How to Design Experiments in Animal Behaviour*

9. Why Do Wasps Fight? Part 1

Raghavendra Gadagkar

Continuing to explore the intriguing world of the Indian paper wasp *Ropalidia marginata*, here we will focus on their fighting behaviour. When wasps fight, there is, as expected, a winner and a loser. The winner is said to have shown dominance behaviour, and the loser is said to have shown subordinate behaviour. What is the function of such dominance-subordinate behaviour? We saw in the 7th article in this series [1] that in the context of founding new nests, wasps fight to decide who would be the queen and who would be the worker. We then saw in the 8th article in this series [2] that when wasps have to decide who would be their next queen in a mature colony, they do not decide by fighting, although they fight for other reasons. We will see in this article that workers continue to show dominance-subordinate behaviour in mature colonies. What is the function of this aggression displayed by the workers? In this article, I will describe two simple experiments that help us answer this question, and show that the function of wasp aggression can be quite different in different contexts .

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How Do the Wasps Fight?

For the reader who might read this article without having read the previous three articles in this series, I should say briefly that in the fourth consecutive article in this series, I am describing simple experiments performed in my laboratory by many bright students passionately interested and curious about how a wasp society functions. We use the Indian paper wasp *Ropalidia marginata*,

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Figure 1. A large nest of the Indian paper wasp *Ropalidia marginata*, showing the nest, brood and adults; the white capped cells are the pupae, a nearly full-grown larva can be seen at the nest periphery at the 1 o'clock position. (Photo: Dr Thresiamma Varghese.)

Keywords
Ropalidia marginata, dominance-subordinate behaviour, regulation of foraging, function of fighting.

whose colonies comprise a single fertile queen and varying numbers of non-reproducing workers, although the queens and workers cannot be distinguished by their morphology. The wasps show efficient division of labour, communication and coordination while at the same time finely balancing the opposing forces of cooperation and conflict. In the preceding articles, we have seen how to perform simple experiments that help us to understand how the wasps decide who will be the queen and who will be the worker when they are starting new nests and how they decide which one of the workers will become the next queen if the original queen dies. In this article, we are seeking to understand why the wasps appear to fight in mature colonies even though the outcome of fighting does not determine who the next queen will be. As in all the articles in this series, the emphasis will be on the design of simple, clever experiments that require little or no sophisticated or expensive facilities. As a side effect, we will also learn many fascinating details of this wasp society.

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I am using 'fight' as a shorthand for a complex and diverse set of behaviours that the adult wasps display in many different contexts. When I first began to study these wasps in the 1970s and

80s, I made a list of the different behaviours they show, in plain English. With nothing more than paper and pencil, I spent many days transported into a whole new world. It was not so different and, in many ways, was more interesting than people-watching. I found the wasps sitting, sitting with raised antennae, sitting with raised antennae and raised wings, walking, inspecting the cells of the nest, building the nest, exchanging food, liquid or building material with each other, feeding the larvae, leaving the nest, returning with food, liquid, building material or nothing, and so on. Classifying these behaviours as finely as common sense dictated, I came up with a list of about 100 behaviours. I did not know at that time that the catalogue of behaviours I had thus produced is called an 'ethogram'. Nor did I know that whatever process I had used to decide where one behaviour ends and another begins, is called 'discretization'. Preparing an ethogram and using an appropriate level of discretization are important first steps in the study of any new species. I will postpone providing a more detailed description of these processes to a future article, so that we can now quickly get back to discussing fighting behaviour of the wasps.

Among the items in the *R. marginata* ethogram, I found some items that seemed agonistic, suggesting a conflict between the interacting pairs of wasps. Sometimes wasps pecked at each other much like birds do when they are expressing dominance over each other. Hens can be arranged in a dominance hierarchy depending on who pecks whom and such a hierarchy is tellingly called a 'pecking order'. I, therefore, considered the wasp who pecked as being dominant over the wasp who was being pecked, the latter I considered subordinate. The wasps showed other forms of dominance-subordinate behaviours. These included chasing and being chased, nibbling and being nibbled, and yet others, to describe which I will need separate sentences! One wasp climbed onto another and attempted to bite its mouthparts, while the latter crouched in an attempt to avoid being so bitten. I labelled these behaviours as 'attack' (dominant) and 'being attacked' (subordinate). At other times a wasp held a body part, such as a leg, an

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antenna, or a wing of another wasp in its mouth and immobilized it. This could last for many seconds, during which the wasp being held in the mouth of another wasp could not move, but it moved away as soon as it was released. Finally, and very rarely, two wasps physically grappled with each other and rolled over, often losing their grip on the nest and falling to the ground. I called this a 'falling fight'—this is the only agonistic behaviour where I could not decide who was dominant and who was subordinate—when counting the numbers of acts of dominance and subordinate behaviours, I considered both wasps engaged in a falling fight as having shown dominance behaviour and none as having shown subordinate behaviour. Even more rarely, one wasp (the dominant, by definition), stings or attempts to sting another wasp (subordinate, by definition).

