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Review

Queen succession in the Indian paper wasp *Ropalidia marginata*: On the trail of the potential queen

RAGHAVENDRA GADAGKAR[1]*, NITIKA SHARMA[1,2] and NOA PINTER-WOLLMAN[2]

[1]*Centre for Ecological Sciences, Indian Institute of Science, Bengaluru 560 012, India*

[2]*Department of Ecology and Evolutionary Biology, University of California, Los Angeles, USA*

**Corresponding author (Email, ragh@iisc.ac.in)*

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Ropalidia marginata is a common primitively eusocial wasp in peninsular India. Their colonies contain a single egg-laying queen and several non-egg-laying workers. Queens and workers are morphologically indistinguishable, and individuals can change from one role to the other. Unlike most primitively eusocial species, queens of *R. marginata* are docile, non-aggressive and non-interactive. Nevertheless, the queens maintain a complete reproductive monopoly mediated by non-volatile pheromones. Upon the death or removal of the queen, one worker becomes temporarily hyper-aggressive and becomes the next queen within about a week; we refer to her as the 'potential queen'. Because only one individual becomes hyper-aggressive and reveals herself as the potential queen, and the other wasps never challenge her, we have been much interested in identifying the potential queen in the presence of the queen. However, we have failed to do so until recently. Here, we recount the four decades of search for the potential queen, ending with the recent resolution that emerged from applying the novel technique of multilayer network analysis. Identifying the potential queen in the presence of the previous queen is now possible by integrating behavioural information from multiple social situations to form a holistic view of the social structure of the wasps.

Keywords. Multilayer network analysis; paper wasp; potential queen; *Ropalidia marginata*; queen succession

1. Insect societies

Many insects species organize themselves into societies that resemble, and potentially outperform, human societies in many ways (Wilson 1971). Insect societies display, in addition to group living, division of labour, communication, coordination, cooperation, conflict, altruism, and more (Hölldobler and Wilson 2009). Perhaps the most fascinating, albeit evolutionarily paradoxical, feature of insect societies is their reproductive caste differentiation. Only one or a small number of individuals reproduce, and the remaining individuals function as non-reproductive workers. The evolution by natural selection of such non-reproductive workers, who can be said to be altruistic, is a major paradox (Wilson 1971; West-Eberhard 1975; Gadagkar 1997; Queller and Strassmann 1998).

One theory that may explain this paradox is the inclusive fitness theory which emerges from Hamilton's rule (Hamilton 1964a, b; Gadau and Fewell 2009; Bourke 2011). According to this theory, the direct Darwinian fitness lost by the sterile workers is more than compensated by the indirect fitness gained by helping close genetic relatives with whom they share genes. Although there have been many attempts to test

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We are pleased to dedicate this paper to Prof. Somdatta Sinha, who has been a dear friend and colleague to one of us (RG) for over 40 years.

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this theory using mathematical models and empirical investigations, much remains to be understood. Unfortunately, we still have inadequate knowledge of the proximate mechanisms of social and altruistic behaviours and do not quite know how to link these mechanisms to the expectations of the theory. We cannot adequately test the theory without understanding and incorporating information about the mechanisms by which the workers pursue their altruistic strategies. Much research continues in this area using a variety of mathematical models and empirical research with a variety of social insect species (Gadagkar 2001; Hunt 2007; Nowak *et al.* 2010; Bourke 2011; Abbot *et al.* 2011; Dew *et al.* 2016; Gadagkar *et al.* 2019; Walton *et al.* 2020).

Social insects display many degrees of social behaviour. The most complex of these, which show reproductive caste differentiation, cooperative brood care, and overlap of generations, are called eusocial. Among the eusocial species, two sub-categories can be recognized. In the so-called advanced eusocial species, such as honey bees, most ants and termites, the reproductive and non-reproductive castes are morphologically differentiated because caste determination happens at the larval stage. Consequently, adults eclose either as fertile queens/kings or as sterile or nearly sterile workers and cannot change their status from one to the other (Wilson 1971).

In contrast, there is no morphological differentiation between queens and workers in the so-called primitively eusocial species, such as those of many wasps and bees, because caste determination happens at the adult stage. Consequently, adults eclose undifferentiated and totipotent, and can perform both queen and worker roles and even switch between the two roles. Primitively eusocial species offer unique opportunities to test the theory of kin selection because workers are altruistic even though they are potentially capable of reproducing and gaining direct fitness (Wilson 1971; Gadagkar 2001; Sumner *et al.* 2010; Hunt 2021; Kennedy *et al.* 2021).

2. The Indian paper wasp *Ropalidia marginata*

Ropalidia marginata (Lep.) (Hymenoptera: Vespidae) is a primitively eusocial wasp commonly found in and around human habitation in peninsular India (Gadagkar 2001). The wasps make paper nests by scraping cellulose fibres from plants and use the hexagonal cells of the nest to rear their brood. Their colonies are relatively small, rarely exceeding 100 individuals (figure 1).

Figure 1. A typical nest of the primitively eusocial Indian paper wasp *Ropalidia marginata* photographed on the Indian Institute of Science campus, Bengaluru. Photo: Thresiamma Varghese.

