How to Design Experiments in Animal Behaviour*

10. Why Do Wasps Fight? Part 2

Raghavendra Gadagkar

Continuing to explore the intriguing world of the Indian paper wasp *Ropalidia marginata* for one last time, here we will focus on the function of fighting behaviour in two additional contexts (i) the hyper-aggression of the potential queen during queen succession and (ii) during encounters with non-nestmate wasps. We will see again that the function of fighting is different in different contexts. We have already seen two different functions of fighting in two different contexts—to decide who will be the queen and who will be the worker in the context of founding new nests, and to regulate foraging in mature colonies by conveying colony hunger levels to foragers. Here we will see that the function of the potential queen's hyper-aggression is to boost her own ovarian development and the function of aggression towards non-nestmates is to keep them away, and if necessary, to kill! As before, our primary focus will be on how to design simple experiments that will help answer a direct question, while minimising the need for expensive equipment or other facilities.

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http://ces.iisc.ac.in/hpg/ragh.
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In the last few articles in this series, we have been using the Indian paper wasp *Ropalidia marginata* (*Figure* 1) to illustrate the design of experiments. Along the way, we have learnt many interesting facts about this remarkable insect society. In the sixth article of this series [1], we saw how simple experiments address the question of why male wasps are lazy. The experiments showed that males can indeed work, at least to feed the larvae, if they have access to enough food and if they have the opportunity to do so. In the seventh article [2], we saw that in the context of founding new nests, the wasps fight to decide who will be the queen

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Figure 1. A nest of the Indian paper wasp *Ropalidia marginata*, showing the nest, some adult wasps and plenty of pupae and large larvae. (Photo: Dr Thresiamma Varghese)

Keywords

Ropalidia marginata, dominance behaviour, hyper-aggression, non-nestmates, potential queen, ovarian development, ovarian index.

In this final article on *R. marginata*, I will describe some more simple experiments designed to understand the function of dominance behaviour in two other contexts, namely (i) the hyper-aggression of the potential queen during queen succession and (ii) the intense aggression shown towards non-nestmates.

and who will be the worker. As might be expected, the winner becomes the queen, and the loser becomes the worker. In the eighth article [3], we saw that wasps continue to fight in mature colonies but do not use fighting to decide who their next queen would be. Instead, we saw that queen succession is based on a long queue of pre-designated potential queens (PQ) who periodically take over the role of the queen without overt conflict. The continued fighting (dominance-subordinate behaviour) (albeit at a lower level) of the wasps in mature colonies is used instead to regulate foraging by conveying hunger levels in the colony to the foragers, as we saw in the ninth article [4]. In this final article on *R. marginata*, I will describe some more simple experiments designed to understand the function of dominance behaviour in two other contexts, namely (i) the hyper-aggression of the potential queen during queen succession and (ii) the intense aggression shown towards non-nestmates.

Figure 2. A Photo gallery of my students who conducted the experiments described in this article. **(From left to right, upper row)** Shakti Lamba, Yasmin Claire Kazi, Sujata Deshpande; **(lower row)** Meghana Natesh, Anindita Bhadra, Arun Venkataraman.

What is the Function of the Potential Queen's Hyper-Aggression?

Background

We have seen from a previous article in this series, that the queens of *R. marginata* are remarkably meek and docile individuals, seldom participating in dominance-subordinate interactions or any other kinds of interactions with their workers. Nevertheless, they maintain complete reproductive monopoly and seem to do this with the help of non-volatile pheromones that they apply to the nest surface. We have also seen that upon the loss or removal of the queen in a colony, one of the workers becomes hyper-aggressive, increasing her levels of dominance behaviour several-fold and that if the queen is not returned, she goes on to become the next queen of the colony in about a week's time. During this period, the potential queen shows dominance behaviour to all the remaining workers, and the workers do not show any dominance behaviour towards her—the hyper-aggressive potential queen is thus unchallenged [3, 5]. Detailed studies of such potential queens from the time they become hyper-aggressive upon the removal of the previous queen until they start laying eggs have shown that in about a week's time, potential queens lose their aggression, develop their ovaries and alter their pheromone profile from that of a worker to that of a queen [6]. The question that

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we are interested in here concerns the hyper-aggression of the potential queen during the first week after the removal of the previous queen. What is the function of this hyper-aggression shown unidirectionally by the potential queen? I was fortunate to be able to put together a large research team to tackle this question, come up with hypotheses, design experiments and interpret the results. The team included Shakti Lamba and Sujata Deshpande, who also conducted the experiments described in the previous article [4], Anindita Bhadra whom we met in a previous article in this series [3], and two undergraduate students Yasmin Claire Kazi from Bangalore and Meghana Natesh from Delhi (*Figure* 2).

