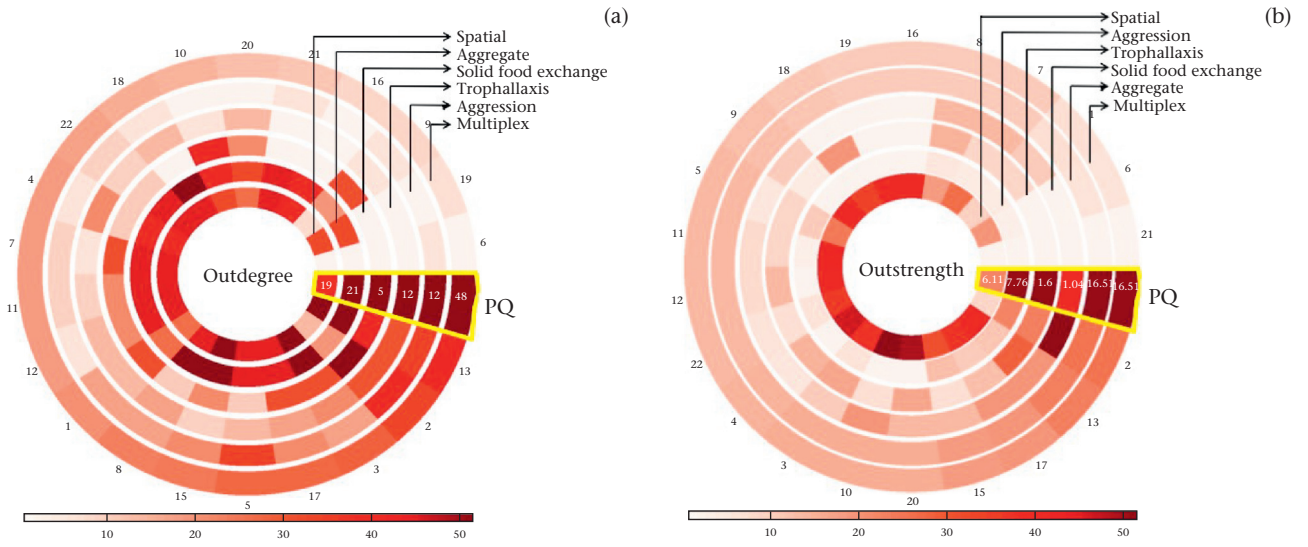


Figure A4. Annular representation of the (a) outdegree and (b) outstrength of individuals in colony v82. Details as in Fig. A2.