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Clearly, the intensity of aggression varies greatly between these different acts of dominance-subordinate behaviours and perhaps even between different repetitions of the same behaviours—attacks can be mild or intense. Nevertheless, and as a first approximation, we simply add up the numbers of times a wasp shows any and all the types of dominance or subordinate behaviours described above, to obtain a quantitative estimate of its rate of dominance or subordinate behaviours respectively. Since the durations of our observations of different wasps vary, we normalize our counts by dividing them by the numbers of hours of observation and compute the frequencies per hour of dominance behaviour and subordinate behaviour [3]. In this article, the reader will repeatedly encounter such estimates, which will be referred to as 'freq/hr of DB'. I will now proceed to describe experiments designed to understand the possible functions of dominance behaviour. We will not be directly using the frequencies of subordinate behaviours. In this article, we will examine the possible functions of dominance behaviour in mature colonies, as opposed to the context of new nest foundation.

Why Do the Wasps Fight?

Background

We have already seen in previous articles that *R. marginata* wasps fight in the context of new nest foundation, in order to decide who would be the queen and who would be the worker [1]. We have also seen from another previous article that in mature colonies, when the wasps have to choose a new queen to replace a lost or dead queen, they do not decide by fighting [2], although the potential queen becomes hyper-aggressive—but that is after she becomes a potential queen, not before. What then is the function of the dominance-subordinate behaviour shown by the workers in mature colonies? A hint comes from asking a different set of questions. If the queen is such a meek and docile individual not showing any dominance behaviour toward her workers, how does she prevent them from developing their ovaries and laying eggs—how does she maintain her monopoly on egg laying in the colony? The answer as we have seen in the previous article in this series [2] is that she does so by producing pheromones from her Dufour's gland and rubbing it on the surface of the nest. That raises another question. How does the queen regulate the foraging and other activities of the workers?—she does not show aggression toward them and pheromones may not be adequate to make the workers work. When we attempted to answer this question, we found no evidence that the queen regulates the foraging and larval feeding behaviours of the workers, in the first place. The workers continue to bring food and feed the larvae at the same rate, whether or not the queen is present. This suggests that the workers must themselves be regulating each other's work, perhaps through a process of decentralized self-organization. But how do they do it?

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We know that honey bee workers self-regulate foraging behaviour, and even indicate the most preferred item of food (nectar, pollen or water) to the foragers by eagerly downloading foragers returning with the preferred item and making the foragers bringing non-preferred items wait [4]. Could *R. marginata* be doing something

like this? Unlike honey bees, our wasps are few in number and the foragers even fewer so that it seems unlikely that making them wait after the act of foraging will be efficient enough. Instead, it may rather require stimulating the wasps to go out and look for food. Might this be accomplished by the dominance-subordinate behaviour shown by the workers? This indeed seems to be the method that queens use to stimulate foraging by their workers in wasp species where queens are dominant and regulate both the reproductive and non-reproductive activities of their workers using physical dominance. So, in *R. marginata*, since the queen is docile and does not regulate worker activity, the workers may have taken over that role, using the same mechanism.

There is a good reason to follow-up on this speculation. In many studies we have found that the amount of aggression received by the foragers is greater than that received by non-foragers, the rates at which foragers forage is positively correlated with the rates of dominance behaviour they receive. Seen in another way, there is a positive correlation between the fraction of the colony's total dominance behaviour received by a forager and the fraction of her contribution to the colony's total foraging effort. Moreover, because the wasps that show dominance behaviour to the foragers are those that feed the larvae, they are expected to have information about the hunger levels of the colony. Based on these speculations and preliminary observations, we proposed the following hypothesis.

Intra-nidal workers (those on the nest) convey information about the hunger levels in the colony by showing dominance behaviour toward the foragers who bring food to the colony.

Hypothesis

The function of worker dominance-subordinate behaviour is to regulate each other's foraging in a decentralized, self-organized manner. Intra-nidal workers (those on the nest) convey information about the hunger levels in the colony by showing dominance behaviour toward the foragers who bring food to the colony. This hypothesis leads to two predictions, which we will test below, one at a time.

Figure 2. A Photo gallery of my students who conducted the experiments described in this article. **(From left to right, upper row)**: Nadia Bruyndonckx, Sujata Deshpande; **(lower row)**: Shakti Lamba, Kannepalli Chandrasekhar.