Males leave their natal nests within a week after eclosion, lead a nomadic life and mate with foraging wasps that they might encounter. Colonies, therefore, consist mainly of females, one of which is the queen, i.e., the sole reproductive of the colony. The remaining wasps function as non-reproductive workers performing all the functions required to maintain the colony, including nest building, defence, brood care, and foraging. Because there is no morphological differentiation among individuals, queens can usually be identified only by their egg-laying behaviour. Female wasps initiate new colonies either solitarily or in small groups. In the single foundress nests, a lone wasp lays eggs and performs all the duties of the workers until her first batch of offspring reaches adulthood. Usually, some of her daughters from the first brood function as workers, helping her rear more brood. In the multiple foundress nests, one of the wasps functions as the queen and the remaining function as non-reproductive workers. The group of foundresses may or may not be related to one another (Shakarad and Gadagkar 1995).

As expected from a primitively eusocial species, workers engage in agonistic interactions and can thus be arranged in a dominance hierarchy. Social dominance appears to be the mechanism by which reproductive division of labour (into queens and workers) and non-reproductive division of labour (into intra-nidal and extra-nidal workers, for example) are established in the early stages of colony founding (Premnath *et al.* 1996; Brahma *et al.* 2018). Later on, agonistic interactions do not appear to play a role in reproductive conflict in the colony cycle, as they do in most other primitively eusocial wasps. Instead, we have argued

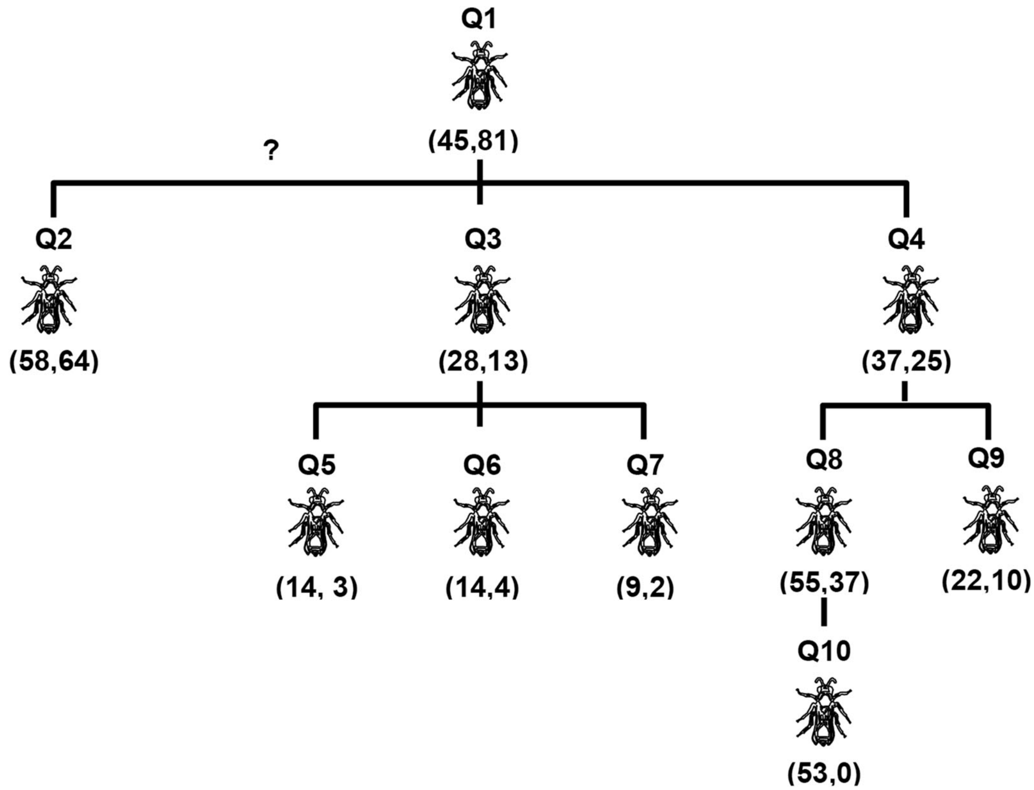

Figure 2. A pedigree of queens in a colony of the social wasp, *Ropalidia marginata*. Note that Q2, Q3 and Q4 were daughters of Ql, and Q5, Q6 and Q7 were daughters of Q3 and so on. The question mark indicates that the relationship of Q2 alone to Ql was somewhat doubtful. Of the two numbers in parentheses, the first one shows the tenure in days of each queen, and the second one indicates the number of offspring she produced during her tenure. [*Source*: Reprinted by permission of the publisher from Raghavendra Gadagkar (1997). *Survival Strategies: Cooperation and Conflict in Animal Societies*. p. 117, Cambridge, Mass.: Harvard University Press, Copyright © 1997 by the President and Fellows of Harvard College.]

that dominance-subordinate interactions have been co-opted to regulate the non-reproductive activities of the workers. For example, foragers appear to get information about colony hunger levels through the agonistic behaviour shown towards them by the intra-nidal workers. All this is quite unusual for a primitively eusocial species (Bruyndonckx *et al.* 2006; Lamba *et al.* 2008).

Ropalidia marginata has turned out to be an unusual representative of primitively eusocial species, in other ways as well. Unlike in other typical primitively eusocial species, the queens of *R. marginata* are strikingly meek and docile, almost never at the top of the dominance hierarchies of their colonies, often not even participating in agonistic interactions. Indeed, the queen is not only non-aggressive but also very non-interactive (Premnath *et al.* 1995; Sumana and Gadagkar 2003). Nevertheless, she is entirely successful in maintaining a strict reproductive monopoly. In the hundreds of hours of observations by dozens of observers, we have never once seen any worker lay an egg in the presence of the queen. The secret of the queen's success seems to be that she uses non-volatile pheromones, which she rubs onto the surface of the nest, to signal her presence and fertility to her workers. Her pheromones are produced in her Dufour's gland, and one can experimentally apply a crude extract of her Dufour's gland and signal her presence to her workers (Bhadra *et al.* 2010).