Hypothesis 1

The hyper-aggression of the potential queen serves to suppress the other workers in the colony while she establishes and consolidates her position as the next queen.

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Predictions

If the potential queen does indeed use hyper-aggression as a mechanism to suppress workers who might constitute a threat to her, then the amount of aggression she shows should increase with the number of workers in the nest, their dominance status in the colony, and the state of their ovarian development.

Experimental Design

This being a large study, we used 45 nests ranging in size from 3 to 14 adult wasps. Because these experiments involved an unusually large number of observers, we took an additional precaution. Each observer made preliminary observations independent of each other, and then we compared data from the different observers and proceeded to the final experiment only after the inter-observer disagreement was less than 5%. Other aspects of the experimental design were similar to what we have seen in the previous four articles in this series. All individuals were marked for unique identification, the presence or absence of wasps was noted through a census every night or every other night, and maps of the nest were maintained to keep track of the brood. The experiment lasted two days per nest, with observations on un-manipulated colonies on day-1 and observations after removing the queen on day-2. All the wasps were collected and dissected after the experiment to measure their ovarian development. We were particularly interested in the rates of dominance behaviour shown by the potential queens on day-2 and in the identity of the individuals to whom such aggression was shown. To test the predictions that the rate of dominance behaviour shown by the potential queen would be related to the number of wasps present on the nest, their dominance rank or their ovarian development, we needed to measure a number of quantities. These are;

1. Dominance behaviour of the potential queen: The frequency per hour of dominance behaviour shown by the potential queen was obtained from our observations on day-2 when we removed the queen and, as expected, one of the wasps revealed herself as the potential queen, by her unmistakable hyper-aggression.

2. Number of wasps present on the nest: We got this from the census data, as described above.

3. Dominance ranks of the wasps (other than the PQ): Dominance ranks could not be measured on day-2 because the potential queen was then hyper-aggressive, and the rest of the workers interacted little with each other. Hence, we used our observations on day-1 when the queen was still present to calculate the dominance ranks of the different workers. We have developed a method of assigning a unique dominance rank for each wasp in the colony. Without going into all the details, I will just say that in assigning this rank, we take into consideration, the amount of dominance behaviour a wasp shows to different wasps, the amount of dominance behaviour her victims show to other wasps, as well as the amount of dominance behaviour she receives and the amount of dominance behaviour received by those who show dominance to her [3–5]. I might also add that the actual dominance ranks of the wasps would, of course, depend on the total number of wasps in the nest, and this number varies from nest to nest. To make the dominance ranks comparable across nests, we divided the ranks by the number of individuals in the nest to obtain a 'normalized' dominance rank for each wasp.

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4. Ovarian indices of the wasps: Over the years, we have developed a method to represent the state of ovarian development of wasps by a single number that we call the ovarian index. We obtain the ovarian index by dissecting the wasps and measuring several aspects of their ovaries such as the length and width of the largest proximal oocyte, average length and average width of all six proximal oocytes, total number of oocytes, total number of mature oocytes and the total number of oocytes with yolk.

These values are then reduced to a single number using a method called 'Principal Components Analysis'. Without going into more details, it suffices to say here that this method allows us to produce a single number for each wasp that has in it the maximum possible information about all aspects of its ovarian development and such that the number is comparable across different wasps in different nests [5]. I should add that the ovarian index obtained in this way can be either a positive or a negative number. Once we computed the ovarian index for each wasp, we ranked all the individuals in each colony by their ovarian indices, and, as with dominance ranks, we normalized the ovarian ranks by dividing them by the number of individuals in the colony. These normalized ovarian ranks can now be compared across nests.

Now, we are ready to test the three predictions. To test the prediction that the potential queen's dominance should increase with the number of wasps on the nest, we simply regressed the frequency per hour of dominance behaviour shown by the potential queen on the number of wasps present on the nest. Testing the predictions that the dominance behaviour of the potential queen was disproportionately directed towards high-ranking individuals and individuals with better-developed ovaries, was a little more complicated. Considering the dominance behaviour actually received by each wasp, we computed the deviation from what we might have expected if the potential queen showed dominance behaviour equally to all wasps. Because the potential queens on different nests may show different absolute amounts of dominance behaviour, such deviations cannot be directly compared across nests. To make the values of such deviations comparable across nests, we divided the deviations by the total number of acts of dominance shown by the potential queen. We then regressed the 'normalized' deviation from expectation in dominance behaviour against the 'normalized' dominance ranks and the 'normalized' ovarian indices of the different wasps.

