More Fun Than Fun: The Wasp Who Would Be Queen

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*A well-grown nest of the Indian paper wasp (*Ropalidia marginata*). Photo: Dr Thresiamma Varghese*

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This article is part of the 'More Fun Than Fun' column by Prof Raghavendra Gadagkar. He will explore interesting research papers or books and, while placing them in context, make them accessible to a wide readership.

- *A question of obvious interest to all evolutionary biologists is who in a colony of wasps gets to be the queen and who serves as a worker.*
- *Among highly eusocial species, when an individual is born as an adult, its role is already decided and irreversibly fixed.*
- *Among primitively eusocial species, all adults are born equal and social interactions during the adult stage decide who becomes the queen and who a worker.*
- *One consequence of all adults being born nearly equal is that queens can be overthrown and some of the workers can become new queens.*
- *Among the many aspects of the lives of the wasps that the author and his colleagues have studied, perhaps the most intriguing is the phenomenon of queen succession.*

In 2010, my Hungarian friend and colleague Tamas Szekely, then at the University of Bath in the UK, invited me and a few others to write short inspiring essays to be interleaved between the technical chapters of the book *Social Behaviour: Genes, Ecology and Evolution* that he was editing along with Allen J. Moore and Jan Komdeur for Cambridge University Press.

I wrote a 1,500-word essay with the title 'In love with Ropalidia marginata: 34 years, and still going strong'. I am still in love, so I should now say '46 years, and still going strong'. Reflecting on the question of how people should choose the topic of their research, I wrote that in my opinion (or prejudice):

> "The research question should come first, and then one should choose a model organism that is best suited to the question. Methods should come last, and should be slaves at the service of the question and the animal, rather than the masters that dictate what we do. Nevertheless, I must confess that the research question and the model animal are hard to prioritise.
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> I think this is primarily because, though of greater importance, the question is abstract and kind of 'dead', but the study animal is alive and often rather cute. It is hard not to fall in love with your study animal. But is that a bad thing? I don't know – but I have been in love with my study animal for over 30 years, and no harm seems to have come of it so far."

The well-known insect physiologist Vincent Dethier, who also wrote science for the public as well as short stories and novels for children and adults, put it simply in his charming little book *To Know a Fly* (1962):

> "…how does one choose a proper species for study? The answer is simple. Let the species choose you."

The Indian paper wasp

I first encountered the Indian paper wasp (*Ropalidia marginata*) in the summer of 1969. The wasps had built several colonies on the windows of the Zoology and Botany Departments in what was then called Central College in Bangalore, where I pursued my BSc and MSc in zoology.

For the next five years, I often watched these wasp colonies with great fascination but with little technical knowledge. I was mesmerised by their coordinated social behaviour, their industriousness, their ability to return to their own nest without being confused by other nests only a few centimetres away, their aggressive mobbing of nestmates returning with food, and much more.

It surprised me greatly that my zoology teachers neither knew nor cared much about these live zoological specimens just outside the classroom; they cared only for the pickled specimens inside!

It was only when I came to the Indian Institute of Science to do a PhD in molecular biology and met Prof Madhav Gadgil at the Centre for Theoretical Studies that I was exposed to the writings of E.O. Wilson, Mary Jane West-Eberhard and others, and realised what a treasure I had been ogling at. With Gadgil's encouragement, I now began to go back to Central College during the weekends to study these wasp colonies as a hobby. The wasps had chosen me and I had fallen in love with them.

By the end of my PhD, the wasps had motivated me enough to shun the idea of going abroad on a post-doctoral fellowship to do more molecular biology and to stay back home to study the social biology of *Ropalidia marginata* as a professional scientist. My goal has been to understand everything humanly possible about this insect society.

I was soon joined by a large number of bright, young students equally enamoured by the goings-on in these wasp colonies. Together we have made many a discovery about the social life of this fascinatingly enigmatic, primitively eusocial wasp.

Not all wasps are social; most lead a solitary or parasitic mode of life. A minority of the species are social, and as a group, the wasps display a fine gradation of complexity in social organisation and colony size.

Those with the most complex social life are termed eusocial, meaning, truly social. Eusocial species live in colonies consisting of members belonging to two or more generations (usually mothers and their daughters). Only one reproduces, i.e. mates and lays eggs, usually the mother, who is called the queen. All the colony labour is performed by the remaining females, called the workers, who cooperatively build, maintain and guard the nest, forage for food and take care of the brood.