3. Queen succession

In natural colonies, the queen is replaced from time to time by one of her workers. The tenure of the queen is highly variable, ranging from a mere 7 days up to 236 days (figure 2) (Gadagkar *et al.* 1993). The process of queen succession is quite remarkable in that the queen disappears and is replaced by a temporarily hyper-aggressive potential queen quite suddenly without any apparent conflict. Indeed, we can rarely predict an impending queen succession, which, of course, is rather inconvenient for many of our experiments (Gadagkar 2021). To better understand the process of queen succession, we have conducted many laboratory experiments by inducing queen succession. It is easy to do so because all we have to do is to remove the existing queen.

Truly remarkable changes happen immediately upon the experimental removal of the queen. The otherwise relatively peaceful colony becomes hyper-aggressive. The levels of dominance-subordinate behaviours seen even within 30 min of queen removal can be many-fold higher than all such behaviours shown during a whole day in the presence of the queen. Even more interestingly, all of this new aggression is shown by a single worker. One and only one worker becomes hyper-aggressive, and none of the other workers challenges her. Instead, most workers temporarily leave the nest and periodically return, only to be aggressed by the potential queen. We refer to this hyper-aggressive individual as the potential queen because she will become the next queen if we do not return the original queen to the colony (figure 3) (Premnath *et al.* 1995, 1996; Sumana and Gadagkar 2003). We do not interpret the hyper-aggression of the potential queen as an indication of intra-colony conflict because the aggression of the potential queen is unidirectional: the workers almost never retaliate. Besides, the aggression of the potential queen appears to serve an altogether different function (see below).

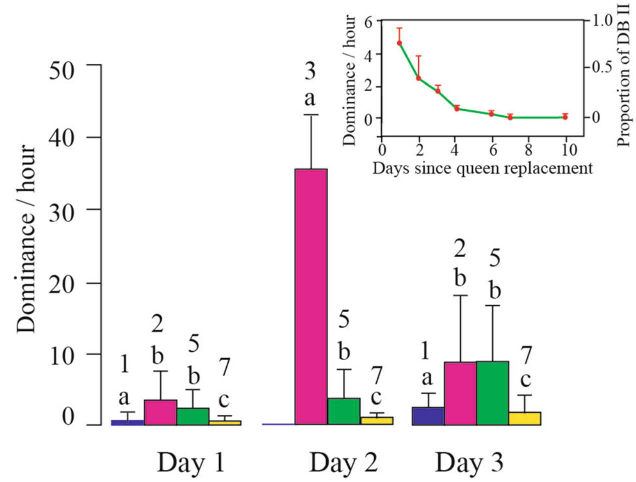

Figure 3. A typical queen removal experiment showing the frequencies per hour of dominance behaviour shown by the queen (blue bars), potential queens (pink bars), maximum workers (those showing the highest value of dominance behaviour) (green bars), and mean levels of dominance behaviour shown by the workers (yellow bars) on days 1–3. Bars that carry different letters are significantly different from each other ($P<0.05$) within each day. Bars that carry different numbers are significantly different from each other ($P<0.05$) among the three days. Comparisons are by the two-tailed Wilcoxon matched-pairs signed-rank test. [Redrawn with permission from Premnath *et al.* 1995 (© Oxford University Press).] (Inset) Dominance acts per nestmate per hour shown by replacement queens from the day of takeover up to 10 days after queen replacement. Means and ± SD are shown for 9 nests for days 1–7 and 6 nests on day 10. [Redrawn with permission from Premnath *et al.* 1996, © Elsevier.]

4. Potential queens

Even though the potential queen is hyper-aggressive towards all the workers in the first few days after the loss or removal of the queen, it is remarkable that other workers continue to forage and feed larvae at rates comparable to those seen before queen removal. If the previous queen is returned within a day or two, the potential queen immediately ceases to be aggressive, and the original queen resumes her queen-like behaviour, including egg-laying and rubbing her abdomen on the nest. It is very striking that there is little or no aggression between the potential queen and the returned queen at this early stage. However, if the original queen is returned more than 2 days after her removal, the potential queen will be aggressive to her.

We were naturally curious about when the potential queen will no longer recognize the old queen and continue to remain aggressive. By periodically returning a removed queen and removing her immediately if she is not attacked, we have shown that the potential queen refuses to accept the old queen after 1–2 days of the original day of queen or potential separation removal (Saha and Gadagkar, in prep.).

Finally, if the queen is not returned, the potential queen gradually reduces her aggression and becomes indistinguishable from a meek and docile queen within about a week. She also develops her ovaries during this period and lays her first egg at the end of about a week. The pheromone profile of the potential queen changes from a worker profile on the day of queen removal to the queen profile at the end of the week.