Results

The results of this experiment are simple and straightforward, although somewhat surprising. We found no evidence that the rate of dominance behaviour shown by the potential queen had any significant relationship either with the number of wasps (nestmates), their dominance ranks or their ovarian indices (*Figure* 3). Hence, we are forced to reject the hypothesis that the potential queen uses her hyper-aggression to suppress workers who might be a threat to her. And this means that we need another hypothesis.

If aggression is necessary for the potential queen to develop her ovaries, then a potential queen who does not have the opportunity to show aggression should take longer to start laying eggs compared to a potential queen who does have the opportunity to show aggression.

Hypothesis 2

The hyper-aggression of the potential queen is necessary for the rapid development of her own ovaries.

Prediction

If aggression is necessary for the potential queen to develop her ovaries, then a potential queen who does not have the opportunity to show aggression should take longer to start laying eggs compared to a potential queen who does have the opportunity to show aggression.

Experimental Design

It is easily possible to create a situation where there is a lone potential queen with no one to show aggression to, by simply

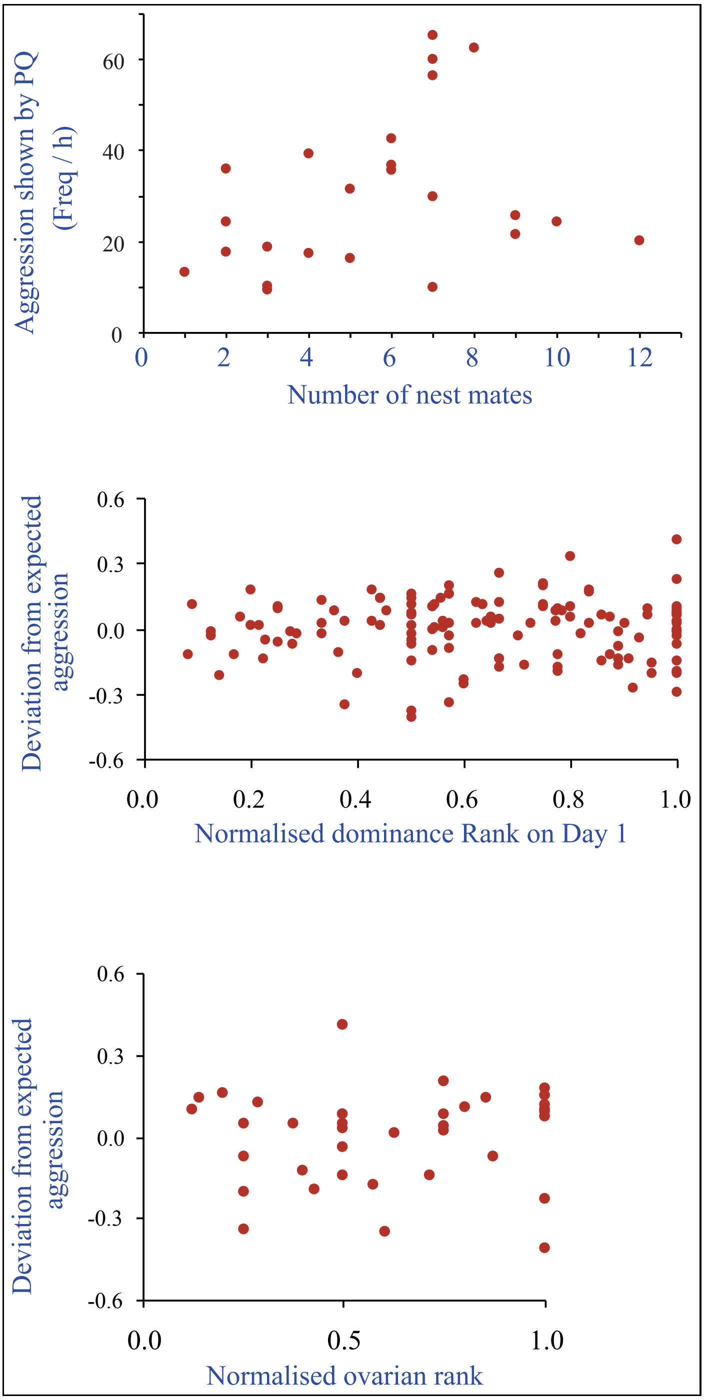

Figure 3. **(Top)** Aggression shown by PQ, plotted against the number of her nest mates. Slope of the linear regression isn't significantly different from 0 (p = 0.17; r^2 = 0.085, N = 24). **(Mid)** Deviation from expected aggression for each wasp plotted against her normalised dominance rank on day 1. Note that a numerically lower rank corresponds to higher dominance status. Slope of the linear regression isn't significantly different from 0 (p = 0.82; r^2 = 0, N = 135). **(Bottom)** Deviation from expected aggression for each wasp plotted against her normalised ovarian rank (where a numerically lower rank corresponds to better developed ovaries). Slopes of the linear regressions are not significantly different from zero (ovarian index: p = 0.62; r^2 = 0.007, N = 36; ovarian rank: p = 0.75; r^2 = 0.003, N = 36). [Reprinted with permission from: S Lamba, Y C Kazi, S Deshpande, M Natesh, A Bhadra and R Gadagkar, A possible novel function of dominance behaviour in queen-less colonies of the primitively eusocial wasp *R. marginata*, *Behavioural Processes*, 74: 351–356, 2007.]