The males usually leave the nest of their birth and mate with virgin females from other nests and do not participate in the colony's social life.

Who the queen and who the worker?

A question of obvious interest to all evolutionary biologists, and indeed, to all humans, is who gets to be the queen and who serves as a worker. There are two kinds of species with regard to the mechanism by which queen and worker roles get allocated.

In the so-called highly eusocial species, the decision is made in the early larval stage through a process of developmental regulation. Thus, when an individual is born as an adult, its role is already decided and irreversibly fixed.

In the so-called primitively eusocial species, on the other hand, this decision is postponed until the wasps complete their metamorphosis and become adults. As a result, all adults are born equal and social interactions during the adult stage decide who becomes the queen and who becomes a worker – a process of social regulation.

R. marginata is a primitively eusocial wasp. There are two consequences of all adults being born nearly equal. One is that all individuals look alike, i.e. the queens and workers cannot be distinguished on the basis of their morphology. The second is that the roles of individuals are flexible so that individuals can switch from one to the other. As a result, queens can be overthrown and some of the workers can become new queens.

As one might imagine, the possibility of such queen succession creates a great potential for conflict and strife in the colony.

Among the many aspects of the lives of the wasps that we have studied and the many mysteries we have uncovered, perhaps the most intriguing concerns the phenomenon of queen succession and the associated behaviours of the queens and their successors.

Very early in my work with *R. marginata*, I was intrigued that the queens cannot be distinguished from the workers by their morphology; one had to witness them laying eggs or cut them open to see their ovaries.

So, I set out to see if they could be distinguished by their behaviour; perhaps the queens displayed some unique behaviour that the workers lacked. I examined all possible behaviours, my list of behaviours exceeded 100, but except for the act of laying eggs, there were no behaviours unique to the queens.

Sitters, fighters and foragers

Suspecting that the behavioural difference between the queen and the workers might be quantitative rather than qualitative, I calculated how each wasp, be it a queen or a worker, allocated its time between different behaviours.

Subjecting such time-activity budgets of all wasps to statistical analysis, my colleague N.V. Joshi and I discovered that the wasps in each colony could be classified into three groups, which we called sitters, fighters and foragers. As their names imply, the sitters were rather lazy individuals, the fighters were particularly aggressive toward other wasps in the colony, and the foragers were often busy going out to fetch food, and plant fibres with which to build nests.

As I have explained on a previous occasion, this classification was the starting point for much of what we discovered about the wasps in the following decades. The first question that arose from such a classification was: ‘where is the queen?’ Was she a sitter, a fighter or a forager?

We had good reason to suspect that the queen would be a fighter. In other primitively eusocial wasps, queens were known to be the most aggressive individuals in their colonies, using sheer physical harassment and intimidation to prevent their workers from reproducing and making sure that they work for the colony.

But our wasps seem not to have read the previous research papers – our queen was a meek and docile sitter. Contrary to what many people think, there is nothing better in research than failure to get the expected result. An unexpected result forces us to ask new questions and often leads to new discoveries. The question we were forced to ask here is: how does a meek and docile queen become a queen in the first place?

It is easy to see why the workers will accept an aggressive, bullying queen, but why accept and respect a non-aggressive, non-interactive individual as their queen? And respect they do, because no worker will ever challenge the queen nor attempt to lay eggs in her presence.

A meek and docile queen

To answer this question, I suggested to my PhD student Sudha Premnath to experimentally remove queens from colonies and witness the process of a worker becoming a queen. In collaboration with my postdoc, Anindya Sinha, she found that the worker who becomes the new queen indeed begins her career as a hyper-aggressive individual. That's why the rest of the workers accept her as their new queen. But curiously, she loses her aggression and becomes a typical meek and docile queen by the time she begins to lay eggs, which can be as soon as a week after the removal of the original queen.

We decided to call the hyper-aggressive worker who becomes the queen after the removal of the previous queen a 'potential queen'. The potential queen is thus 'the wasp who would be queen', in the title of this essay.

Good science never runs out of questions. The answer to every question spawns new questions, often hitherto unimaginable. At this stage of our research, we had the opportunity to unravel not one but two mysteries: the curious non-aggressive behaviour of the queen and the equally curious aggressive behaviour of the potential queen.