Even more interestingly, we can clearly recognize three consecutive periods. First, a pre-conflict period, when the potential queen accepts the old queen. Second, a period of conflict, when the returned queen and the potential queen will fight, and might even kill each other. Finally, a post-conflict period, when the returned queen appears to voluntarily leave the nest, allowing the potential queen to function as the new queen. This pattern is remarkably consistent with Trivers' theory of parent–offspring conflict (Trivers 1974). We have evidence that a somewhat similar situation occurs during natural queen turnover. In some cases of natural queen turnover, however, the potential queen might begin to show slightly increased aggression even before the natural death of the old queen and, in such cases, her period of hyper-aggression and time to lay her first egg is somewhat reduced (Saha *et al.* 2018).

5. The function of the potential queen's hyper-aggression

We naturally wondered about the function of the hyper-aggression shown by the potential queen. As mentioned before, the potential queen's hyper-aggression is unidirectional: the recipients of her aggression almost never retaliate. We tested whether the function of this hyper-aggression is to temporarily suppress the rest of the workers during the week that the potential queen is still developing her ovaries and building up her queen-like pheromone profile. However, we did not find strong evidence supporting this hypothesis. The level of aggression shown by the potential queen is not correlated with the number of workers on the nest, the dominance ranks of the workers, nor the residual levels of ovarian development of the workers. Instead, we have evidence supporting the rather counterintuitive hypothesis that the potential queen's hyper-aggression helps her develop her own ovaries rapidly. The implication of this counterintuitive finding is that the lone potential queen with no one to aggress should take longer, rather than less time, to develop her ovaries and lay her first egg, as compared to a potential queen who has to spend time and effort to aggress several nestmates. Indeed, we found that when a lone potential queen has no one to aggress, she lays her first egg significantly later than potential queens in colonies with several other wasps. We have, therefore, argued that we may have discovered a novel function of aggression, namely, to facilitate rapid ovarian development of the aggressor (Lamba *et al.* 2007).

6. We cannot identify the potential queen

The most intriguing part of this fascinating story is that we are unable to predict the identity of the potential queen before removing the queen. Not only is the timing of the queen succession unpredictable, but the identity of the successor is equally unpredictable. This seems all the more surprising because we can unambiguously identify the potential queen within 30 min of removing the queen. We, therefore, undertook several studies with the explicit aim of trying to identify the potential queen even in the presence of the old queen. We found, however, that the potential queens are indistinguishable from other workers. They are not unique in body size, dominance rank, ovarian development or overall behavioural profile (figure 4) (Deshpande *et al.* 2006). Even in studies that examined the properties of the social interaction networks of the wasps, the potential queen did not appear to occupy any unique position in the networks, nor do they have any unique network properties (Bhadra *et al.* 2009; Bhadra and Jordan 2013). We also studied the spatial organization of wasps on the colonies but failed to find any unique spatial location for the potential queen with reference to the nest or the queen (Sharma and Gadagkar 2019). The identity of the potential queen, in the presence of the old queen, has thus remained an enduring mystery, somewhat frustrating but at the same time adding thrill to our investigations (Gadagkar 2009).

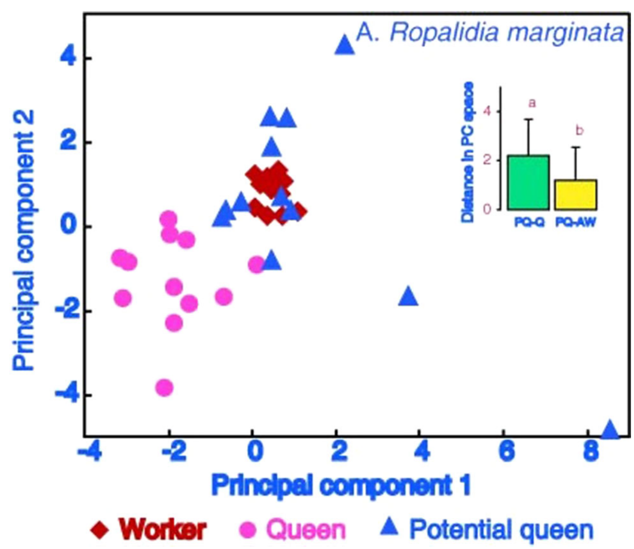

Figure 4. Relative positions of queens (pink circles), potential queens (blue triangles) and average workers (red diamonds) in the space of the first two principal components. (Insets) Mean and standard deviation of the distance between potential queen and queen (PQ–Q) and potential queen and average worker (PQ–AW) in the principal component (PC) space. PQ–Q distance is significantly greater than PQ–AW distance (see Deshpande *et al.* 2006 for details). [Redrawn with permission from Deshpande *et al.* 2006.]

7. The wasps know their potential queen

Our inability to identify the potential queen was made even more intriguing because of our suspicion that the wasps themselves seem to know her identity. We have been able to put this suspicion to a rigorous test. We have tested the hypothesis that there is an heir-designate whose identity is known to the other wasps even though it is unknown to us (figure 5). To test such a hypothesis, we split nests into two halves, separated by a wire-mesh screen and introduced the queen and half the workers (randomly chosen) on one side of the mesh and the remaining half on the other side. Because the queen pheromone is non-volatile, we know from previous experiments that the wasps on the queen-less side will behave as if they do not have a queen. One of the workers on the queen-less side will become hyper-aggressive and go on to become a full-fledged queen. We reasoned that if the heir-designate is on the queen-less side, not only will she become a potential queen on her side, but she will also be acceptable as the new queen to the wasps on the opposite side if they lose the original queen.