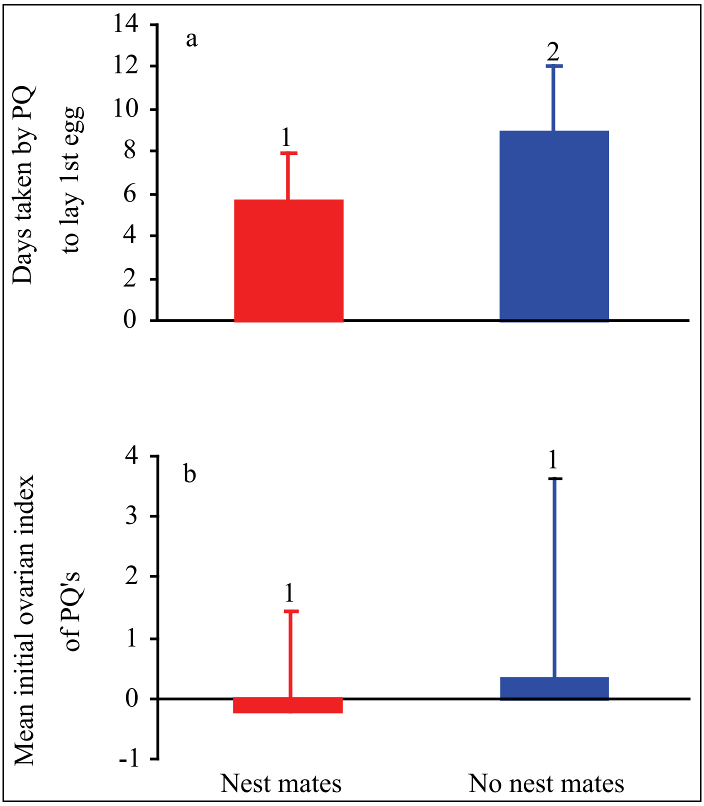

Figure 4. Means and standard deviations of **(a)** the time taken to lay their first eggs, by PQ's with nest mates (red bar: M = 5.62, S.D. = 2.29, N = 13), and those without nest mates (blue bar: M = 8.85, S.D.= 3.18, N = 7) and **(b)** ovarian indices of PQ's with nest mates (red bar: M = -0.23, S.D. = 1.69, N = 8), and without nest mates (blue bar: M = 0.31, S.D. = 3.31, N = 6), on the day of queen removal. Bars with different numbers are significantly different from each other. (See [8] for more details. Reprinted with permission from: S Lamba, Y C Kazi, S Deshpande, M Natesh, A Bhadra and R Gadagkar, A possible novel function of dominance behaviour in queen-less colonies of the primitively eusocial wasp *Ropalidia marginata*, *Behavioural Processes*, 74: 351–356, 2007.)

removing the queen from a nest with two wasps; after the queen is removed the lone remaining wasp is by definition a potential queen. Thus, we removed the queens from 13 nests with two wasps each and 7 nests with more than two wasps each and waited until the potential queens laid their first eggs. A comparison of these times should permit us to test this hypothesis.

Results

Potential queens that had no one to show aggression to, indeed took significantly longer to lay their first eggs compared to the potential queens who had the company of wasps to whom they could show aggression (*Figure* 4 upper panel). Before we conclude that this confirms our prediction and supports the hypothesis that the hyper-aggression of the potential queen serves to hasten the development of her own ovaries, we need to exclude a possible alternate interpretation of the results. It could be that the lone potential queens took longer to develop their ovaries and lay their first eggs because their ovaries were in a poorer condition at the time of queen removal, as compared to those of the potential queens who had the company of other wasps and who laid eggs sooner. This is plausible because the lone potential queen came from a colony with only two wasps and she must have had the full brunt of her queen's inhibition of her ovarian development. Potential queens who had the company of other wasps, on the other hand, came from colonies with several wasps and the effect of the queen may have been diluted. To rule out this possibility, we compared the ovarian indices of lone potential queens and potential queens with company, on the day of queen removal. Needless to say, we did this in a separate set of nests because we needed to dissect the potential queens on the day of queen removal rather than wait to see how long they will take to lay their first eggs. In any case, it turned out to be a false alarm. There was no significant difference in the ovarian indices of potential queens with nestmates and those without nestmates, on the day of queen removal (*Figure* 4, lower panel) [7].