Before we come to the mystery of the potential queen, let me briefly describe some remarkable things we have discovered about the queen herself. Our finding that the queen begins her career as a very aggressive individual may have solved the problem of how she becomes a queen, but it raised the question of how she continues to maintain her status for many weeks, even months, after a mere one week of aggression. There are really two questions here. How does a meek and docile queen prevent workers from becoming new queens? And how does she ensure they actually work?

To explore how our queens prevent their workers from reproducing, we tested the hypothesis that, like the queens of highly eusocial species such as honey bees and ants, *R. marginata* queens may have defied their primitively eusocial label and evolved chemical weapons to control worker reproduction. Such chemicals are called pheromones.

My PhD student Annagiri Sumana took the lead to test this hypothesis and worked closely with my other students, Sujata Deshpande and Anindita Bhadra. They performed a clever experiment in which they separated the queen and some workers from the rest of the workers using a wire mesh screen. They found that the workers on the queen-less side of the wire mesh could not detect the queen's presence across the mesh. This suggests that any chemicals that the queen might use must be non-volatile.

Some of the many students who performed many of the experiments described here. Clockwise: Alok Bang, Anindita Bhadra, K. Chandrasekhar, Annagiri Sumana, Shakti Lamba and Nadia Bruyndonckx. Photos: RG Lab Collection

The non-volatile nature of the queen pheromone raised the question of how the workers perceive it when the queen is around. It was a lot of effort and a lot of fun to answer this question, and here we were greatly helped by a brilliant young mathematics undergraduate from IIT, Kanpur, Priya Iyer.

We wanted to test the obvious hypothesis that the workers obtained some of the queen's non-volatile pheromone whenever they physically interacted with her. But we did not know what the pheromone was and therefore had no way of detecting it directly.

Resorting to an indirect method, we reasoned that if the workers got the pheromone during interactions with the queen, the rate of interaction between the queen and the PQ (short for potential queen) must be faster than the time taken by the PQ to realise the absence of the queen and become hyper-aggressive. We thus measured the rates at which all individuals in the colony interacted with each other.

Using a fairly sophisticated mathematical procedure called Dijkstra's algorithm, Priya Iyer calculated the minimum time required for information to flow from the queen to the PQ.

It turned out that physical interaction was not the answer. The PQ realises the queen's absence much sooner than the time required for her to get the pheromone through direct interaction with the queen or even by interacting with a worker who might have had more frequent interactions with the queen. The PQ was really quick in realising the queen's absence; she needed less than 30 minutes to do so. The queen being a particularly non-interactive individual, there had to be another way for the workers to get the queen pheromone.

Another undergraduate, Saubhik Ghosh, then took the lead in discovering that the queen actually applied her pheromone onto the nest surface from where the PQ and other workers could pick it up.

Priya Iyer (1982-2021), a brilliant mathematical ecologist, my neighbour, student and colleague who tragically passed away on January 25, 2021. Photo: Vishwesha Guttal lab

There remained at least one more big mystery. The queen can possibly regulate worker reproduction with the help of a pheromone because the pheromone can inhibit the development of the ovaries of the workers. But how can the queen make sure that her workers actually work? There is no reason why the workers should obey just a pheromone and work. There had to be another way.

We had a great deal of fun solving this mystery, with the participation of a large number of additional students and interns, including Shakti Lamba visiting from the University of Oxford, Nadia Bruyndonckx visiting from the University of Lausanne, and a postdoc, K. Chandrasekhar, who had graduated from the Jawaharlal Nehru University in Delhi.

We showed that the workers themselves regulated the work of the colony – at least the work of foraging for food and feeding the larvae, without the involvement of the queen.

