Landscapes of Collectivity in the Life Sciences

edited by Snait B. Gissis, Ehud Lamm, and Ayelet Shavit

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5 Choosing a New Queen: Consensus without Conflict in a Social Wasp Colony

Raghavendra Gadagkar

The Insect Societies

Many species of insects, such as ants, bees, and wasps, organize themselves into societies that parallel if not better human societies in many respects. These insects organize themselves into social units called colonies, characterized by efficient communication and coordination and division of labor. A particularly remarkable property of insect societies is their reproductive division of labor—only one or a small number of individuals reproduce (the queens), while the rest remain sterile and work for the welfare of the colony (workers) (Wilson 1971). However, the queen may die at some stage and so will have to be replaced. Such queen succession in the primitively eusocial wasp *Ropalidia marginata* is the focus of this chapter. Space prevents me from describing the many remarkable details of collective behavior in many other social insect species discovered by a large number of social insect researchers (see, e.g., Gadau and Fewell 2009).

Ropalidia marginata

Ropalidia marginata is a tropical, primitively eusocial wasp, widely distributed in peninsular India. It is called primitively eusocial because, unlike among honeybees, ants, and termites, there is no morphological differentiation between queens and workers. This means that the queen cannot be distinguished from workers except through her behavior. It also means that queens and workers can potentially exchange roles. The process of becoming a queen or a worker does not happen in the early larval stage, as it does in honeybees and other highly eusocial insects. In primitively eusocial insects all or most adults are largely totipotent at eclosion and subsequent differentiation into queens and workers is based, among other things, on social interactions. As one can imagine, this makes the process of queen succession particularly interesting. The fact that *R. marginata* occurs in the tropics seems to have a significant influence on the process of queen succession. Similar species of wasps that live in the temperate regions of the world undergo a so-called annual colony cycle—their colonies are abandoned at the onset of winter, newly born individuals hibernate and new colonies are started the following spring. In contrast, because there is no severe winter in the tropics, colonies are not necessarily abandoned at any particular time; they can be aseasonal and perennial and in principle immortal, with a succession of queens and workers turning over. As one can imagine, this creates many more possibilities for cooperation and conflict to be carried forward over long periods of time. All these features—long-lived perennial colonies, totipotent adults, queenship decided by social interactions, and periodic queen succession—make *R. marginata* an attractive model system to begin to understand the landscapes of collective behavior in animals (Gadagkar 2001).

Queens and Workers of *R. marginata*

When I first began studying this fascinating species I was interested in understanding how the morphologically indistinguishable queens and workers are behaviorally different from each other. Early observations, which were qualitative in nature, indicated that there were no significant differences in the behavior of the queens and the workers. I therefore began to suspect that the difference between the behavior of queens and workers must be quantitative. In order to investigate this possibility, we constructed time–activity budgets (the proportion of time spent in each behavior) of all the individuals in a colony. The resulting time–activity budget data were then subjected to multivariate statistical analysis. This study revealed that in each colony there are three kinds or three behavioral castes of wasps, which we called sitters, fighters, and foragers. The sitters spend most of their time in the nest, but doing almost nothing. The fighters also spend most of the time in the nest, but they are constantly involved in what we call dominance–subordinate interactions with the other wasps in the colony. The foragers spend a lot of time outside the nest, presumably in search of food and building material. But where is the queen in this classification? Prior literature on such primitively eusocial wasps suggested that the queens should be fighters. This is because in primitively eusocial species queens are known to use physical aggression both to suppress workers from reproducing and to ensure that the workers actually work. We found, however, that in *R. marginata* queens were not fighters; they were almost always in the sitter caste. The queen in the *R. marginata* colony is thus a nonaggressive, noninteractive, meek, and docile sitter. This result was rather surprising and led to many questions, as we will see below (Gadagkar and Joshi 1983).

If the Queen Is Such a Meek Sitter, How Does She Become a Queen in the First Place?

It is surprising that a meek sitter is accepted by the rest of the workers as their queen. To understand why they accept her, we decided to witness the process of queen formation.