We compared the ovarian indices of lone potential queens and potential queens with company, on the day of queen removal. There was no significant difference in the ovarian indices of potential queens with nestmates and those without nestmates, on the day of queen removal.

Conclusion

We, therefore, concluded that the function of the hyper-aggression of the potential queen is not to behaviourally suppress workers who may be a threat to her, until she is ready with a pheromone bouquet required for her to chemically suppress workers, but that it serves to develop her own ovaries rapidly. I must confess that the hypothesis that the function of the aggression of the potential queen is to suppress other workers seemed the most reasonable hypothesis. On the other hand, the hypothesis that the potential queen needs hyper-aggression for the development of her own ovaries seemed unlikely and counterintuitive. However, since we found no evidence for the reasonable hypothesis, we brought on board the only other hypothesis we could think of, despite its apparent unreasonableness; and it paid off. Our confidence came from the fact that the counterintuitive hypothesis made a counterintuitive prediction. Intuition suggested that if the potential queen had no one to show aggression to, it meant that she had no competition and no need to waste time and energy aggressing anyone; she should be able to develop her ovaries rapidly and lay eggs sooner than a potential queen who had competition. But the hypothesis made the opposite prediction that a lone potential queen should take longer to develop her ovaries and lay eggs. And when the results upheld this counterintuitive prediction, we were convinced that we had hit upon something new. We published a paper with the title 'A possible novel function of dominance behaviour in queen-less colonies of the primitively eusocial wasp *Ropalidia marginata*' [7]. We see once again that dominance behaviour seems to serve yet another function in yet another context. Admittedly, this is the first piece of evidence in support of a new hypothesis and a counterintuitive one at that. We should be cautious and tentative in our conclusion. At the same time, there may well be something to it, and we should not lose the opportunity of following up on a potentially important new lead. The hyper-aggressive potential queens are so impressive and so physically active during the short period of their hyper-aggression that it may well change their physiology. One possible analogy is the effect of exercise on our physiology. These results I hope will persuade people to focus their attention on the physiology of not just the victims of aggression but also on the perpetrators. It is possible that such a shift in attention may yield new insights. As long as we don't become enamoured by our hypothesis and become blind to any counterevidence, we are entirely justified in taking this as far as it can go. Indeed, ignoring it may be foolish. I am, therefore, hoping to launch a new research program on the physiology of the wasps, not only focusing on the victims and perpetrators of aggression but also on the physiology of the queens and workers.

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What is the Function of the Aggression Directed to Non-Nestmates?

Background

The wasps, as expected of any social species, display much more conflict toward members of their species that do not belong to their group, in this case, colony, than to those who belong to their own group. Such low in-group conflict and high out-group conflict is a well-known phenomenon in social animals and is believed to be a fundamental characteristic of sociality and perhaps a facilitator of social evolution.

Having found that aggression, or dominance behaviour, as we refer to it more technically, serves three different functions in three different contexts, we should not lose the opportunity to consider yet another context in which the wasps show aggression, and try to understand its function in that context. The wasps, as expected of any social species, display much more conflict toward members of their species that do not belong to their group, in this case, colony, than to those who belong to their own group. Such low in-group conflict and high out-group conflict is a well-known phenomenon in social animals and is believed to be a fundamental characteristic of sociality and perhaps a facilitator of social evolution. Our wasps are no exception. Indeed, *R. marginata* is probably a particularly striking example of such differential aggression towards insiders and outsiders. Their propensity to suppress conflict and exhibit cooperation within their colonies is truly remarkable. Once the wasps founding new colonies have used a mild form of dominance-subordinate behaviour to decide who will be the queen and who will be the worker, there is very little further evidence of intra-colony conflict. As we have seen, queens are meek and docile and do not need aggression to maintain their reproductive monopoly. Workers similarly use a mild form of dominance behaviour, which can barely be called aggression, to regulate each other's foraging. Even in the context of queen succession, where one might expect the greatest conflict, these wasps, as we have seen in the previous article in this series, are strikingly peaceful [3]. They form long reproductive queues, and a single heir-designate takes over the colony unchallenged when the previous queen dies or is experimentally removed. Our research, not described here, shows that there is much potential conflict even within the colony, but that conflict is so well managed and suppressed that it does not come to the fore [8]. And to see real, violent conflict, one must consider a different context—

encounters between members of different colonies. I will describe below an experiment that we performed to study what might be the most violent form of dominance behaviour that *R. marginata* is capable of showing. And here again, we can inquire about the function of such extreme aggression.