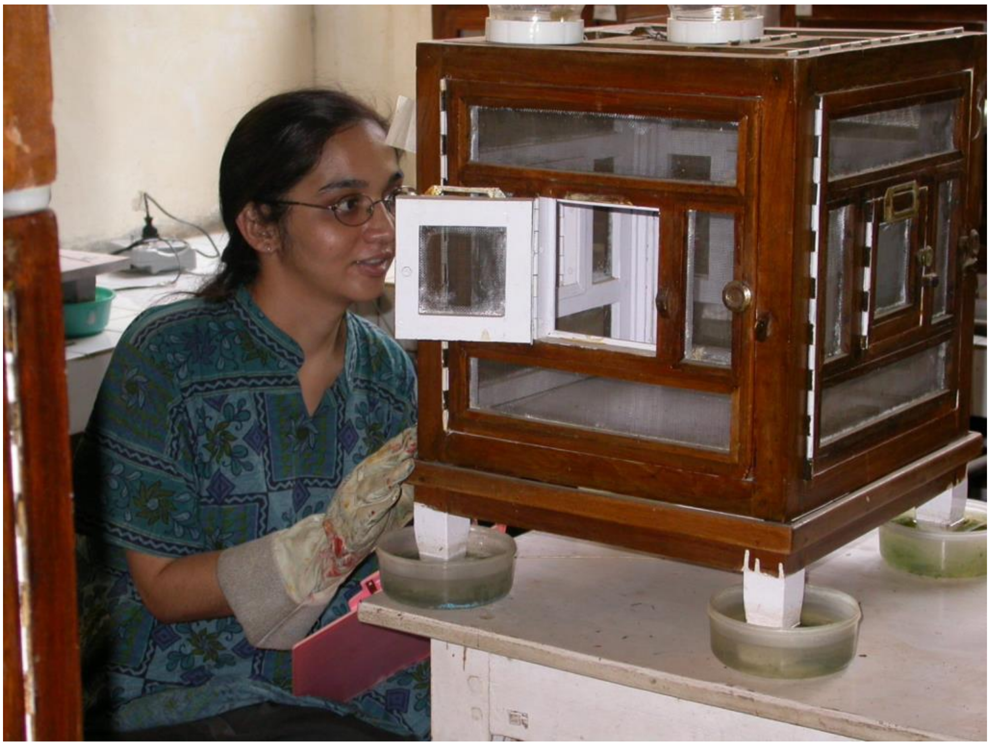
The potential queen

There were at least two mysteries that concerned the PQ. Why did she become hyper-aggressive during the first week of her tenure? Was it to suppress all other workers through aggression until she had time to make the queen pheromones? We found no evidence for this obvious hypothesis. Instead, we found that the hyper-aggression of the PQ was needed for her to develop her ovaries rapidly.

The biggest mystery was the identity of the PQ. As soon as we remove the queen – and we have done this scores of times – one and only one individual becomes hyper-aggressive and becomes the next queen if the original queen is not returned soon enough. Whenever I spoke of our studies of the wasps, people always wanted to know if we could predict the identity of the PQ even before removing the queen.

The short answer was 'no', we could not predict it. But the question became persistent and the answer a bit embarrassing. So I suggested to my student Sujata Deshpande that she take up this challenge and make it a goal of her PhD. She did, and working with many others in the lab, especially Sumana and Anindita, she performed many experiments and taught us a great deal about the wasps.

But the goal of identifying the PQ in the presence of the previous queen remained elusive. The PQ appeared not to be specialised in any way: she was not unique in her body size, dominance rank, any other behaviour or even by the level of her ovarian development. She seemed to be a nondescript worker.



Sujata Deshpande at work in the laboratory attempting to identify the PQ.
Photo: RG Lab Collection

Could it be that the PQ was undecided until we actually removed the queen? This was very unlikely because there was no apparent conflict, no scramble competition among the workers for obtaining the extremely important position of the next queen of the colony. One and only one individual promptly became hyper-aggressive as if she knew that was her role, and she was never challenged by any of the other workers as if they all knew whose turn it was.

Indeed, my student Anindita Bhadra showed by means of a very clever experiment that there was a heir-designate whose identity was known to all the wasps even though she was cryptic to us. The cleverest part of this experiment was to make a wasp who was not the heir designate to *believe* that she was the heir designate.

This Anindita did by isolating some wasps from the queen as well as the true heir-designate. Now, one of the isolated wasps became hyper-aggressive and what we might call a 'false PQ'. Then we brought the false PQ and the actual heir-designate face-to-face. At this point, the false PQ seemed to realise her mistake and voluntarily gave up her aggression and went back to work. And the true heir designate became hyper-aggressive and a true PQ, without ever being challenged by anyone, not even the false PQ.

Evidently, the identity of the true heir-designate was known to all the wasps, including the false PQ.