Prediction 1

A reduction in the demand for food should cause a reduction in the levels of dominance-subordinate behaviour among the workers.

Experimental Design

My PhD student Sujata Deshpande (then Sujata Kardile) and Nadia Bruyndonckx (*Figure* 2, upper row), a visiting student from the University of Lausanne, Switzerland, participated in this study, which we conducted on 11 nests. Each nest was used only once and, as is our usual practice, all the wasps were marked for individual identification. The experiment lasted three days. On day 1, Sujata and Nadia observed the unmanipulated colonies. Their behavioural observation involved recording every occurrence of bringing food, feeding the larvae and dominance-subordinate behaviour, in 30–40 5-min blocks of time with one-minute breaks in between two consecutive blocks. In total, they observed for

5–6 hrs between 8 am and 6 pm. Our aim was to study the effect of reducing the demand for food in the colony. On day 2, Sujata and Nadia, therefore, hand-fed the wasps, in addition to the food that they brought and consumed on their own. They offered two final instar *Corcyra cephalonica* larvae, per every 10 wasp larvae present in the nest, every hour, from 8 am to 6 pm. They offered the food to the adult wasp who readily took it, distributed it among themselves and also fed the larvae. On day 3, they made behavioural observations as on day 1. From these observations, we obtained the frequencies per hour of bring food, feed larvae and dominance behaviour, for each wasp, separately for days 1 and 3.

Results

Our prediction was that the rates of dominance behaviour in the colony should have come down on day 3 compared to day 1. But before we are entitled to make and interpret that comparison, some precautions are necessary. First, we confirmed that the total number of wasps on the nest was the same on day three as it was on day 1. Next, we wanted proof that our feeding the wasps on day two had made a difference. Indeed, it had—on day 3, significantly less food was brought to the nest and the larvae were fed significantly less often, compared to day 1. Finally, as predicted, the frequency per hour of dominance behaviour on day three was significantly less than it was on day one. Then we focused specifically on the foragers. In the 11 nests put together, 49 wasps had acted as foragers on day one, a forager being defined as an individual who had brought back food, building material or water to the nest at least twice. Considering only these 49 wasps, we found once again that they had significantly reduced the rate of bringing food and they received significantly less dominance behaviour directed towards them on day three as compared to day 1 (*Figure* 3). Thus, our prediction was clearly borne out—a reduced demand for food resulted in reduced dominance behaviour. The fact that this result was also true when we only considered the foragers, further strengthened the case. It does appear that worker

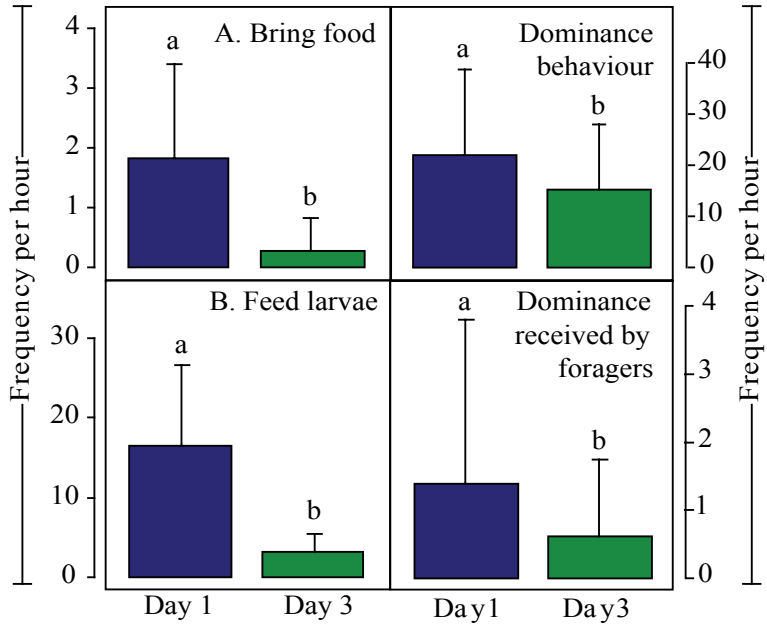

Figure 3. Excess feeding experiment. Comparisons of the rates of bring food, feed larvae, and dominance behaviour among workers and dominance received by foragers, on day 1 (normal colony) and day 3 (1 day after excess feeding). Bars shown are the means and SDs across 11 nests. For all variables, values on day 1 are, as indicated by the different letters on the bars, significantly greater than the corresponding values on day 3 (two-tailed Wilcoxon matched-pairs, signed-rank tests; $P < 0.05$). [Redrawn with permission from N. Bruyndonckx, S. P. Kardile and R. Gadagkar, Dominance behaviour and regulation of foraging in the primitively eusocial wasp *Ropalidia marginata* (Lep.) (Hymenoptera: Vespidae), *Behavioural Processes*, 72, pp.100–103, 2006 (Copyright 2006, Elsevier).]

dominance behaviour is used to regulate foraging—workers do seem to signal colony hunger levels to the foragers by directing dominance behaviour towards them [5]. But there is a second prediction and that too needs to be borne out before we can really have confidence in our hypothesis.