Question: How Do Wasps Treat Non-nestmate Conspecifics?

We set up a simple experiment in an attempt to answer this question. I want to emphasize that in setting up this experiment, we had no hypothesis and no predictions—just a clever experiment and wide-eyed curiosity about what might happen!

Experimental Design

This experiment was performed by my then PhD student Arun Venkataraman (*Figure* 2, lower panel, right). Arun brought three pairs of healthy colonies to the laboratory and transplanted one each into a wood and wire mesh cage, of the kind we have seen in previous articles in this series [2–5]. He ensured that in each pair of nests, the two nests were collected from locations that were at least 10 km apart from each other, to minimise the probability that they were genetically related to each other. In this case, he kept the doors of the cages closed and fed the wasps with the diet of *Corcyra cephalonica* larvae, honey and water. In some experiments, we keep the doors of the cages open and allow the wasps to fly out freely and forage for themselves. But not in this case, and you will soon see why. Once the wasps had established themselves in the cages and had flourishing nests, he made behavioural observations of all the wasps in all the nests. After this, he subjected each pair of nests to the following treatment. One of the nests in a pair of nests was designated as the donor nest, and the other was designated as the recipient nest. He collected all the adult wasps from the donor nest and introduced them into the cage of the recipient nest. Now the cage with the recipient nest thus contained the resident wasps with their own nest and free-floating wasps from the donor nest who were in an alien territory

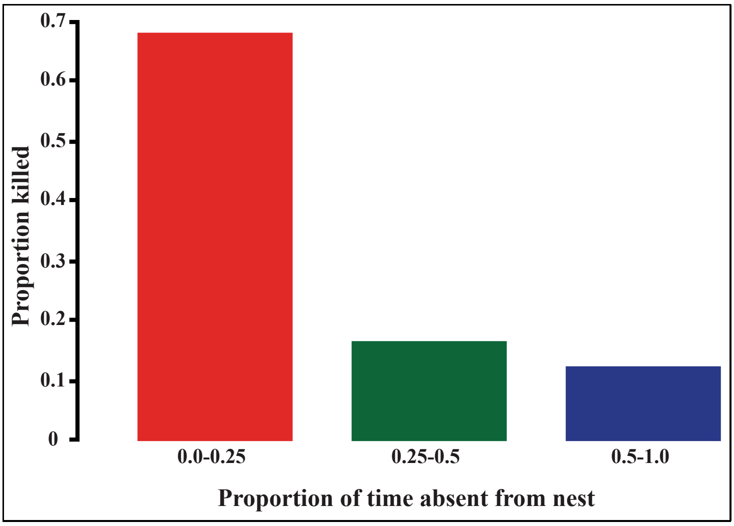

Figure 5. Proportion of wasps killed in each class. The classes are based on the proportion of their time that the wasps spent in being absent from their natal nests prior to introduction. The proportion of animals killed is a function of the class to which they belong ($\chi^2 = 9.05$, $P < 0.05$). The more time the alien wasps had spent away from their natal nests, the less likely they were to be killed by the resident wasps. (Reproduced with permission from: A B Venkataraman and R Gadagkar, Differential aggression towards alien conspecifics in a primitively eusocial wasp, *Current Science*, 64: 601–603, 1993.)

and did not have their own nest—let us call them the alien wasps.

Results

Our interest was to see how the residents treated the aliens. Consistent with our previous knowledge, the young aliens were readily accepted onto the nest of the residents. We know from subsequent experiments [10] that the accepted young aliens will go on to become indistinguishable members of the resident colonies and that they can go on to become foragers or even future queens in these resident nests. There is no evidence that they are subsequently treated differently or treat the residents any differently—they seem to get completely integrated, losing their alien identity altogether. The older aliens were treated very differently. Among them, the alien queen was selectively attacked, dismembered and killed. This treatment of the alien queen, which was witnessed in one of the resident nests, could not, unfortunately, be confirmed in the other two resident nests; in the other two cases, either the queen died before introduction or no queen could be discerned, at the time of introduction. But the behaviour of the residents to the alien queen was striking and unmistakable in the one nest where Arun had the opportunity to observe it.