To accomplish this, we studied a healthy colony and measured the time–activity budgets of the queen and all the workers. Subsequently we removed the queen and remeasured the time–activity budgets of all the workers without the queen. It turned out that at the end of this phase of the experiment we could return the queen and, therefore, were once again able to measure the time–activity budgets of all the wasps in the reunited colony. This experiment led to a very surprising result. *R. marginata* is a reasonably peaceful society with low rates of dominance–subordinate interactions among the workers. However, as soon as we removed the queen, one and only one worker became extremely aggressive, increasing her levels of aggression several fold compared to what they had been in the presence of the queen. If we returned the queen, this individual stopped being aggressive and returned to her normal activities. If we did not return the queen, however, this individual gradually (over the course of about a week) lost her aggression, developed her ovaries, and became the next (meek and docile) queen of the colony. Hence we call this hyper-aggressive individual a potential queen, until she lays her first egg. In other words *R. marginata* queens begin their careers as very aggressive individuals and only later become meek and docile, and this probably explains why the workers accept them as their queens in the first place (Premnath et al. 1995; 1996).

How Does the Queen Inhibit Worker Reproduction?

This is an intriguing question because the queen is aggressive only for about a week and yet maintains perfect reproductive monopoly throughout her tenure, which may last many months. How does she manage to inhibit worker reproduction throughout her tenure without overt aggression? We hypothesized that although *R. marginata* is a primitively eusocial species the queen produces a pheromone to regulate worker reproduction. In order to test whether the pheromone she might produce is volatile or nonvolatile, we designed a different experiment. After observing a normal colony with the queen and her workers, we now, instead of removing the queen, cut the nest in half, inserted a wire mesh in between (to allow volatiles to pass through but not nonvolatiles or the wasps themselves), and randomly released half the workers on one side of the wire mesh and the other half on the other side. The queen was also randomly assigned to one side of the wire mesh. We now observed the queen and all the workers on both sides of the wire mesh. On the following day, we moved the queen from one side to the other without disturbing the workers. The predictions of this experiment were that if the queen pheromone is volatile, then it should pass through the wire mesh so that workers on both sides of the wire mesh should behave as if they have a healthy queen. On the other hand, if the pheromone produced by the queen is nonvolatile, then it will not pass through the wire mesh, and the workers on the queen-less side should behave as if they do not have a living queen, meaning that one of them should become a hyper-aggressive potential queen. We have now performed this experiment several times, and in every case the second prediction is upheld. In other words, workers on the queen-less side behave as if they do not have a queen. When a queen is moved from one side to the other, the hyper-aggressive individual in the formerly queen-less side now (upon the return of the queen) curbs her aggression and resumes normal activities. A different individual in the new queen-less side now becomes hyper-aggressive. We conclude, therefore, that *R. marginata* queens appear to use nonvolatile pheromones to signal their presence to their workers (Sumana et al. 2008).

How Do the Workers Perceive the Nonvolatile Queen Pheromone?

The obvious hypothesis was that the workers perceive the nonvolatile pheromone produced by the queen every time they come into physical contact with her. We tested this hypothesis by comparing the time taken by the workers to realize the absence of the queen and their rate of interaction with the queen. If physical contact with the queen is the method of acquiring the queen pheromone, then the workers should interact very frequently with the queen because they realize the absence of the queen within 30 minutes of her loss. We measured the rates of physical interaction of every individual in the colony with every other individual and found that the workers cannot possibly be perceiving the queen's pheromone through physical interaction with her. We now have evidence that the queen applies her pheromone to the surface of the nest by rubbing her abdomen on the nest as she walks along (Bhadra et al. 2007).

What Is the Source of the Queen Pheromone?

Considering the way that the queen rubs her abdomen on to the surface of the nest, we suspected that the source of the queen pheromone might be the Dufour's gland, because the Dufour's gland is connected to a duct that opens on the underside of the abdomen. To test this hypothesis, we made crude extracts of the queen's Dufour's gland and a worker's Dufour's gland and performed a bioassay. The bioassay consisted of measuring the drop in aggression by the potential queen upon the return of the queen or upon our applying the queen or worker pheromone crude extract on the nest. We already knew that when we remove the queen, the potential queen greatly intensifies her aggression, and when we return the queen, she drops her aggression significantly. Instead of returning the queen, we applied the crude extract of the queen's Dufour's gland to the nest yet witnessed the same significant reduction of aggression in the potential queen. When we applied the worker Dufour's gland extract, however, no such reduction in aggression was seen. In other words, our application of the queen's Dufour's gland extract to the nest surface mimicked the return of the queen. We therefore conclude that at least one source of the queen's pheromone is her Dufour's gland (Bhadra et al. 2010).