We tested this prediction by interchanging the queen and the potential queen across the wire mesh without disturbing the rest of the wasps. But we reasoned that the potential queen should be acceptable to wasps on both sides of the mesh only in about half of the experiments. In the other half of the experiments, the heir-designate should find herself on the queen-right side just by chance. In this case, the 'best' wasp on the queen-less side, who is neither the queen nor the true heir-designate, should become the potential queen on that side. However, this potential queen should be

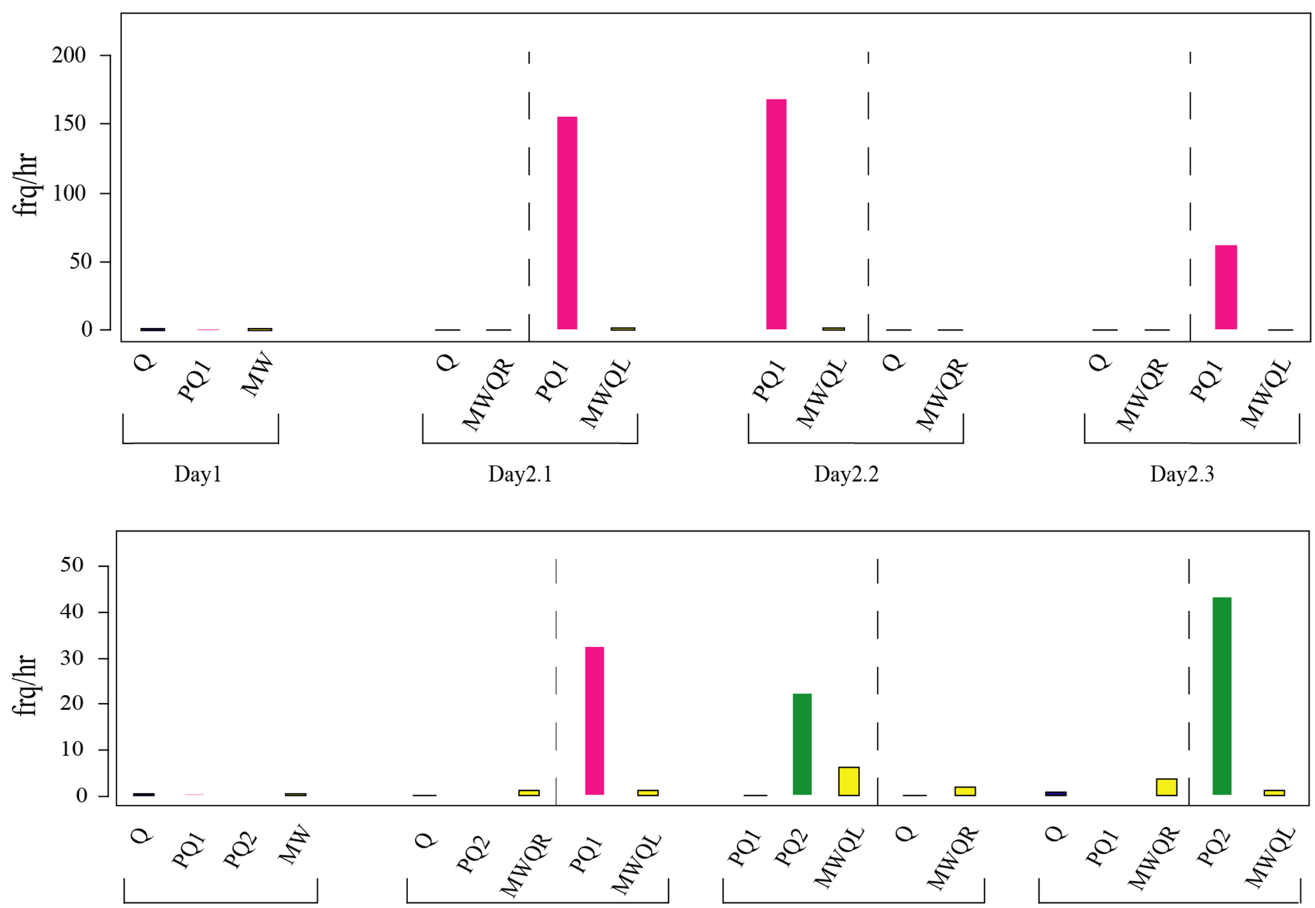

Figure 5. The queen (Q)–PQ exchange experiment. (Upper) A typical experiment in which the PQ1 was the cryptic successor. The frequency per hour of dominance behaviour exhibited by the queen, PQ1, and Max worker (defined as the worker showing maximum aggression) on day 1 in the normal colony and on the queen-right and queen-less fragments in the three sessions on day 2 is shown. (Lower) A typical experiment in which the PQ2 was the cryptic successor. The frequency per hour of dominance behaviour exhibited by the queen, PQ1, PQ2, and Max worker on day 1 in the normal colony and on the queen-right and queen-less fragments in the 3 sessions on day 2 are shown. [Redrawn with permission from Bhadra and Gadagkar 2008 (© The Royal Society).]

unacceptable to the wasps on the other side where the true heir-designate is present. We confirmed this prediction by once again interchanging the potential queen and the queen from one side to the other. We argued that the potential queen should stop being aggressive when moved to the other side, and a different individual, the true heir-designate, should become hyper-aggressive.