It was not only the queen that was killed. Of the 81 aliens introduced, 8 were accepted, 13 were killed, and the remaining 60 were allowed to live as long as they were away from the nest of the residents. We investigated the reasons why some individuals were killed while others were spared. Of all the factors we examined, the only significant factor turned out to be the proportion of their time that the alien wasps had spent on their own nests prior to the introduction. Those aliens who had spent more time on their own nests had a higher probability of being killed compared to those wasps who had spent more time away from their own nests (*Figure* 5). There are several mutually overlapping interpretations of this differential treatment that the aliens got. At the proximate level, wasps that spend more time on their nests may acquire stronger cues (smell) that identify them with their nests, and wasps that spend more time away from their nests may have a weaker cue. When introduced into alien territory, the wasps that have a strong smell of their own nest may be more easily recognised as aliens compared to wasps that have a weak smell of their own nest. At the ultimate, evolutionary level, wasps who have spent more time away from their nests may be more likely to be foragers and perhaps less of a threat to the resident wasps; they may even be perceived as useful. Thus it appears that the resident wasps treated aliens in proportion to the threat that they might experience from them, accepting the young aliens, showing extreme aggression to the alien queen, killing some of the alien workers who might pose a greater threat and tolerating others who might pose a lesser threat or whose alien-ness may not be that obvious.

In the context of our discussion in the last few articles in this series, this experiment reveals yet another function of aggression, or dominance behaviour. Dominance behaviours seen among nestmates, either in the context of new nest foundation, in the context of workers in mature colonies and even in the context of a hyper-aggressive potential queen, all appear to be rather ritualized and almost never cause physical injury. Dominance behaviour towards aliens is quite different—it is designed to kill. We are not entirely sure about the specific behaviours used to kill, but I believe it is reasonable to think of the highly ritualized dominance

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behaviour shown by workers while regulating each other's foraging as being at one extreme and the behaviour used to kill aliens posing a threat as being at the other extreme, of a continuum of aggressiveness.

In summary, dominance behaviour is used in four different contexts for four different functions—to decide who would be the queen during new nest foundation, to convey hunger signals and regulate foraging in mature colonies, to boost the ovarian development of the aggressor in the context of the potential queen and to kill aliens wasps in the context of interaction with non-nestmates.

In summary, we have so far seen that dominance behaviour is used in four different contexts for four different functions—to decide who would be the queen during new nest foundation, to convey hunger signals and regulate foraging in mature colonies, to boost the ovarian development of the aggressor in the context of the potential queen and to kill aliens wasps in the context of interaction with non-nestmates.

My goal now is to make a detailed description of the behaviours used in these different contexts and to understand how dominance behaviour is subtly modified to suit the required function. As you can guess, I am looking for a student who will help me conduct this study with the kind of passion that all my students have taught me to expect.

Reflections

In addition to flagging the by now familiar criteria of being simple and low cost, I wish to take this opportunity to reflect on two additional lessons we can learn from the experiments described in this article, one from each experiment.

The experiment designed to understand the function of the potential queen's hyper-aggression involved testing the predictions of clear-cut hypotheses. The first and most plausible hypothesis we tested was that the PQ's hyper-aggression serves to suppress other workers and prevent them from becoming queens and is used temporarily until she has an adequate pheromone profile. All three predictions of this hypothesis were not supported. At this point, we had two things to consider. First, there may be other, even better predictions that we have not tested, but we could not immediately come up with any. Second, the alternate hypothesis that we brought to the table was very counter-intuitive. It proposed that the PQ's hyper-aggression was less meant to suppress other workers and more important for the PQ herself to boost her ovarian development rapidly. We justified this hypothesis to ourselves, saying that it is not so unreasonable after all and likening it to the effect of exercise on physiology. We also reassured ourselves that we were testing very counter-intuitive predictions and therefore it justified a counter-intuitive hypothesis. The counter-intuitive prediction was that a lone PQ with no one to show aggression to should take longer rather than less time, to develop her ovaries and lay her first egg. This single counter-intuitive prediction of this counter-intuitive hypothesis was in fact upheld.

Now, how much confidence should we have in ruling out the first intuitive hypothesis and accepting the second counter-intuitive hypothesis? If the second hypothesis was, in fact, correct, we would have made an important discovery, namely a novel function of aggression. We could also potentially move the field of the physiology of aggression in a new direction by suggesting that more attention should be paid to the physiology of the perpetrators of aggression and not just of the victims. It would be silly to miss this opportunity. In science, we are often faced with this dilemma—a trade-off between the need to be cautious and the fear of missing the boat. There will always be pressure, real or imagined, to throw caution to the wind, bury the intuitive hypothesis, hype up the counter-intuitive hypothesis as a bold discovery and publish in a high-profile journal. And yet, the dilemma is easy to resolve—cautiously and tentatively reject the intuitive hypothesis and even more tentatively and cautiously accept and examine the consequences of the discovery, even if it means publishing in a low-profile journal. There is no real dilemma if we accept that science is always work in progress—new facts and new research in the hands of new people can always overturn previous findings. In the present case, our current thinking is that the hyper-aggression of the PQ might serve both functions—to suppress nestmates as well as to boost her own ovarian development. I have recently proposed a model that posits both functions, and we are now ready to embark on a new program of testing the model by beginning to investigate the physiology of social behaviour in *R. marginata* [11].