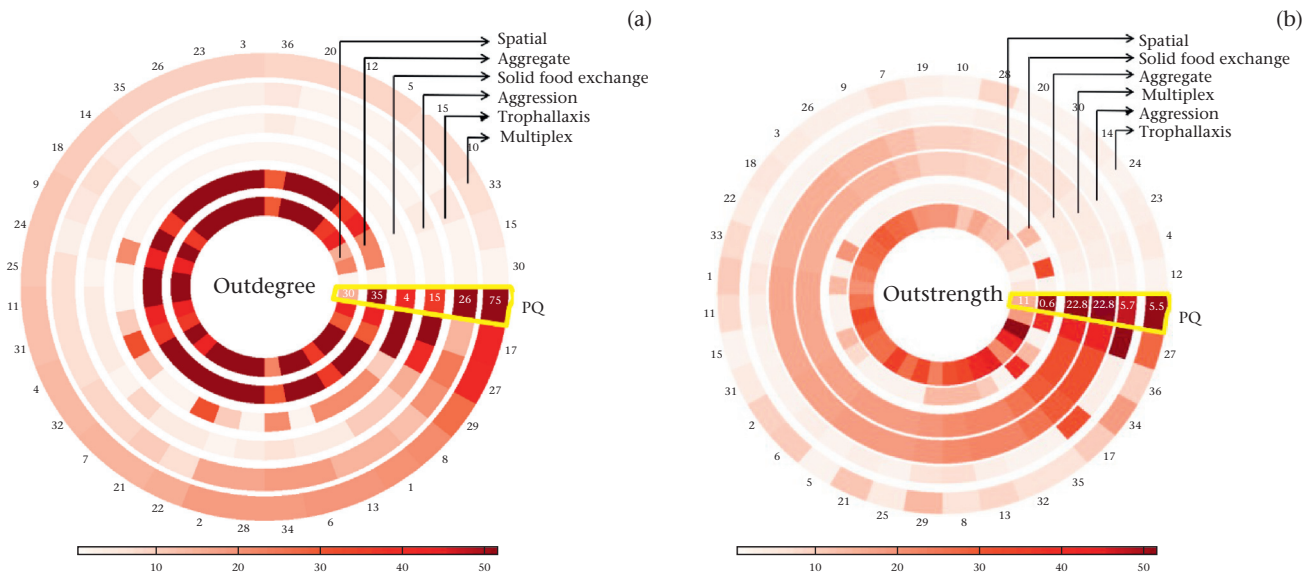


Figure A5. Annular representation of the (a) outdegree and (b) outstrength of individuals in colony v99. Details as in Fig. A2.

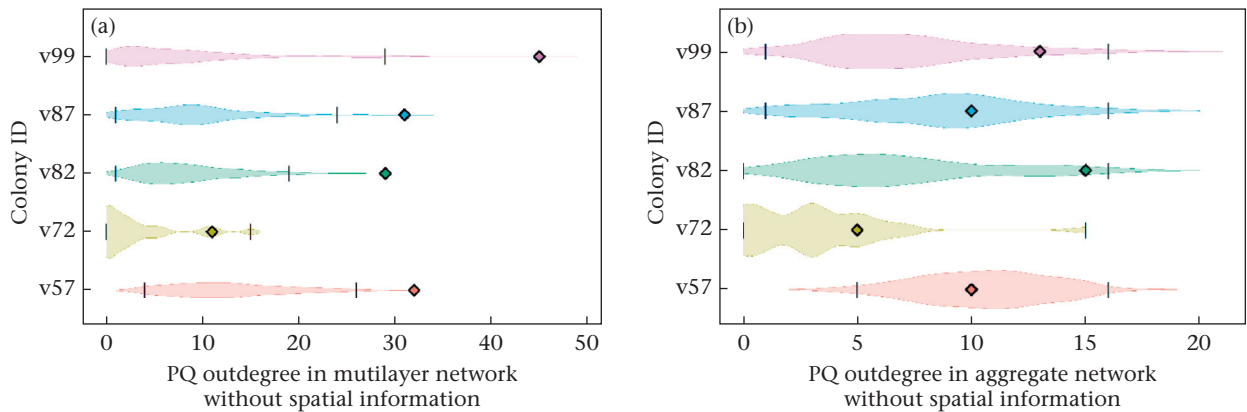


Figure A6. Observed and simulated outdegrees of the potential queen (PQ) in the (a) multilayer and (b) aggregate networks with aggression, trophallaxis and solid food exchange layers and without the spatial proximity layer. Observed values are depicted as diamonds. Distributions depict randomized measures based on 1000 runs in which we randomized node identities (IDs) while maintaining the network structure (edges). Vertical black lines indicate 95% quantiles of the randomized distribution. Each row within a plot corresponds to one of the five colonies.

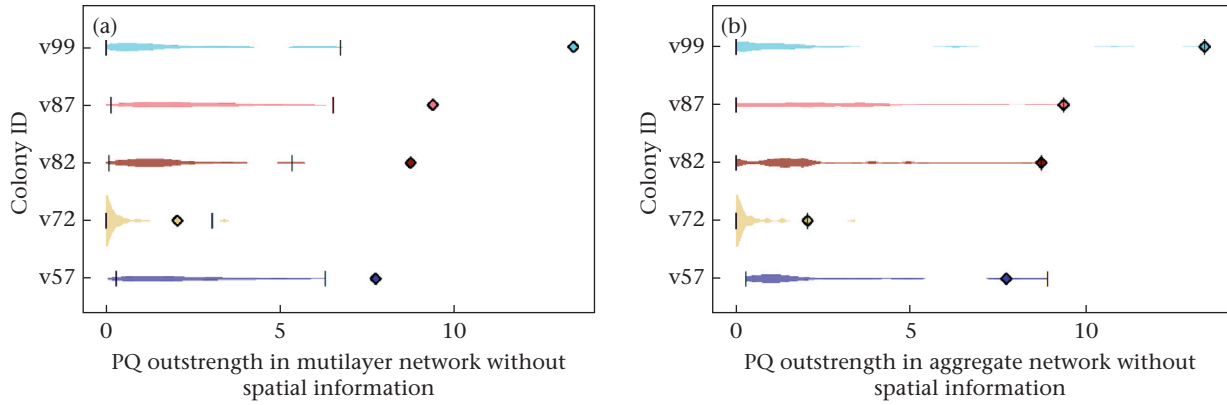


Figure A7. Observed and simulated outstrength of the potential queen (PQ) in the (a) multilayer and (b) aggregate networks with aggression, trophallaxis and solid food exchange layers and without the spatial overlap layer. Details as given in Fig. A6.