We also argued that the new hyper-aggressive individual, let us call her potential queen #2, should be acceptable to wasps on both sides of the mesh and that there should never be a third potential queen in such an experiment. All these predictions were upheld in our experiments. In three out of eight experiments, the first individual to become hyper-aggressive was unchallenged on both sides. But in the remaining five experiments, the first individual to become hyper-aggressive voluntarily ceased to be aggressive when moved to the opposite side. Instead, a second individual became hyper-aggressive who was unchallenged on both sides. Remarkably, the second hyper-aggressive individual did not receive a single act of aggression either when she first became hyper-aggressive in the presence of potential queen #1 or when she was moved to the opposite side. The identity of the heir-designate thus appears to have been known to all the wasps, including the hyper-aggressive individual #1. We, therefore, conclude that there is indeed an heir-designate whose identity is known to all the wasps, even in the presence of a healthy queen, and even though she remains cryptic to us (Bhadra and Gadagkar 2008).

In subsequent experiments, we have shown that there is not just one potential queen but that there is a reproductive queue of at least five cryptic potential queens who only become hyper-aggressive when their turn arises, i.e., when the queen and all potential queens higher up in the queue have been removed. In each experiment, when the queen or the potential queen is removed, one and only one individual becomes hyper-aggressive, and she is almost never challenged. These so-called serial PQ removal experiments provided us with another opportunity to try to predict the identity of potential queens. Because we had a large sample of over a hundred potential queens in these experiments, we could look for statistically significant correlates of the position of potential queens in the reproductive queue. We examined the frequency of dominance behaviour, dominance rank, building behaviour, feeding larvae, the proportion of time spent on the nest, body size, ovarian development, productivity and age. Only age was significant — the older the potential queen, the higher up she was in the reproductive queue, statistically speaking. Age is thus a statistically significant predictor of a wasp's position in the reproductive queue. However, even age is not a perfect predictor: subsequent experiments showed that the wasps often jump the age queue and become hyper-aggressive potential queens out of turn without being challenged. Thus, the potential queens are drawn from among the oldest individuals in the colony, but which will become the next potential queen remains a mystery (Bang and Gadagkar 2012).

8. Success at last?

We may now have finally solved the mystery of the identity of the potential queens (Sharma *et al.* 2022). In the past, when looking at the behaviour of the wasps, each social situation was examined in isolation. We have now used the novel analytic approach of multilayer networks that combines multiple social situations into a single mathematical object (Kivelä *et al.* 2014; Silk *et al.* 2018). In doing so, a multilayer network accounts for the differential impacts of different social situations on each other as well as on the overall global social dynamics of the society (Finn *et al.* 2019; Finn 2021; Hasenjager *et al.* 2021). Network measures used to identify well-connected, i.e., central, individuals in traditional social networks might overlook individuals who are not important in one social situation but are important in another. However, a multilayer network measure, 'versatility', reports the importance of an individual in the society while accounting for all social situations simultaneously (De Domenico *et al.* 2015; Beisner *et al.* 2020).

When we used a measure of social connectedness that accounts for four social situations simultaneously, before the original queen was removed, we were finally able to distinguish the potential queen from other workers according to her central social position. The four situations we used were aggression, trophallaxis (liquid food exchange), solid food exchange, and spatial overlap (figure 6). The potential queen was observed to have the most central role in the society of the wasps only when considering all four situations together in four of five colonies, and it was the second-highest in the fifth colony. To confirm that this highly central social position could not be expected by chance alone, we permuted the node identities randomly 1000 times within each layer of the multilayer network and recalculated the number of unique individuals a potential queen interacted with in each iteration. We found that the number of individuals the potential

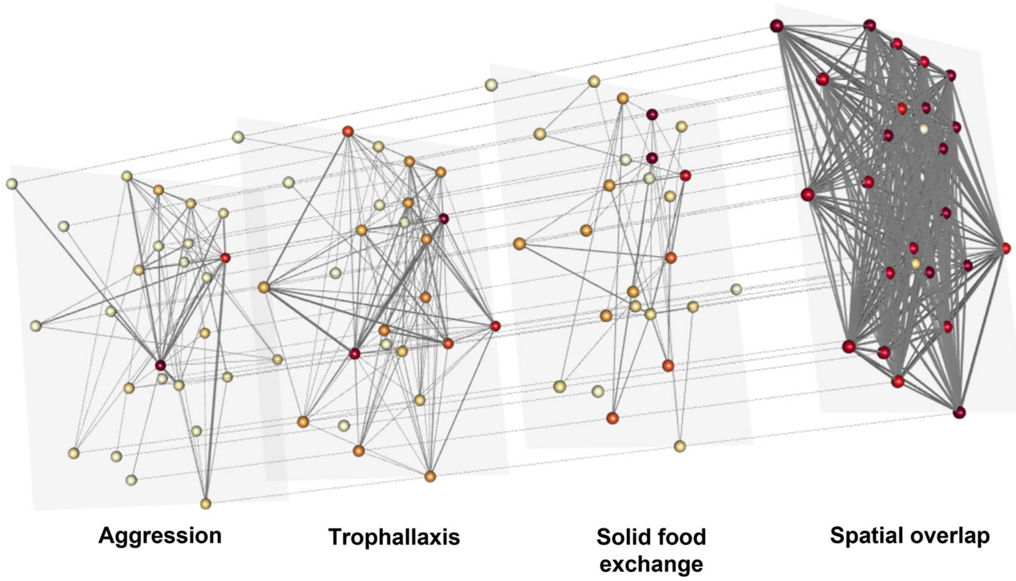

Figure 6. Multilayer network of one of the colonies depicting different social situations as different layers. From left to right, the layers include aggression, trophallaxis, solid food exchange, and spatial overlap. Spheres (nodes) depict wasps; darker node colours indicate wasps who interact with more unique individuals (higher out-degree) in a social situation. Grey lines connecting nodes in the same layer (within a grey plane) are intra-layer edges — the line thickness corresponds to edge weight. Grey lines connecting nodes across layers are inter-layer edges connecting the same individuals. [Reproduced with permission from Sharma *et al.* 2022.]

queens interacted with in all five colonies were significantly higher than 95% of the values obtained in the randomized scenarios. When examining each social situation separately, we did not find such a strong pattern (figure 7).