There are different ways of doing science. We can list at least four of them—(i) Hypothesis testing, (ii) Discovery science, (iii) Inventorying and (iv) Exploration. We need different strategies to understand Nature—one size does not fit all.

The experiment designed to understand the function of aggression towards non-nestmates was of a very different kind. In contrast to the previous experiment, it did not test any hypothesis at all. Is this a problem? Does that make it poor science, or bad science? There are different ways of doing science. We can list at least four of them—(i) Hypothesis testing, (ii) Discovery science, (iii) Inventorying and (iv) Exploration. We need different strategies to understand Nature—one size does not fit all. Unfortunately, there is a prevailing social hierarchy among these different ways of doing science. Hypothesis testing has become far too fashionable and prestigious to be good for the healthy growth of science. Hypothesis testing is often considered the most exalted way of doing science and those who practice this method sometimes look down upon other methods and consider anything else to be inferior and perhaps not science at all. Lower down in the pecking order is discovery science. This involves attempting to answer well-defined questions but with no clue about what the answer might be and hence with no a priori hypotheses and no predictions to test. Our experiment to understand the function of aggression towards non-nestmates falls in this category. Still, lower down the prestige scale is inventorying, which everyone likes to trample on and disparagingly call it "mere stamp collecting". But a little reflection will tell us that taxonomy is in this category. No biology is possible without taxonomy. Lowest in the prestige scale is exploration. This involves, as the name implies, exploration. Let alone hypotheses and predictions, there are not even well-defined questions. Much of natural history, which we all recognise as the backbone of biology, falls in this category—let's explore the forest and see what we might find!

The most appropriate strategy for a given situation depends on the state of our knowledge in that domain.

The truth of the matter is that we need all these strategies to do good science. The most appropriate strategy for a given situation depends on the state of our knowledge in that domain. If we know nothing, we cannot but begin with exploration. When we do know something, inventorying becomes useful and necessary. Exploration and inventorying will often give us enough knowledge to begin to ask questions but not yet any hypotheses or predictions. At this juncture, we need discovery science. As discoveries accumulate, we can begin to come up with testable hypotheses and predictions. It is, therefore, reasonable to expect that research in a new branch of science begins with exploration, followed by inventorying and then discovery science and finally, hypothesis testing. This is perhaps the reason why prestige increases as we go from exploration to inventorying, to discovery science, to hypothesis testing. The difference in prestige for these different kinds of science is very real—it is reflected in the ease of getting funding, getting jobs and the journals in which we can publish. But this differential prestige is misguided, and it serves to establish the hegemony of well-endowed research groups capable of doing expensive research and drive less-financially endowed research groups out of business.

However, it is important to advance the frontiers of human knowledge, but it matters less where one does so. Why should further advancing knowledge in an already advanced field be more prestigious than doing so in a nascent field of research? If anything, advancing knowledge in a nascent field should be more prestigious. More importantly, we should use the method that is suitable to the domain we are working in. If everybody is in a mad rush to do what is more prestigious, and what is more expensive, then several problems arise—some nascent areas of science get neglected (think taxonomy and natural history); those who do not have adequate resources and facilities attempt to do expensive science and end up doing poor science; we end up applying more prestigious methods such as hypothesis testing in a nascent field of research where we do not have adequate background knowledge to be able to come up with good hypotheses and robust predictions, and so on. For the healthy growth of science and for equal opportunities for many different researchers, institutions and nations to participate in cutting-edge science, we need to reassign prestige to the production of significant new knowledge without regarding to which method is used to do so how much money is spent in doing so.

For the healthy growth of science and for equal opportunities for many different researchers, institutions and nations to participate in cutting-edge science, we need to reassign prestige to the production of significant new knowledge without regarding to which method is used to do so how much money is spent in doing so.

This article concludes the set of five articles devoted to the Indian paper wasp *Ropalidia marginata* and have drawn upon experiments conducted in my laboratory. You might recall that the set of five articles prior to these were drawn from the wider literature but they also had insects as their protagonists. This may give the impression that simple inexpensive experiments requiring no sophisticated laboratory and other facilities can only be performed on insects. Admittedly, insects are wonderfully convenient organisms to conduct cutting-edge experimental research at a trifling cost. And yet, equally simple and inexpensive experiments can also be conducted with higher animals. Perhaps one may have to think harder to meet the criteria of 'cutting-edge' and 'trifling cost'. I will, therefore, devote the next several articles to experiments conducted on vertebrates, from fish to humans.

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Suggested Reading

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