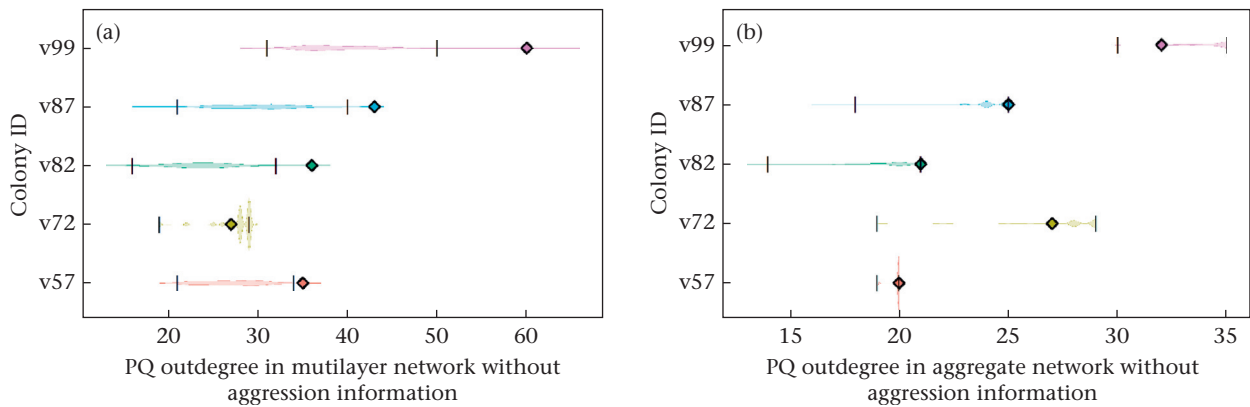


Figure A8. Observed and simulated outdegree of the potential queen (PQ) in the (a) multilayer and (b) aggregate networks with spatial overlap, trophallaxis and solid food exchange layers, and without the aggression layer. Details as given in Fig. A6.

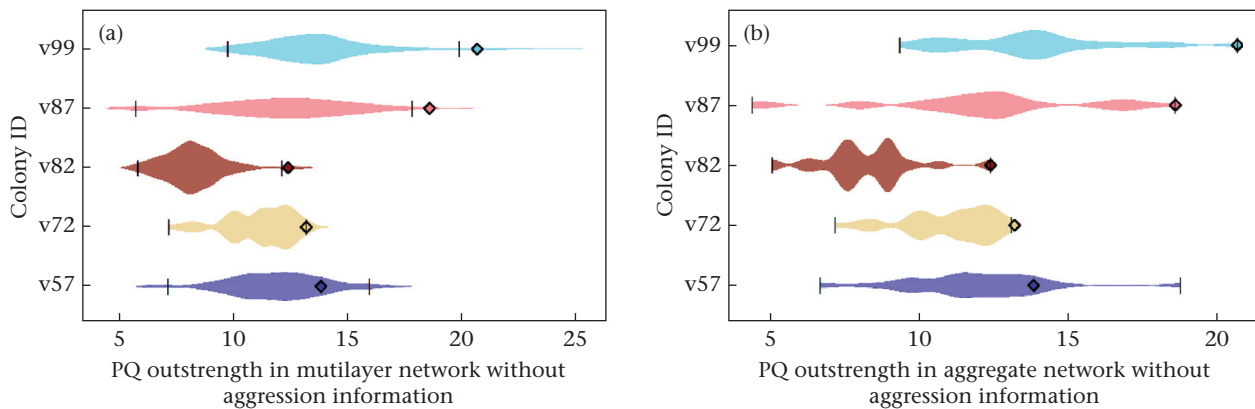


Figure A9. Observed and simulated outstrength of the potential queen (PQ) in the (a) multilayer and (b) aggregate networks with spatial overlap, trophallaxis and solid food exchange layers and without the aggression layer. Details as given in Fig. A6.