Aggressive and trophallactic interactions were the most important for identifying the potential queen (Sharma *et al.* 2022). Aggressive interactions might be important because of their role in accelerating ovarian development of the potential queen after queen removal (Lamba *et al.* 2007). Trophallactic interactions were more frequent with the potential queen than with the queen and could have therefore provided a mechanism for the other wasps to identify the potential queen (figure 7D). While spatial overlap on its own cannot identify the potential queen (Sharma and Gadagkar 2019), it is likely that spatial overlap with many wasps facilitate chemical communication even if they do not directly interact during rare instances of aggression or food exchange (Sumana *et al.* 2008; Bhadra *et al.* 2010; Mitra 2014).

The potential queen consistently influenced more social partners than any other wasps only when accounting for multiple situations simultaneously. We confirmed that the potential queen was not similarly unique when we constructed networks using one of the four social situations at a time and even when we constructed an aggregate network by lumping all the four social situations together as if they were the same. This finding suggests that social relationships are not built on just one social situation. Humans do not decide whom to become friends with only based on interactions in pleasant situations. Interactions during trying times provide important information about the strength of a relationship. We also do not select our partners based only on email correspondences, or Twitter interactions, for example. Instead, we combine many types of interactions that occur in different social situations to determine who our friends truly are. It appears as though wasps, too, account for social interactions in more than one situation to find out who the potential queen may be.

Our success in identifying the potential queen (at least in four out of five colonies) using a multilayer network analysis and our failure to do so when examining aggregate networks suggests that the variation in the roles played by wasps in different social situations are retained in multilayer networks but are lost in aggregate networks. The indiscriminate summing of interactions, irrespective of the situation in which they occurred, led to the appearance of uniform connectivity across all wasps in a colony in the aggregate networks (figure 8B). On the other hand, in the multilayer networks, the non-uniform connectivity