Prediction 2

An increase in the demand for food should cause an increase in the levels of dominance-subordinate behaviour among the workers.

Experimental Design

Shakti Lamba who was taking a break between her Masters degree in the University of Oxford and a PhD degree in the University College, London and spending time in my laboratory, and my post-doc Kannepalli Chandrasekhar (Chandu) (*Figure* 2, lower row) participated in this study, which we conducted on another set of eight nests. This experiment also lasted for three days. On day 1, Shakti and Chandu allowed the wasps to forage freely by

keeping the doors of the cages open. On day 2, they closed the doors of the cages and did not provide any food to the wasps. On day 3, they once again opened the doors of the cages and allowed the wasps to forage freely. Thus, on the second day, instead of feeding the wasps, they starved them. As might be expected, this was easy to do—they simply closed the doors of the cages and did not provide any food to the wasps. I should emphasize that in the previous 'excess feeding' experiment, where the wasps foraged on their own on days 1 and 3 and Nadia and Sujata had hand-fed the wasps, in excess of what the food they brought and consumed on their own on day 2. But in this experiment, Shakti and Chandu did not hand-feed the wasps on any day. They allowed the wasps to forage on their own on days 1 ad 3, and they deprived them even of that possibility on day 2. Unlike in the previous experiment where Nadia and Sujata could not make observations on day 2 when the wasps were being hand-fed, in this experiment, Shakti and Chandu took turns to make observations on all three days. We used these observations to calculate their rates of foraging and dominance behaviour on all three days.

Results

As in the previous experiment, we justified our comparison of the behaviour of the wasps on different days of the experiment by showing that the number of wasps present on the nest did not differ significantly between days 1, 2 and 3. Again, as in the previous experiment, we verified that our treatment—in this case, starvation—had some effect on the wasps. It did indeed. The number of times the wasps left their nests, presumably in search of food, increased significantly on day 2, and once again dropped on day 3, to become comparable to the corresponding rates on day 1. While no food was obviously brought to the nest on day 2, the rates at which food was brought back to the nest on day 3 was not significantly different from the corresponding rates on day 1. Finally, and as per our prediction, the moderate rates of dominance behaviour shown by the wasps on day 1 increased significantly on day 2, when the wasps were being starved. And they came down

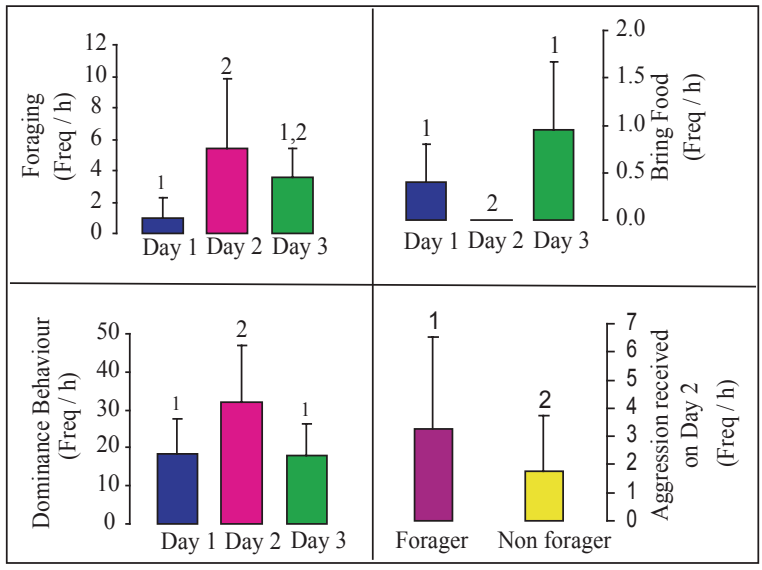

Figure 4. Food deprivation experiment. Comparison of mean and SD of frequency per hour of dominance behaviour, foraging attempts, and bring food on day 1 (normal colony), day 2 (food deprived by preventing foraging), and day 3 (foraging permitted). Different numbers on the bars indicate a significant difference between the bars (two-tailed, Wilcoxon matched-pairs test; n=8). [Redrawn with permission from S. Lamba, K. Chandrasekhar and R. Gadagkar, Signaling hunger through aggression—the regulation of foraging in a primitively eusocial wasp, *Naturwissenschaften*, 95, pp.677–680, 2008, DOI 10.1007/s00114-008-0369-9 (Copyright 2008, Springer).]