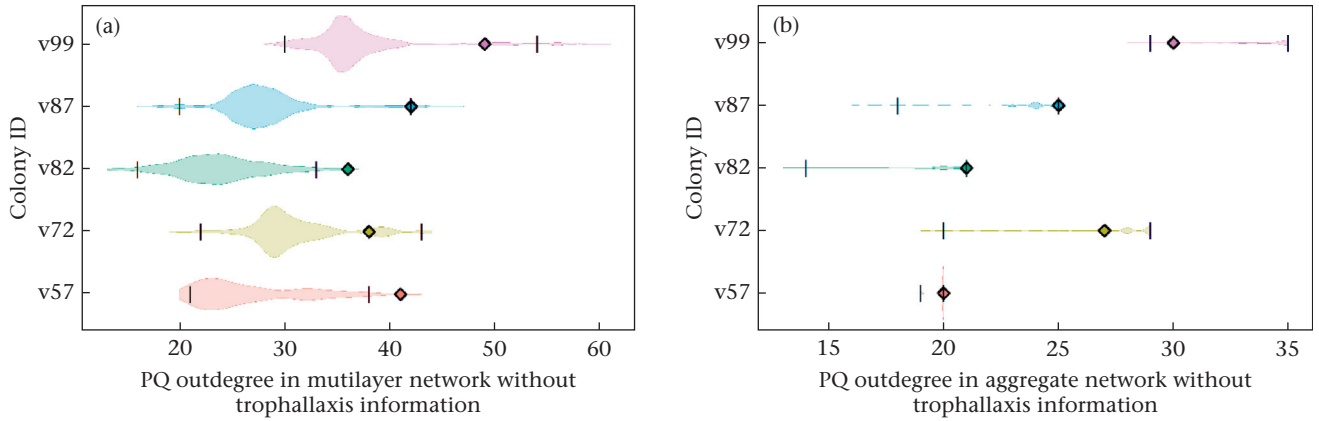


Figure A10. Observed and simulated outdegree of the potential queen (PQ) in the (a) multilayer and (b) aggregate networks with spatial overlap, aggression and solid food exchange layers and without the trophallaxis layer. Details as given in Fig. A6.

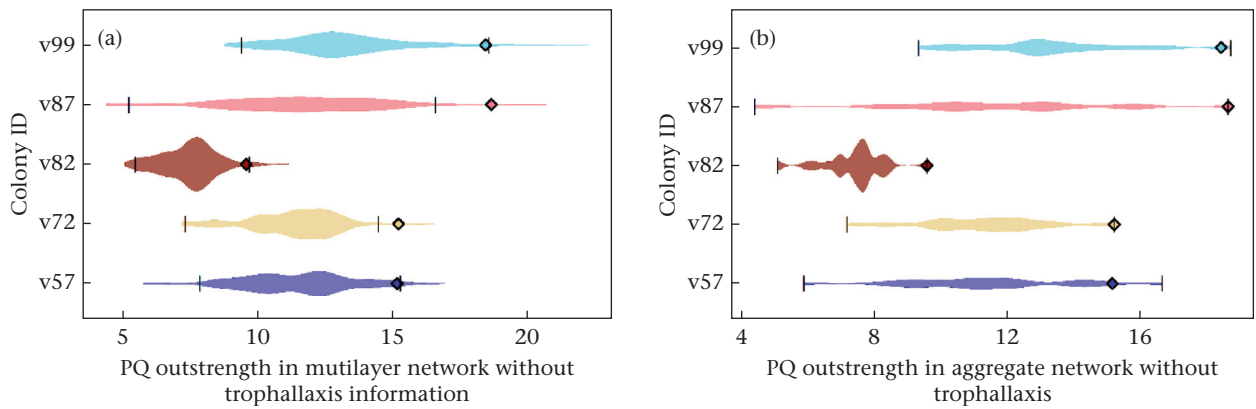


Figure A11. Observed and simulated outstrength of the potential queen (PQ) in the (a) multilayer and (b) aggregate networks with spatial overlap, aggression and solid food exchange layers and without the trophallaxis layer. Details as given in Fig. A6.