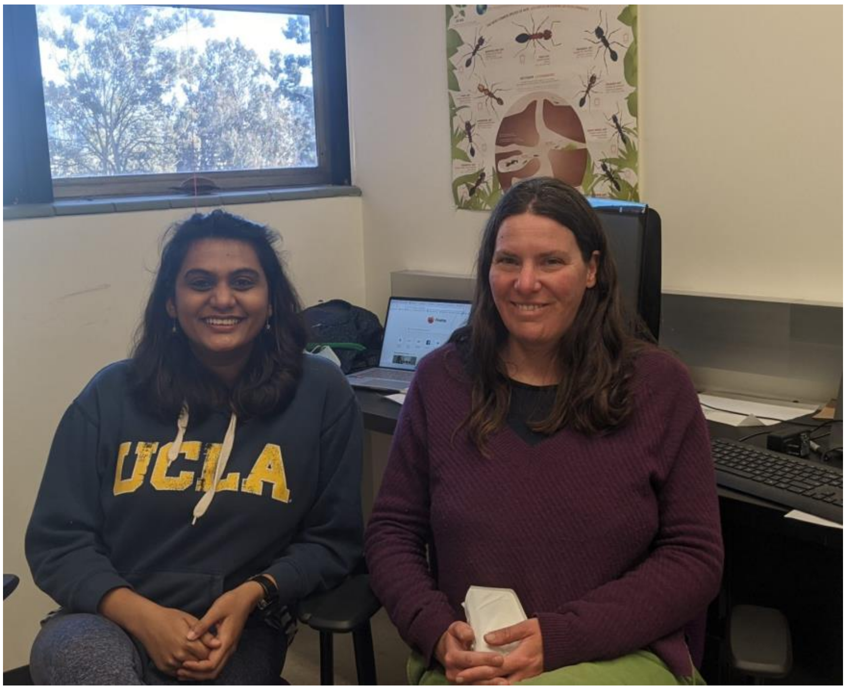
Another student, Alok Bang, performed what we called a 'serial PQ removal' experiment and showed that there is not just one but a series of heir designates who patiently await their turn until the previous one in the line is removed.

All this makes it even more embarrassing that we cannot identify the PQ in the presence of the previous queen. Over the years, I had come to accept defeat and had even begun to joke that I was proud of my wasps I so love because they keep some secrets to themselves! Maybe, this is one possible harm that comes from falling in love with your study animal.

What's special about the PQ?

But my youngest student, Nitika Sharma, did not accept defeat so easily. Although the topic of her thesis was to examine whether the wasps had any preferred locations where they would sit on the nest, apparently she also kept thinking about how we could identify the PQ.

I was not quite aware that our inability to identify the PQ was smarting her. After going to the University of California, Los Angeles, to work as a postdoctoral researcher with Prof Noa Pinter-Wollman on such creatures as ants and vultures, Nitika came up with a plan to avenge our defeat at the hands of the *Ropalidia* PQ.



Postdoc Nitika Sharma (left) and her mentor Noa Pinter-Wolman. Photo: Guo Xiaohui

Prof Wollman is a world authority and one of the pioneers in establishing a novel statistical technique called 'multilayer network analysis'.

We usually construct separate networks for each social situation. For example, we may construct a network of feeding behaviour considering all the acts of mutual feeding by all individuals in the colony. In such a network, a central individual is one who feeds most other individuals or is fed by the largest number of other individuals. Alternatively, we may construct a network of grooming by considering who grooms whom and how often. Here, a central individual is one who grooms the most number of individuals or is groomed by the most number of individuals. But this method cannot tell us who is most central to the network considering both feeding and grooming simultaneously and such a method did not reveal the identity of the PQ.

A multilayer network, on the other hand, "accounts for the differential impacts of different social situations on each other as well as on the overall global social dynamics of the society". Using this technique, one can calculate a mathematical quantity called 'versatility', which captures "the importance of an individual in the society while accounting for all social situations simultaneously".

Thus, Nitika applied multilayer network analysis to the *R. marginata* colony, simultaneously considering the four social situations: aggression, liquid food exchange, solid food exchange and spatial overlap, on the nest. Sure enough, she found that in all five colonies she studied, the PQ is the most 'versatile' individual in the colony. Thus we may have finally solved the mystery of the identity of the wasp who would be queen.

Does this mean all previous efforts to predict the PQ were a waste of time, and we should be embarrassed about those experiments? Not in the least. As I said in the previous article of this column, "For the scientist-detective, every wrong turn, every misguided chase up a blind alley means more knowledge to gain." That's the joy of science.

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