How and When Is the Queen's Heir Decided?

As human observers, we cannot help being intrigued by the fact that as soon as we remove the queen, or the queen dies naturally, one of the workers immediately takes over as the next queen. An obvious question is how and when is the queen's heir decided? In spite of great efforts and many years of experiments, I must confess that to this day we are unable to answer this question. Because the potential queen is indistinguishable from a typical worker, we are unable to predict the successor before we actually remove the queen (Deshpande et al. 2006). Lest the reader think that we can always answer every question by doing experiments and finding a clear, unambiguous answer, I want to emphasize that this is one aspect of the behavior of these wasps that we are simply unable to understand. Collective behavior is indeed complex. But even though we cannot predict the identity of the queen's successor in her presence, is it possible that there is an heir-designate, a cryptic heir-designate, and is it possible that the wasps actually know who this heir-designate is? It may seem far-fetched to imagine that the wasps know who their successor is, but we were emboldened to make this speculation because we found that every time the queen is removed, one and only one worker becomes hyper-aggressive and she is not challenged by any of the other wasps. In order to test the possibility that there is indeed a cryptic heir-designate known to all the wasps though unknown to us, we performed a different experiment. Here we cut the nest in half, inserted a wire mesh and, as before, randomly distributed the workers between the two sides and placed the queen on one side. We reasoned that if there is indeed a cryptic heir-designate, by chance alone she should end up on the opposite side to the queen in about half the experiments. Now, because the workers cannot perceive the presence of the queen on the opposite side, the heir designate should become hyper-aggressive and begin to behave like a potential queen on her side. However, if she is indeed the heir-designate for the entire colony and not just for her side, she should be acceptable to the wasps on the opposite side. To test if this is true, we exchanged the queen and the potential queen from the previous side to the opposite side and confirmed that this hyper-aggressive individual is indeed acceptable (i.e., she is not challenged) to the workers on both sides of the mesh. We obtained this result in about half our experiments. However, in about half the experiments the heir-designate ends up on the same side as the queen. Now she cannot become hyper-aggressive because the queen is on her side and she perceives her presence. But on the opposite side, where there is no queen, one of the individuals, perhaps the best among those on that side, should become hyper-aggressive and begin to act like a potential queen. She may be acceptable as the next queen on her side, but she should not be acceptable on the opposite side because the true heir-designate is on that opposite side. We again exchanged the queen and the potential queen from one side to the other and showed that in about half the experiments the first individual to become hyper-aggressive, although accepted on her side, is not accepted on the opposite side. When she is moved to the opposite side, another individual becomes hyper-aggressive and goes on to become the potential queen while the first individual voluntarily loses her aggression and goes back to work. We therefore argue that there is a cryptic heir-designate; although we do not know who the successor is, we know that the wasps know who their successor is! It is especially remarkable that the second individual to become hyper-aggressive was never challenged. She did not receive a single act of aggression from the first potential queen or from any other worker, either when she emerged in the presence of the potential queen or when she was moved to the opposite side. The heir-designate was thus obviously "known" and acceptable to all the wasps, including the potential queen (Bhadra and Gadagkar 2008). Next, we wondered whether there is a single heir-designate or whether there is a queue of individuals waiting their turns to become subsequent queens. This was easy to test. Upon removal of the queen, within 30 minutes we can identify a hyper-aggressive individual who will go on to become the next queen if we do not return the original queen. Once we identify this hyper-aggressive individual, instead of waiting for her to become the next queen, we removed her immediately and found that one and only one of the remaining workers becomes hyper-aggressive once again. By such experimentation we have been able to identify queues of up to at least five potential queens. What is most remarkable is that at any given time one and only one individual becomes hyper-aggressive and she is not challenged by any other individual. Everyone seems to await their legitimate turn (Bang and Gadagkar 2012).