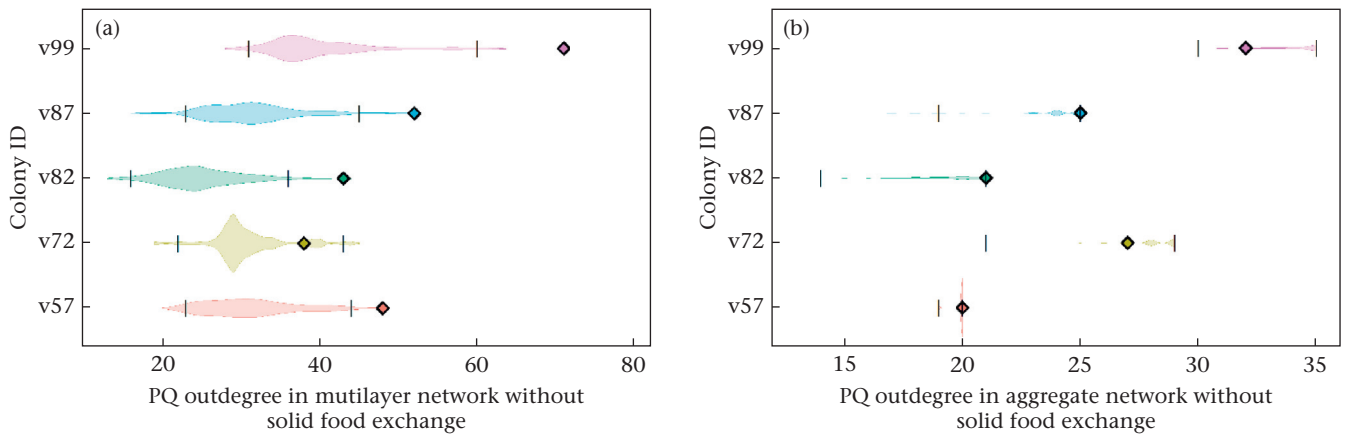


Figure A12. Observed and simulated outdegree of the potential queen (PQ) in the (a) multilayer and (b) aggregate networks with spatial overlap, aggression and trophallaxis layers and without the solid food exchange layer. Details as given in Fig. A6.

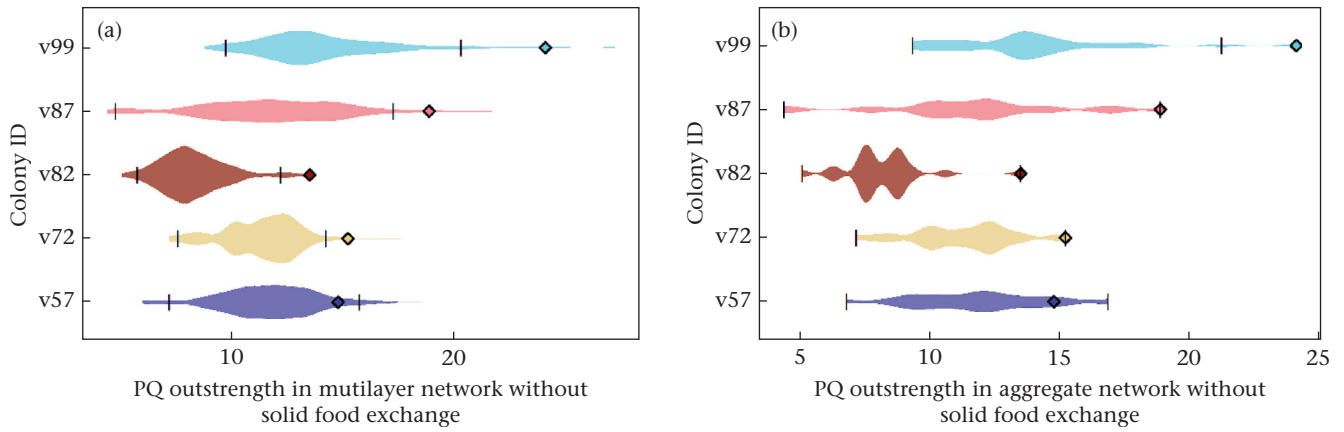


Figure A13. Observed and simulated outstrength of the potential queen (PQ) in the (a) multilayer and (b) aggregate networks with spatial overlap, aggression and trophallaxis layers and without the solid food exchange layer. Details as given in Fig. A6.