again, when the doors to the cages were opened and the wasps resumed foraging on day 3, to become comparable to the rates on day 1. The rates of dominance behaviour on day 2, that were directed toward individuals identified by us as foragers on day 1 were significantly greater than the rates of dominance behaviour directed toward individuals we had identified as non-foragers on day 1 (*Figure* 4). This means that on day 2, the hungry wasps directed their aggression more specifically to those wasps which were known to have previously brought food to the nest [6].

Conclusion

The results of the two experiments described above, strongly support our hypothesis that dominance-subordinate behaviour shown by the workers in *R. marginata* is used to regulate each other's foraging in a decentralized, self-organized manner and that intranidal workers convey information about the hunger levels in the colony by showing dominance behaviour toward to the foragers who bring food to the colony. Let us recall that in the context of new nest foundation, dominance-subordinate behaviour is used by these same wasps to decide who will be the queen and who will be the worker [1]. Now we see that in mature colonies work-

ers use dominance behaviour to regulate each other's foraging. Thus, this is the second function of aggression in this species—function being dependent on the context, making aggression a multifaceted signal.

Reflections

As has been my practice, I will attempt some reflection at the end of describing these experiments. The two experiments described in this article exemplify the various themes that I have been exposing in this series—low-cost or no cost, no sophisticated equipment, just thinking and an abundant supply of passionate and competent students.

But there is one additional point that I would like to reflect upon. The two experiments described in this article were designed to test the hypothesis that worker dominance-subordinate behaviour is used for the decentralized self-regulation of foraging. This hypothesis arose from the observation of a positive correlation between two variables, namely, the amount of dominance behaviour received by wasps and their foraging effort. Just because there is a correlation between dominance received and foraging effort, it does not necessarily mean that receipt of dominance behaviour causes the wasps to forage. Both dominance behaviour and foraging may be caused, i.e., may be independently correlated with some other common factor, giving the illusion that one of them causes the other. It is a well-known and yet, frequently committed mistake to infer causation from correlation. Let us consider a simple imaginary example. There may well be a strong positive correlation between the number of hospitals and the number of deaths. This does not mean that hospitals caused deaths. Both the number of hospitals and the number of deaths are likely to be independently correlated with a third variable namely, population size. Higher population size leads to more hospitals, and independent of hospitals, or, despite hospitals, higher population sizes will witness more deaths. If we hold the population size constant, the positive correlation between hospitals and deaths might break

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down and indeed, we might even find a negative correlation between the number of hospitals and the number of deaths. In different cities with the same population size, there may well be fewer deaths in those cities with more hospitals. In this case, we could disentangle the correlation between the number of hospitals and the number of deaths because we could guess the third variable namely, population size, and hold it constant. In most cases, this is not possible.

The next best option is to change the value of the variable expected to be the causative factor keeping everything else constant and see whether the other variable changes as expected. This strategy was possible in our situation. But the situation was a little more complicated. Let us analyse it in some detail. Our original hypothesis was that receiving dominance behaviour induces the wasps to forage. According to the logic we have just outlined, we should change the level of dominance behaviour and see a corresponding change in the foraging effort. But how can we change the rates at which the wasps show dominance behaviour? So, we elaborate our argument a little more and hypothesize that wasps staying at the nest use dominance behaviour to convey hunger signals to the wasps who act as foragers. This means that dominance behaviour should increase when there is a greater demand for food and should decrease when there is less demand for food. In other words, we can increase or decrease the rates of dominance behaviour indirectly, by changing the demand for food. Now, the demand for food is more easily manipulated. Hence, we fed the wasps excessively and thereby reduced the demand for food, and we expected, and we found, a decrease in dominance behaviour, especially that directed towards foragers. Conversely, we increased the demand for food by starving the wasps, and we expected, and we found, an increase in dominance behaviour, especially that directed towards foragers. Only based on the results of both these experiments can we conclude that there is a causal relationship between demand for food and dominance behaviour and thus, have confidence in our hypothesis that the function of worker dominance behaviour is to regulate foraging in a decen-

tralized self-organized manner.

There are two more contexts in which *R. marginata* wasps show dominance behaviour, and in the next article, I will describe experiments designed to understand their respective functions.

Acknowledgements

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Suggested Reading

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