Is There No Conflict at All?

The most striking feature of all these experiments is the lack of overt conflict within the colony. Everything seems to be very orderly, and even queen succession seems to happen with no conflict—nobody challenges the one and only one individual who becomes hyper-aggressive and goes on to become the next queen. Is it really true that there is no conflict, or do the wasps have such an efficient method of managing conflict and keeping it under control that we are unable to see the conflict in the normal course of events? To test this second possibility, we attempted to devise experiments where we could uncover potential underlying conflict. One approach we have tried is to prevent the queen from applying her pheromone even though she is healthy. In such a situation the queen should perceive that she is perfectly healthy to lead the colony but the workers should perceive that she is failing and therefore unable to lead it. We used the following experimental design to achieve this. We collected all the wasps—queen and workers—from their natural nest, and we released them in a laboratory cage without the nest. In the absence of the nest the queen could not apply her pheromone on to the nest surface and advertise her status (and presumably her health) to the workers. We predicted that in such a situation the queen will be overthrown and another individual will become the potential queen. However, this did not happen. In most of the experiments the queen survived and continued to be the queen—there was no queen succession. To understand why this was so, we made careful observations and found to our surprise that, although the queen did not have a nest to apply her pheromone, she frantically rubbed her abdomen all over the laboratory cage surface. This is perhaps how she could still advertise her presence and was therefore not overthrown. However, we reasoned that applying the same amount of pheromone all over the surface of a large cage should result in a much lower concentration of the pheromone as compared to applying all of it on the surface of a small nest. Indeed, we found evidence of such a lowered concentration of pheromone. For the first time we found that in this situation, although the workers did not overthrow the queen, the workers nevertheless physically attacked the queen, something we never see in normal colonies. Even more interestingly, we found that the queen physically retaliated and showed aggression to the workers, again something that we never see in normal colonies. Thus, the queen normally maintains her status by applying the pheromone to the nest, but if there is a problem with this, she is able to use a combination of pheromones and aggression to assert her position. More importantly, workers can in principle show aggression to the queen and the queen can in principle retaliate. Even though she is a meek, docile sitter, she has not lost the ability to be physically aggressive. This suggests that there is underlying conflict, but that the wasps manage this conflict so efficiently that we cannot see it unless we find a way to uncover it (Saha et al. 2012).

Why Is Conflict So Well Managed?

Perhaps the most remarkable feature of these wasps is that they do not display overt conflict. All aspects of colonial life seem to be peaceful and well-organized. The queen is not an aggressive individual; rather, she is a meek, docile sitter. She conveys her presence (and presumably her health) to her workers by applying a chemical pheromone on the surface of the nest. No overt conflict is displayed upon the death or loss of the queen. Queen succession happens without any overt conflict, and this is probably why we cannot identify the successor before the removal of the old queen. There is a cryptic heir-designate, indeed a series of cryptic heir-designates waiting "patiently" for their turn to become the next queen and never jumping the queue. Why is this society so peaceful, so well-organized? Why is conflict so well managed? We argue that it is especially important for a wasp species that lives in the tropics to be so well organized and to manage conflict so efficiently. These wasps face a number of threats, not only from predatory wasps and parasites but, most importantly, from conspecific usurpers. Managing their conflict and always functioning in an organized, peaceful, cooperative manner may be very important for their ability to face the challenge of conspecifics who are trying to enter and take over their colonies. Perhaps I must add that such peaceful management of conflict is not universal—intense conflict over queen succession is known in many species and has been especially well-studied in fire ants (Tschinkel 2006) and honeybees (Winston 1987). I might also add that the potential for conflict is to be expected in the tropics, where colonies and therefore the potential for conflict exist throughout the year rather than for only a short period of time. Nevertheless, I should add that to date very few tropical species have been sufficiently studied.

The wasp colony is a collective of nonclonal individuals with divergent genetic interests, and yet their fitness is tied up with the reproduction of the queen, which in turn is contingent upon the efficient functioning of the colony. It is therefore not surprising that the collective behavior of *R. marginata* is well designed to manage conflict. Nevertheless, such peaceful queen succession is a remarkable example of self-organization that may need new methods of study.

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