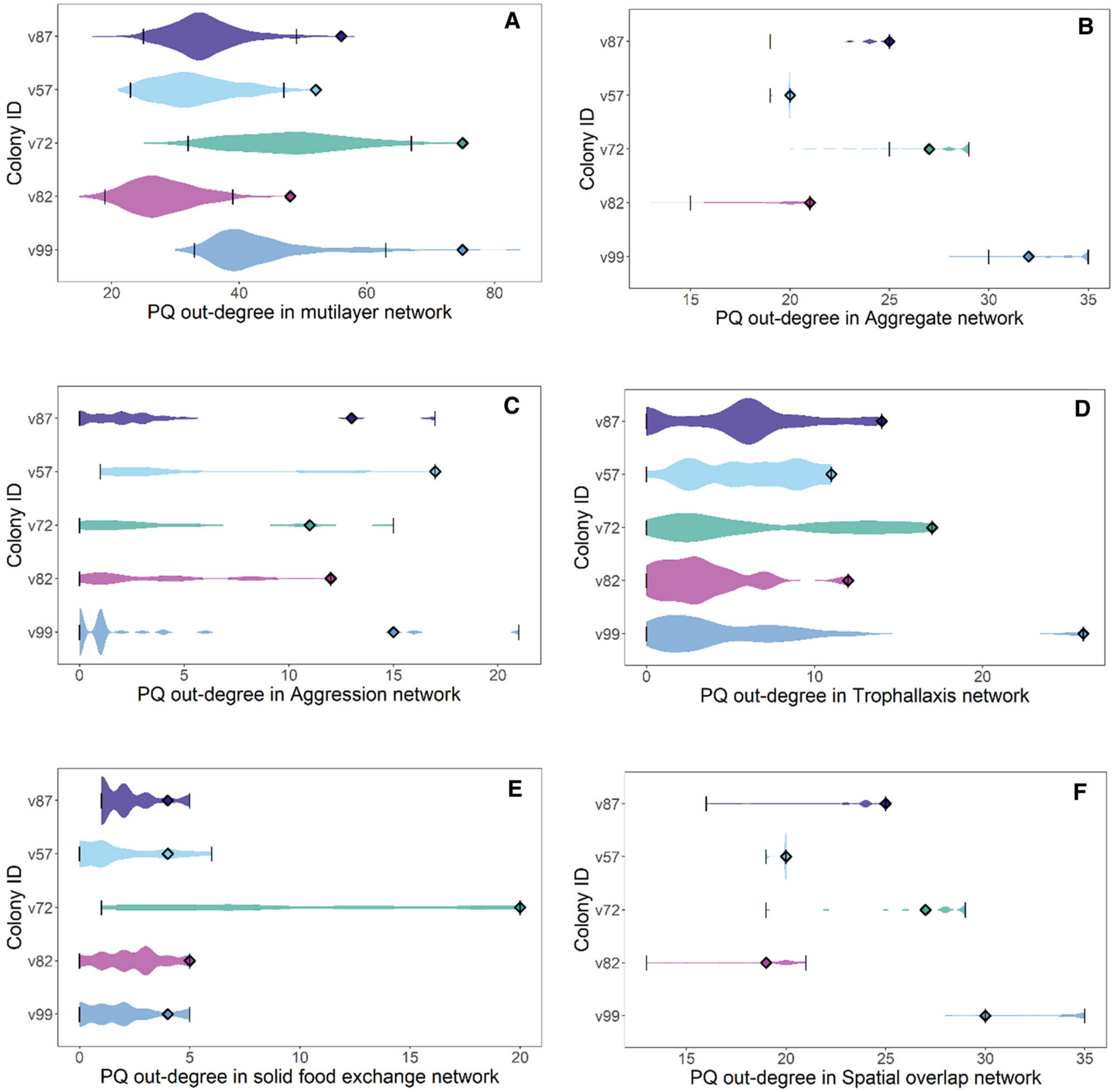

Figure 7. Observed and randomized out-degrees (number of unique individuals one interacts with) of the potential queen (PQ) in the (**A**) multilayer, (**B**) aggregate, (**C**) aggression, (**D**) trophallaxis, (**E**) solid food exchange, and (**F**) spatial overlap networks. Observed out-degree values are depicted as diamonds. Distributions depict simulated out-degree based on 1000 runs in which we randomized node 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. [Reproduced with permission from Sharma *et al.* 2022.]

across different wasps was made apparent, indicating that there were few highly interacting individuals and most others had few social partners (figure 8A). Thus, in the multilayer networks, the slightly different pieces of information provided by the four social scenarios are retained and assembled such that new information about the social interactions of all wasps in the colony arises. In fact, the potential queen cannot be identified consistently if any one of the four social situations is missing from the multilayer network or if all the social networks are lumped in an aggregate.

We have thus been able to identify the potential queen even in the queen's presence by incorporating and retaining the diversity of social situations that

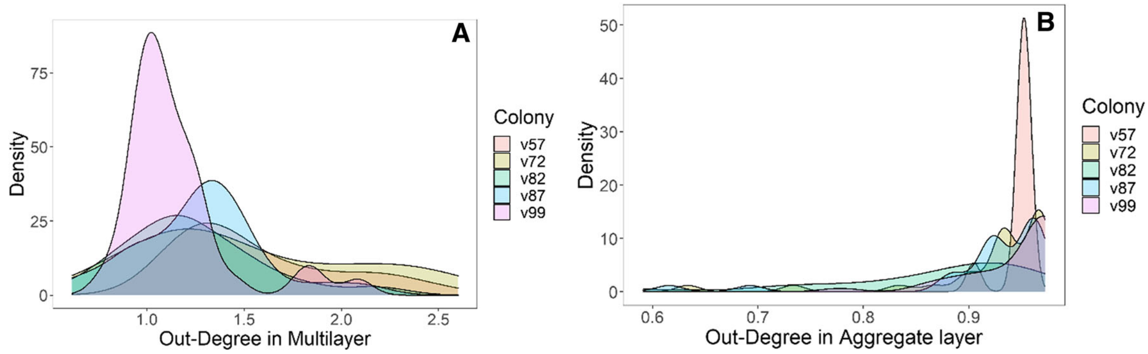

Figure 8. Distributions of standardized out-degree (number of unique individuals one interacts with) for the multilayer (**A**) and aggregated (**B**) networks for each of the five colonies are depicted in different colours. The out-degree (x-axis) was standardized by the total number of wasps in each colony. [Reproduced with permission from Sharma *et al.* 2022.]

individuals in complex societies display. Such identification implies that the potential queen in *R. marginata* colonies may signal her heir status to the other wasps in the colony through a combination of multiple social situations. Indeed, we have evidence that the wasps in the colony 'know' who their potential queen is, even though she was hitherto cryptic to us (Bhadra and Gadagkar 2008). How other workers integrate these various signals from different social situations to recognize the potential queen as the queen's successor remains to be studied. Even though individuals may often interact in a myriad of social situations, many traditional studies overlook this complexity and miss out on understanding the social roles of prominent individuals. The approach of viewing individuals as embedded in complex social fabrics instead of a standalone thread has important implications for uncovering previously overlooked ecological and evolutionary dynamics of social systems.

9. Conclusion and words of caution

We are pleased that our four-decade journey on the trail of the potential queen of *R. marginata* has likely reached its destination, with success in identifying the potential queen even in the presence of the queen. But we wish to end with some words of caution. Although the potential queen turned out to be unique when considering multiple social situations simultaneously, this was true only in four colonies and was not true in one colony. A sample size of four is small and is on account of the incredible amount of painstaking work it takes to manually track the behaviour of each individual wasp in a colony for many days. Perhaps new automated data acquisition methods (e.g., reviewed by Smith and Pinter-Wollman 2021) will allow increasing the sample size. However, most automated tracking of social insect behaviour is restricted to inferring behaviour from spatial proximity, without providing the nuanced detail required to identify functional behaviours and distinguish between certain behaviours, such as liquid and solid food exchange. Apart from increasing the sample size, we need to perform new experiments, such as identifying the potential queen using multilayer network analysis and only then removing the queen to see if our prediction of the identity of the potential queen was upheld. Some success and such a clear indication of the road ahead is the best possible outcome of any research project.

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Corresponding editor: Mohit Kumar Jolly